Masuoka S*, Nishio J*, Yamada S*, Saito K, Kaneko K*, Kaburaki M*, Tanaka N*, Sato H*, Muraoka S*, Kawazoe M*, Mizutani S*, Furukawa K*, Ishii-Watabe A, Kawai S*, Saito Y, Nanki T*: Relationship Between the Lipidome Profile and Disease Activity in Patients with Rheumatoid Arthritis.

Inflammation. 2024;47(4):1444-1458. doi: 10.1007/s10753-024-01986-8.

Lipid mediators have been suggested to play important roles in the pathogenesis of rheumatoid arthritis (RA). Lipidomics has recently allowed for the comprehensive analysis of lipids and has revealed the potential of lipids as biomarkers for the early diagnosis of RA and prediction of therapeutic responses. However, the relationship between disease activity and the lipid profile in RA remains unclear. In the present study, we performed a plasma lipidomic analysis of 278 patients with RA during treatment and examined relationships with disease activity using the Disease Activity Score in 28 joints (DAS28)-erythrocyte sedimentation rate (ESR). In all patients, five lipids positively correlated and seven lipids negatively correlated with DAS28-ESR. Stearic acid [FA(18:0)] ($r = -0.45$) and palmitic acid [FA(16:0)] ($r = -0.38$) showed strong negative correlations. After adjustments for age, body mass index (BMI), and medications, stearic acid, palmitic acid, bilirubin, and lysophosphatidylcholines negatively correlated with disease activity. Stearic acid inhibited osteoclast differentiation from peripheral blood monocytes in *in vitro* experiments, suggesting its contribution to RA disease activity by affecting bone metabolism. These results indicate that the lipid profile correlates with the disease activity of RA and also that some lipids may be involved in the pathogenesis of RA.

Keywords: biomarker, lipidomics, rheumatoid arthritis

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Takahashi K*, Kawaguchi S*, Ikeda T*, Tomonari Y*, Funakoshi T*, Nakai K*, Fujimoto T*, Yamamoto D*, Okamura T*, Uchida H*, Saito Y, Otake S*: Effects of microsampling on toxicity evaluation of 1-naphthylisothiocyanate (ANIT), a hepatotoxic substance, in a mouse toxicity study.

J Toxicol Sci. 2023;48(11):607-615. doi: 10.2131/jts.48.607.

ICH S3A Q&A focused on microsampling (MS) was published to help accelerate the use of MS and states that MS is useful because toxicokinetic (TK) evaluation with conventional blood sampling volume requires many animals for TK satellite groups; however, there are few reports of MS application in mice. We investigated the influence of MS on toxicity evaluation in mice by comparing the toxicity parameters with and without MS after a single oral administration of 1-naphthylisothiocyanate (ANIT), a hepatotoxic substance. Blood samples (50 μL/point) were collected from the tail vein of 3 mice per group at 2 or 3 time points during a 24-hr period, and toxicity was evaluated 2 days after administration. ANIT-related changes suggesting liver or gallbladder injury were noted in blood chemistry and histopathology. Some of these changes such as increases in focal hepatocyte necrosis and inflammatory cell infiltration in the liver as well as mucosal epithelium necrosis in the gallbladder were apparently influenced by MS. A tendency to anemia was noted in animals with MS but not without MS, which was also noted in the vehicle-treated controls, suggesting influence of blood loss. The current results indicate that ANIT hepatotoxicity could be evaluated in mice in which blood samples were collected by MS for most parameters; however, parameters in anemia and pathology in the liver and gallbladder were influenced by MS in this study condition with ANIT. Therefore, MS application in mice should be carefully considered.

Keywords: microsampling, mouse, toxicity

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Koinuma K*[1], Noto K*[1], Morita T, Uekusa Y*[1], Kikuchi H*[1], Shimoji M*[2], Seki H*[1], Yamazaki H*[3], Guengerich FP*[4], Nakamura K*[5], Yamamoto K*[1], Imaoka A*[1], Akiyoshi T*[1], Ohtani H*[1,6]: Kinetics of the inhibition of CYP3A4 and CYP2C19 activity by jabara juice and identification of the responsible inhibitory components.

J Pharm Sci. 2025;114(2):849-856. doi: 10.1016/j.xphs.2024.10.037.

Some citrus fruits are known to cause clinically significant drug interactions by inhibiting intestinal

cytochrome P450 (CYP) enzymes. This *in vitro* study aimed to investigate the kinetics of the inhibition of CYP3A4 and CYP2C19 by the juice of jabara, a Japanese citrus fruit that does not contain furanocoumarins such as 6',7'-dihydroxybergamottin, and to identify the inhibitory compound(s). CYP3A4 and CYP2C19 activity levels were determined *in vitro* using recombinant CYP preparations and their respective substrates. The ethyl acetate extract (EAE) of jabara juice was separated to isolate and identify the compound(s) that inhibited CYP3A4. Then, the time-dependent kinetics of the inhibition of CYP3A4 and CYP2C19 by the EAE and its inhibitory compound(s) were analyzed. The EAE of jabara juice was found to inhibit CYP3A4 in a time-dependent manner. Two flavonoids, 3,3',4',5,6,7,8-heptamethoxyflavone (HpMF) and 3,3',4',5,6,7-hexamethoxyflavone (HxMF), were identified as the responsible compounds. HpMF and HxMF inhibited CYP3A4 activity in a concentration- and time-dependent manner, with inhibition constants (K_I) of 10.0 and 7.90 μM and maximal inactivation rate constants ($k_{inact,max}$) of 0.00856 and 0.0134 min^{-1}, respectively. The EAE did not inhibit CYP2C19, even when preincubation was employed. These findings imply that jabara juice may cause food-drug interactions via time-dependent inhibition of intestinal CYP3A4.

Keywords: cytochrome P450, food interactions, inhibition

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Morita T, Yoshida H, Tomita N, Sato Y: Comparison of *in vitro* screening methods for evaluating the effects of pharmaceutical excipients on membrane permeability.
Int J Pharm. 2024;665:124727. doi: 10.1016/j.ijpharm.2024.124727.

The effects of pharmaceutical excipients on intestinal drug absorption have been highlighted and careful excipient selection is required to develop biologically equivalent formulations. This study aimed to evaluate the effects of excipients on drug permeability and compare the characteristics of *in vitro* screening methods. Three *in vitro* models, the commercial precoated parallel artificial membrane permeability assay (PAMPA), PermeaPad™, and Caco-2 monolayer, were used to evaluate the effects of 14 excipients on the permeability of several drugs with different biopharmaceutical classification system classes. Concentration-dependent effects were analyzed to distinguish non-specific effects. The permeability of low-permeability drugs was increased by excipients such as hydroxypropyl cellulose and povidone K30 in the precoated PAMPA model, whereas PermeaPad™ maintained membrane integrity at higher concentrations. Conversely, croscarmellose sodium and sodium lauryl sulfate (SLS) decreased the permeability of highly permeable drugs in both precoated PAMPA and PermeaPad™ assays in a concentration-dependent manner. In Caco-2 monolayer assays, most excipients showed minimal effects on drug permeability. However, SLS significantly reduces the permeability of highly permeable drugs at concentrations above the critical micelle concentration, thereby compromising the integrity of the cell monolayer. Our results suggested that most of excipients, except SLS, did not affect the membrane permeation of drugs at clinically used concentrations. The pre-coated PAMPA model demonstrated high sensitivity to excipient effects, making it suitable for conservative evaluation. The PermeaPad™ and Caco-2 models allowed assessment at higher excipient concentrations, with PermeaPad™ being particularly useful for excipients that cause toxicity in Caco-2 cells.

Keywords: drug interaction, membrane permeability, pharmaceutical excipient

Izutsu K*, Yoshida H, Abe Y, Yamamoto E, Sato Y, Ando D: Application of the thermal analysis of frozen aqueous solutions to assess the miscibility of hyaluronic acid and polymers used for dissolving microneedles.
Pharmaceutics. 2024;16(10):1280. doi:10.3390/pharmaceutics16101280.

Background: The combination of multiple polymers is anticipated to serve as a means to diversify the physical properties and functionalities of dissolving microneedles. The mixing state of components is

considered as a crucial factor in determining their suitability. Objectives: The purpose of this study was to elucidate whether thermal analysis of frozen aqueous solutions can appropriately predict the miscibility of hyaluronic acid (HA) and other polymers used for dissolving microneedles prepared by a micromolding method. Methods: Aliquots of aqueous polymer solutions were applied for thermal analysis by heating the samples from −70℃ at 5℃/min to obtain the transition temperature of amorphous polymers and/or the crystallization/melting peaks of polymers (e.g., polyethylene glycol (PEG)). Films and dissolving microneedles were prepared by air-drying of the aqueous polymer solutions to assess the polymer miscibility in the solids. Results: The frozen aqueous single-solute HA solutions exhibited a clear Tg′ (the glass transition temperature of maximally freeze-concentrated solutes) at approximately −20℃. The combination of HA with several polymers (e.g., dextran FP40, DEAE-dextran, dextran sulfate, and gelatin) showed a single Tg′ transition at temperatures that shifted according to their mass ratio, which strongly suggested the mixing of the freeze-concentrated solutes. By contrast, the observation of two Tg′ transitions in a scan strongly suggested the separation of HA and polyvinylpyrrolidone (PVP) or HA and polyacrylic acid (PAA) into different freeze-concentrated phases, each of which was rich in an amorphous polymer. The combination of HA and PEG exhibited the individual physical changes of the polymers. The polymer combinations that showed phase separation in the frozen solution formed opaque films and microneedles upon their preparation by air-drying. Coacervation occurring in certain polymer combinations was also suggested as a factor contributing to the formation of cloudy films. Conclusions: Freezing aqueous polymer solutions creates a highly concentrated polymer environment that mimics the matrix of dissolving microneedles prepared through air drying. This study demonstrated that thermal analysis of the frozen solution offers insights into the mixing state of condensed polymers, which can be useful for predicting the physical properties of microneedles.

Keywords: hyaluronic acid, freeze concentration, microneedle

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Yoshida H, Teruya K*1, Abe Y, Furuishi T*1,2, Fukuzawa K*1,3, Yonemochi E*1,4, Izutsu K*4: Effects of Glass Bead Size on Dissolution Profiles in Flow-through Dissolution Systems (USP 4).
AAPS PharmSciTech. 2024;25(8):251. doi:10.1208/s12249-024-02972-x.

The effects of glass bead size in the conical space of flow-through cells on the dissolution profiles were investigated in a USP apparatus 4. Dissolution tests of disintegrating and non-disintegrating tablets in flow-through dissolution systems were performed using semi-high precision glass beads with diameters ranging from 0.5 mm to 1.5 mm. Computational fluid dynamics (CFD) was used to evaluate the effect of shear stress from the dissolution media flow. The use of smaller glass beads in a larger cell resulted in a faster dissolution of the model formulations under certain test conditions. The effect on the dissolution was highly dependent on the size of the beads in the top layer, including those in contact with the tablets. The absence of a bead-size effect on the dissolution of an orodispersible tablet in a small cell can be explained by the floating fragments during the test. CFD analysis showed that smaller bead diameters led to greater shear stress on the tablet, which was correlated with the dissolution rate. Hence, fluid flow through the narrow gaps between the small beads generated strong local flows, causing shear stress. The size of the glass beads used in flow-through cells affects the dissolution rate of tablets by altering the shear stress on the tablets in certain cases (e.g., direct deposition of the formulation on glass beads, large cells, and very low flow rates). Thus, glass bead size must be considered for a robust dissolution test in a flow-through cell system.

Keywords: flow-through cell system, glass bead size, hydrodynamics

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Sato T*, Haneishi K*, Hisada H*, Fujii M*, Koide T, Fukami T*: Real-time drug quantitative evaluation

in liposome preparation process using probe-type Raman spectrometer.

Langmuir. 2024;40:7962-73. doi:10.1021/acs.langmuir.3c03872.

During the manufacturing process of liposome formulations, it is considered difficult to evaluate their physicochemical properties and biological profiles due to the complexity of their structure and manufacturing process. Conventional quality evaluation is labor-intensive and time-consuming; therefore, there was a need to introduce a method that could perform in-line, real-time evaluation during the manufacturing process. In this study, Raman spectroscopy was used to monitor in real time the encapsulation of drugs into liposomes and the drug release, which are particularly important quality evaluation items. Furthermore, Raman spectroscopy combined with partial least-squares (PLS) analysis was used for quantitative drug evaluation to assess consistency with results from UV-visible spectrophotometry (UV), a common quantification method. The prepared various ciprofloxacin (CPFX) liposomes were placed in cellulose tubes, and a probe-type Raman spectrophotometer was used to monitor drug encapsulation, the removal of unencapsulated drug, and drug release characteristics in real time using a dialysis method. In the Raman spectra of the liposomes prepared by remote loading, the intensities of the CPFX-derived peaks increased upon drug encapsulation and showed a slight decrease upon removal of the unencapsulated drug. Furthermore, the peak intensity decreased more gradually during the drug release. In all Raman monitoring experiments, the discrepancy between quantified values of CPFX concentration in liposomes, as measured by Raman spectroscopy combined with partial least-squares (PLS) analysis, and those obtained through ultraviolet (UV) spectrophotometry was within 6.7%. The results revealed that the quantitative evaluation of drugs using a combination of Raman spectroscopy and PLS analysis was as accurate as the evaluation using UV spectrophotometry, which was used for comparison. These results indicate the promising potential of Raman spectroscopy as an innovative method for the quality evaluation of liposomal formulations.

Keywords: liposome, Raman spectroscopy, ciprofloxacin

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Miyazaki T, Takeda Y*, Ando D, Koide T, Sato Y, Yamamoto E: Measurement of the particle density of small amounts of pharmaceutical powders using high-contrast micro X-ray computed tomography.

Powder Technol. 2025;457:120929. doi: 10.1016/j.powtec.2025.120929.

Particle density is a fundamental and important physical property of powders. However, the widely used gas displacement pycnometry (GDP) method typically requires sample volumes in the gram range. In this study, we developed a method for evaluating the density of milligram-scale samples using X-ray computed tomography (XRCT). We used pharmaceutical powders, consisting of organic and light metallic elements, as subjects. The volumes of 24 pharmaceutical powders (2-160 mg) with various particle sizes and shapes were measured using an XRCT device with a resolution of 0.65-2.6 μm (field of view: 1.33-5.32 mm). Copper and molybdenum targets were used as X-ray sources, providing high-contrast imaging for materials with low electron densities. The densities obtained using XRCT correlated well with those obtained using GDP, as indicated by a linear regression line with a slope of 1.0 passing through the origin. The coefficient of variation for six sequential measurements was 0.0070, suggesting high repeatability. Additionally, we investigated optimal experimental conditions, such as spatial resolution, X-ray sources, and measurement time, to enhance the quality of three-dimensional XRCT images. We found that images with a grayscale histogram peak separation of approximately one between the sample and other components (sample tube and air) yielded optimal results. This non-destructive technique has the potential to accurately measure the densities of small sample quantities and can contribute not only to the pharmaceutical field but also to other industries handling organic and light metallic powders.

Keywords: particle density, X-ray computed tomography, volume measurement

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Takahashi K*, Akiyama K*, Horita K*, Sakamoto T, Satozono H*: Real-time monitoring of Hydration

reaction of Theophylline Anhydrous via Terahertz Attenuated Total Reflection Time Domain Spectroscopy.

J Infrared Millim Terahertz Waves. 2024;45:444-453. doi:10.1007/s10762-024-00986-x

In pharmaceuticals, pseudo-polymorphism, e.g., the existence of hydrate and anhydrous forms, affects their physicochemical characteristics. Therefore, the evaluation of pseudo-polymorphism is one of the most important quality analyses. In this research, we investigate the real-time monitoring of the hydration reaction of theophylline using terahertz attenuated total reflection time domain spectroscopy (THz-attenuated total reflection (ATR)-TDS). We continuously measured a mixture of hydroxypropyl cellulose solution and theophylline anhydrous (TPA) while keeping it pressed to the ATR surface. We observed that the absorption peaks derived from TPA decreased and those derived from theophylline monohydrate (TPM) increased with time, demonstrating that the hydrate reaction of TPA can be monitored. Subsequently, we performed an accurate and quantitative evaluation of the hydration reaction by calculating the temporal changes in the crystal form ratio of TPM based on the changes in its second derivative peak intensity followed by a curve fitting. In addition, we performed real-time monitoring of the reaction using two different pressure mechanisms, finding that using a weight to apply pressure provided better reproducibility than using a screw. This study demonstrates that THz spectroscopy is a useful method for the evaluation of pseudo-polymorphism in pharmaceuticals.

Keywords: terahertz spectroscopy, attenuated total reflection, pseudo-polymorphism

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Nakayama K*1, Sahara J*2, Fujimoto M*1, Yagisawa Y*1, Kobata K*2, Kawagoe H*2, Ikarashi A*2, Yokoyama T*2, Sakamoto T: Quantification of API content in pharmaceutical tablets within milliseconds by time-stretch near-infrared transmission spectroscopy.

J Pharm Biomed Anal. 2024;249:116372. doi: 10.1016/j.jpba.2024.116372

We explored the feasibility of high-speed and high-accuracy quantification of active pharmaceutical ingredient (API) content in tablet products by near-infrared (NIR) spectroscopy to improve the reliability of pharmaceuticals. For this purpose, we employed a high-power NIR time-stretch transmission spectrometer recently developed by us. By using this transmission spectrometer with a multivariate calibration model, we demonstrated the ability to quantify API content with a short measurement time of 3.9 ms per tablet for model pharmaceuticals. For the model tablet, the quantification ability of our spectrometer was comparable to that achieved by a commonly used Fourier-transform NIR (FT-NIR) spectrometer with a measurement time of several seconds. We also confirmed that the effect of irradiating tablets with the NIR pulses used in our spectrometer was negligible.

Keywords: near-infrared spectroscopy, high-speed measurement, time-stretch spectroscopy

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Shimura K*1, Sasaki T*2, Ono T*1, Mohara, M. *1, Aiko, K*1, Sakamoto T: Terahertz Frequency-Domain Spectroscopy with a Method for Suppressing Water Vapor Absorption Peaks for Analysis of Pharmaceutical Hydrate Samples.

J Infrared Millim Terahertz Waves. 2024;45:868-882. doi:10.1007/s10762-024-01004-w

Measurements of terahertz absorption spectra of pharmaceutical hydrate samples are achieved under a normal humidity condition by combining terahertz frequency-domain spectroscopy with a newly proposed method for suppressing absorption peaks caused by water vapor. In this method, only simple mathematical operations such as subtraction, thresholding, interpolation, and smoothing are applied to extinction (or absorbance) data obtained by locating samples under a normal humidity condition. By considering the difference in spectral line width between narrow absorption peaks caused by water vapor and the relatively wide absorption peaks caused by active pharmaceutical ingredients (APIs) in solid forms, the absorption peaks caused by water vapor can be effectively suppressed without affecting the absorption peaks of the APIs in the samples. In the present study,

levofloxacin hydrates were used as samples to investigate the performance of the proposed method. Spectra were obtained under both dry and normal humidity conditions. The temperature of the samples was raised from 300 to 363 K to dehydrate them and brought back to 313 K to observe hydration under the normal humidity condition. Spectra obtained under the normal humidity condition were processed with the proposed method. The spectra of the hydrates obtained under the dry condition were slightly different from those obtained under the normal humidity condition and processed by our method. Dehydration during the measurements under the dry condition was suggested. Stable and reliable results are expected by measuring spectra under normal humidity conditions and applying the proposed method to suppress absorption peaks by water vapor.

Keywords: terahertz frequency-domain spectroscopy, water vapor, hydrates

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Akiba H[*1,2], Ise T[*2], Satoh R[*2], Abe Y, Tsumoto K[*3], Ohno H[*1,2], Kamada H[*1,2], Nagata S[*2]: Generation of antagonistic biparatopic anti-CD30 antibody from an agonistic antibody by precise epitope determination and utilization of structural characteristics of CD30 molecule.

Antib Ther. 2025;8(1):56-67. doi: 10.1093/abt/tbaf002.

Background: CD30 is a member of the tumor necrosis factor receptor superfamily. Recently, blocking CD30-dependent intracellular signaling has emerged as potential strategy for immunological regulation. Development of antibody-based CD30 antagonists is therefore of significant interest. However, a key challenge is that the bivalent form of natural antibody can crosslink CD30 molecules, leading to signal transduction even in the absence of specific ligand, CD153. Biparatopic antibodies (BpAbs) offer a solution, using two different variable fragments (Fvs) to bind distinct epitopes on a single antigen molecule. BpAbs format is an attractive alternative of natural antibody by potentially avoiding unwanted crosslinking and signaling induction.

Methods: We systematically characterized 36 BpAbs, each designed with pairs of Fvs binding to nine distinct epitopes across the CD30 extracellular domain. We first identified the precise epitope sites of the nine antibodies by assessing the binding to multiple orthologous CD30 proteins and mutants. We then produced the 36 BpAbs and analyzed their biological activities and binding modes.

Results: Among 36 BpAbs, we identified both potent ligand-independent agonists and ligand-blocking antagonists, with many displayed reduced signal activation, including 1:1-binding antagonists derived from AC10, a strong agonist developed for lymphoma therapy. Epitope dependency in reduced signaling activity was observed and associated with the flexible nature of CD30 protein.

Conclusions: We successfully developed antagonistic BpAbs against CD30 by controlling the stoichiometry of antibody-antigen binding mode. This study elucidated the mechanism of signaling induction, informing the design strategies of the development of biparatopic antibodies.

Keywords: CD30 receptor, biepitopic antibody, biparatopic antibody

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Yamamoto T[†], Nakayama J[*1,2,†], Urabe F[*1], Ito K[*1], Nishida-Aoki N[*3], Kitagawa M[*4], Yokoi A[*4,5], Kuroda M[*1], Hattori Y[*6], Yamamoto Y[*1], Ochiya T[*7]: Aberrant regulation of serine metabolism drives extracellular vesicle release and cancer progression.

Cell Rep. 2024; 43: 114517. DOI: 10.1016/j.celrep.2024.114517

Cancer cells secrete extracellular vesicles (EVs) to regulate cells in the tumor microenvironment to benefit their own growth and survive in the patient's body. Although emerging evidence has demonstrated the molecular mechanisms of EV release, regulating cancer-specific EV secretion remains challenging. In this study, we applied a microRNA library to reveal the universal mechanisms of EV secretion from cancer cells. Here, we identified miR-891b and its direct target gene, phosphoserine aminotransferase 1 (PSAT1), which promotes EV secretion through the serine-ceramide synthesis pathway. Inhibition of PSAT1

affected EV secretion in multiple types of cancer, suggesting that the miR-891b/PSAT1 axis shares a common mechanism of EV secretion from cancer cells. Interestingly, aberrant PSAT1 expression also regulated cancer metastasis via EV secretion. Our data link the PSAT1-controlled EV secretion mechanism and cancer metastasis and show the potential of this mechanism as a therapeutic target in multiple types of cancer.

Keywords: extracellular vesicles, exosome

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Tominaga M*1, Shima Y*1, Nozaki K*1, Ito Y*1, Someda M*2, Shoya Y*2, Hashii N, Obata C, Matsumoto-Kitano M*1, Suematsu K*1, Matsukawa T*1, Hosoya K*1, Hashiba N*1, Kondo A*1,3, Ishii J*: Designing strong inducible synthetic promoters in yeasts.

Nat Commun. 2024 15(1):10653. DOI: 10.1038/s41467-024-54865-z

Inducible promoters are essential for precise control of target gene expression in synthetic biological systems. However, engineering eukaryotic promoters is often more challenging than engineering prokaryotic promoters due to their greater mechanistic complexity. In this study, we describe a simple and reliable approach for constructing strongly inducible synthetic promoters with minimum leakiness in yeasts. The results indicate that the leakiness of yeast-inducible synthetic promoters is primarily the result of cryptic transcriptional activation of heterologous sequences that may be avoided by appropriate insulation and operator mutagenesis. Our promoter design approach has successfully generated robust, inducible promoters that achieve a > 103-fold induction in reporter gene expression. The utility of these promoters is demonstrated by using them to produce various biologics with titers up to 2 g/L, including antigens designed to raise specific antibodies against a SARS-CoV-2 omicron variant through chicken immunization.

Keywords: expression systems, applied microbiology, synthetic biology

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Hashii N, Obata C, Okada M*1, Nakamura S*2, Fukazawa K*2, Watanabe S*3, Ishii-Watabe A: Multi-attribute method analysis of therapeutic monoclonal antibodies using an automated sample preparation system.

J Pharm Biomed Anal. 2025:253:116542. DOI: 10.1016/j.jpba.2024.116542

The multi-attribute method (MAM) has attracted increased attention as an alternative strategy for evaluating structural heterogeneity using conventional separation techniques such as ion-exchange chromatography and capillary electrophoresis of therapeutic monoclonal antibodies (mAbs). One of the remaining challenges for the practical use of the MAM is reliable and robust continuous monitoring. In this study, we successfully established an automated sample preparation system as a solution to this issue. Through method optimization, we confirmed that the peptide purification step using a solid-phase extraction column, which is usually performed after the digestion step, was not mandatory and that the addition of methionine as an oxidation inhibitor was able to significantly reduce artificial oxidation. Importantly, the use of our system enabled high-precision analysis for targeted peptide monitoring without relying on the operator's knowledge and experience with peptide mapping using liquid chromatography/mass spectrometry (LC/MS). Our system could also be useful as a platform approach for targeted peptide monitoring in MAM workflow of traditional IgG-type mAbs. Furthermore, using common samples that were prepared using the automated system, we assessed the compatibility of LC/MS system in the targeted peptide monitoring via a collaborative study with MS vendors. The results showed that there was no significant difference in the mass accuracy, repeatability of the peak retention time and variations of intermediate precision of the measurement values among the four LC/MS systems, suggesting sufficient compatibility

among the LC/MS systems. MAM system using our automated sample preparation method, which had high intermediate precision, will be useful for release and stability testing as well as characterization and manufacturing process development.

Keywords: multi-attribute method, automated sample preparation, monoclonal antibody

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Sawada H*1,2, Hattori I*1,2, Hashii N, Saito T*3: Involvement of Metalloproteases in the Fertilization of the Ascidian Halocynthia roretzi.

Biomolecules. 2024;14(12):1487. DOI: 10.3390/biom14121487

We previously reported that five astacin-like metalloproteases with thrombospondin type-1 repeats (Tasts) located on the sperm surface are a promising candidate as the protease involved in sperm penetration of the vitelline coat (VC) during fertilization of the ascidian Ciona intestinalis type A (Phlebobranchia). However, whether such a protease is involved in the fertilization of other ascidians is unknown. Here, we investigated the effects of four metalloprotease inhibitors on the fertilization of the ascidian Halocynthia roretzi (Stolidobranchia). Three metalloprotease inhibitors, GM6001, TAPI-0, and TAPI-1, strongly inhibited fertilization at 33 and 11 μM, whereas TAPI-2 weakly inhibited fertilization at 33 μM. In contrast, GM6001NC (negative control) had no effect on fertilization at 100 μM. Furthermore, GM6001 had no inhibitory effect on the fertilization of VC-deprived eggs. The metalloprotease appears to function at the middle or late stage of fertilization. Ten Tast genes were identified in the H. roretzi genome database, among which four genes (HrTast1, HrTast2b, HrTast2c, and HrTast3c) possessed a single transmembrane domain in the N-terminal region. These four genes are transcribed in the testis and ovary, as revealed by RT-PCR. Anti-HrTast2c IgG raised against a peptide corresponding to the Zn-binding consensus sequence weakly inhibited fertilization at 0.5 mg/mL. These results led us to propose that sperm astacin-like metalloproteases may be involved in sperm penetration of the VC during H. roretzi fertilization.

Keywords: ascidian, fertilization, metalloprotease

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Nishimura H, Hashii N, Yamamoto T, Sun Y, Miura T, Sato Y, Ishii-Watabe A: Usefulness of Size-Exclusion Chromatography-Multi-Angle Light Scattering to Assess Particle Composition and Protein Impurities for Quality Control of Therapeutic Exosome Preparations.

Pharmaceutics. 2024;16(12):1526. DOI: 10.3390/pharmaceutics16121526

Extracellular vesicles (EVs), including exosomes, are promising pharmaceutical modalities. They are purified from cell culture supernatant; however, the preparation may contain EVs with the desired therapeutic effects and different types of EVs, lipoproteins, and soluble proteins. Evaluating the composition of particulate impurities and the levels of protein impurities in final preparations is critical for quality control. However, few analytical methods can detect these impurities. We established and evaluated an analytical method using size-exclusion chromatography-multi-angle light scattering (SEC-MALS) for particle and protein impurity analyses of EV samples. In the particle size distribution analysis of EV samples, SEC-MALS showed higher resolution compared with nanoparticle tracking analysis (NTA) and dynamic light scattering (DLS). MALS showed comparable accuracy and precision to that of other methods for particle size evaluation using polystyrene standard beads with 60, 100, or 200 nm diameter. Coupling SEC-MALS with UV detection quantitatively evaluated soluble protein impurities. Proteomic analysis on the SEC-MALS-fractionated samples identified different EV and lipoprotein marker proteins in different fractions. SEC-MALS can characterize EV preparations obtained from human adipose-derived mesenchymal stem cells, suggesting that it can evaluate the particle component composition in various EV samples and therapeutic exosome preparations.

Keywords: extracellular vesicles, size-exclusion chromatography, multi-angle light scattering

Hashimoto N[*1,2], Ito S[*1], Harazono A, Tsuchida A[*3], Mouri Y[*2], A Yamamoto A[*2], Okajima T[*1], Ohmi Y[*4], Furukawa K[*4], Kudo Y[*2], Kawasaki N[*5], Furukawa K[*1,4]: Bidirectional signals generated by Siglec-7 and its crucial ligand tri-sialylated T to escape of cancer cells from immune surveillance.

iScience. 2024;27:111139. DOI: 10.1016/j.isci.2024.111139

Siglec-7, an inhibitory receptor expressed on natural killer (NK) cells, recognizes sialic acid-containing glycans. However, the ligand glycan structures of Siglec-7 and its carrier proteins have not been comprehensively investigated. Here, we identified four sialyltransferases that are used for the synthesis of ligand glycans of Siglec-7 and two ligand O-glycan-carrier proteins, PODXL and MUC13, using a colon cancer line. Upon binding of these ligand glycans, Siglec-7-expressing immune cells showed reduced cytotoxic activity, whereas cancer cells expressing ligand glycans underwent signal activation, leading to enhanced invasion activity. To clarify the structure of the ligand glycan, podoplanin (PDPN) identified as a Siglec-7 ligand-carrier protein, was transfected into HEK293T cells using sialyltransferase cDNAs. Mass spectrometry of the products revealed a ligand glycan, tri-sialylated T antigen. These results indicate that Siglec-7 interaction with its ligand generates bidirectional signals in NK and cancer cells, leading to the efficient escape of cancers from host immune surveillance.

Keywords: Siglec-7 ligand, tri-sialylated T antigen, podoplanin

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Aoyama M, Tada M, Yokoo H, Ito T, Misawa T, Demizu Y, Ishii-Watabe A: Linker and Conjugation Site Synergy in Antibody-Drug Conjugates: Impacts on Biological Activity.

Bioconjugate Chem. 2024;35:10, 1568-1576. DOI: 10.1021/acs.bioconjchem.4c00348

Antibody-drug conjugates (ADCs) produced using general conjugation methods yield heterogeneous products containing mixtures of species with different numbers of payloads per antibody (drug-antibody ratios) conjugated at multiple sites. This heterogeneity affects the stability, efficacy, and safety of ADCs. Thus, various site-specific conjugation methods have been developed to achieve homogeneity in ADCs. It was reported that linker structures and conjugation sites generally affected the characteristics of site-specific ADCs such as stability, efficacy, and safety. However, the combined effects of conjugation sites and linker structures on the physicochemical and biological characteristics of site-specific ADCs have remained unclear. In this study, we generated 30 homogeneous site-specific ADCs with a combination of six conjugation sites and five linker structures using THIOMAB technology and evaluated the characteristics of these homogeneous ADCs. We found that both conjugation sites and linker structures affected characteristics unique to ADCs (linker stability as well as target-dependent and target-independent cytotoxicity) in site-specific ADCs. Especially, conjugation to the constant regions of the light chain and the presence of polyethylene glycol structures in the linker are important for those ADC-specific characteristics. Interestingly, we also found that the effects of linker structures on the target-independent cytotoxicity of homogeneous ADCs at certain conjugation sites differed from those seen in conventional heterogeneous ADCs. Our results suggest that optimizing linker structures based on the conjugation site may be necessary for site-specific ADCs.

Keywords: antibody-drug conjugates, site-specific conjugation, characteristics

Yamamoto T, Urabe F[*2,3], Yoshioka Y[*1], Yamamoto Y[*2], Ochiya T[*1]: Protocol for extracellular vesicle secretion-related gene screening via ExoScreen technique.

STAR Protoc. 2025;14;6:103569. DOI: 10.1016/j.xpro.2024.103569

Extracellular vesicles (EVs) play a key role in cancer development and cellular homeostasis by transferring the biological cargo to recipient cells. Here, we describe steps for screening EV secretion-related genes by combining a microRNA (miRNA) library and ExoScreen, a highly sensitive EV detection

technique. We also detail procedures for screening the direct target genes regulated by miRNAs. This protocol provides a useful tool for understanding complex intracellular communications involved in EV secretion.

Keywords: extracellular vesicles, exosome

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Vaccine immunogenicity is influenced by the vaccinee's genetic background. Here, we perform a genome-wide association study of vaccine-induced SARS-CoV-2-specific immunoglobulin G (IgG) antibody titers and T cell immune responses in 1,559 mRNA-1273 and 537 BNT162b2 vaccinees of Japanese ancestry. SARS-CoV-2-specific antibody titers are associated with the immunoglobulin heavy chain (IGH) and major histocompatibility complex (MHC) locus, and T cell responses are associated with MHC. The lead variants at IGH contain a population-specific missense variant (rs1043109-C; p.Leu192Val) in the immunoglobulin heavy constant gamma 1 gene (IGHG1), with a strong decreasing effect ($\beta = -0.54$). Antibody-titer-associated variants modulate circulating immune regulatory proteins (e.g., LILRB4 and FCRL6). Age-related hematopoietic expanded mosaic chromosomal alterations (mCAs) affecting MHC and IGH also impair antibody production. MHC-/IGH-affecting mCAs confer infectious and immune disease risk, including sepsis and Graves' disease. Impacts of expanded mosaic loss of chromosomes X/Y on these phenotypes were examined. Altogether, both germline and somatic mutations contribute to adaptive immunity functions.

Keywords: genome-wide association study, COVID-19 vaccine, immunogenicity

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Microorganisms. 2025;13:641. DOI: 10.3390/microorganisms13030641

Several Ephedra Herb-containing Kampo medicines are common initial treatments for various infections; however, the ephedrine alkaloids in Ephedra Herb can cause side effects by stimulating adrenergic receptors. Accordingly, an ephedrine alkaloids-free Ephedra Herb Extract (EFE) has been developed. This study aimed to evaluate whether EFE can be used effectively and safely in patients with mild coronavirus disease 2019 (COVID-19). We randomized patients with mild COVID-19 to receive EFE equivalent to 6 g of Ephedra Herb per day or a placebo for 14 days. The primary efficacy endpoint was the non-aggravation rate up to Day 15. We allocated 41 and 40 patients to the EFE and placebo groups, respectively. All participants were included in the mITT and safety analysis populations [male ratio, mean age: 31.7%, 42.0 years (EFE); 17.5%, 43.2 years (placebo)]. The non-aggravation rate up to Day 15 for the primary endpoint was 100.0% and 94.6% in the EFE and placebo group, respectively, with no between-group difference. The number of days to the improvement in nausea symptoms was significantly shorter in the EFE group. One patient in the placebo group discontinued the trial due to a side effect. Although EFE demonstrated safety in patients with mild COVID-19, it did not show superior efficacy compared to placebo for symptoms other than nausea.
Keywords: COVID-19, Ephedra Herb, randomized comparative trial

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Y[*1], Morikawa T[*2], Ito M: Novel compounds isolated from health food products containing beni-koji (red yeast rice) with adverse event reports.
J Nat Med. 2024;78:845-848. doi: 10.1007/s11418-024-01827-w

Recently, health hazards, such as kidney damage, have been reported owing to the ingestion of a health food product, so-called "foods with functional claims (FFC)", containing beni-koji (red yeast rice). Although not an expected compound in the FFC, the detection of puberulic acid has also been reported. Further investigations of these health food products, such as the identification of other unintended compounds and clarifying the health impacts of puberulic acid, are required. To clarify the causes of these health issues, we investigated the presence of unintended compounds in the FFC containing beni-koji using comprehensive instrumental analyses. Using differential analysis, novel compounds **1** and **2** were detected as unexpected components between the samples with and without adverse event reports. Although limited to the samples available for analyses in this study, both compounds **1** and **2** were detected in all the samples that also contained puberulic acid. Compounds **1** and **2**, with molecular formulas of $C_{23}H_{34}O_7$ and $C_{28}H_{42}O_8$, respectively, may be lovastatin derivatives. Their structures were confirmed using NMR analyses and are novel natural compounds. For definitive confirmation, we are in the process of synthesizing compounds **1** and **2** from lovastatin. The route of contamination of these compounds are currently under investigation. The findings of this study could be used to address the growing health hazards associated with health food products.
Keywords: health hazard, beni-koji, comprehensive analysis

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生薬学雑誌. 2024;78:139-146.

Orengedokuto (OGT) is a Kampo prescription characterized by a strong bitter taste, consisting of Coptidis Rhizoma (CR), Phellodendori Cortex (PC), Scutellariae Radix (SR), and Gardeniae Fructus (GF). OGT is a multicomponent drug and one of the constituents contained is the alkaloid berberine, which is widely recognized for its bitter taste. Berberine is known to bond with baicalin, one of the constituents of SR, in the decoction to form a precipitate. However, the extent to which this precipitation is responsible for the bitter taste of OGT is unknown. In this study, we quantitatively evaluated the bitter taste of OGT extracts utilizing a taste-sensing system that can objectively measure taste intensity and investigated the effects of the constituents of OGT on bitter taste.
Keywords: orengedokuto, berberine-baicalin complex, taste evaluation

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生薬学雑誌. 2024;78:147-159.

The family name for the botanical origin of crude drugs in the Japanese Pharmacopoeia (JP) is based on the modified Engler system. However, the Angiosperm Phylogeny Group (APG) system, which is a novel classification system for Angiospermae using DNA information, was reported in 1998 and revised three times to date. Now, the APG system is widely accepted in not only plant systematics but also other fields. In this study, we prepared the comparison table between the two systems for family names of crude drug items in the JP. The resultant table was provided for General Information for the JP.
Keywords: Japanese pharmacopoeia, Family name, APG IV

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馬場まり子，水谷佐久美，徳本廣子，伊藤美千穂：半合成カンナビノイドが検出される植物系危険ドラッグ製品の基原植物について.
生薬学雑誌. 2024;78:160-171.

In Japan, it is illegal to sell herbal products and

powders that contain psychoactive compounds. Many of these illegal herbal products are presumed to be manufactured by artificially adding synthetic cannabinoids to plant pieces that do not naturally have any psychoactive properties, particularly products that are expected to have cannabis-like effects. In recent years, semi-synthetic cannabinoids prepared by performing simple chemical transformations of *Cannabis* plant extracts have appeared on the illegal drug market and are often distributed as vape products. Although semi-synthetic cannabinoids are known not to be detected in plants, plant pieces containing the semi-synthetic cannabinoid tetrahydrocannabinol-*O*-acetate（THC-O）were found in this study. Therefore, we investigated the botanical origin of this illegal herbal product by performing GC-MS and liquid chromatography-mass spectrometry analysis, DNA sequence analysis, and morphological observation with a stereoscopic microscope. Cannabidiol（CBD）, THC-O, and cannabidiolic acid, which is the inactive precursor of CBD in plants, were detected in this product. DNA sequence analysis revealed that most of the plant pieces were from *Cannabis sativa* L. In combination with the results from the morphological observation, most of the plant pieces were identified as coming from the *Cannabis* plant. Accordingly, it was suggested that the investigated illegal herbal product consisted of *Cannabis* plant pieces, the main component of which was CBD, with THC-O artificially added to the pieces.

Keywords: illegal herbal product, semi-synthetic cannabinoid, *Cannabis sativa* L.

田中理恵, 花尻（木倉）瑠理：インターネット上で流通するオイル製品中のΔ^8-tetrahydrocannabinol（THC）及びΔ^9-THCのC-3位のアルキル鎖の長さが異なるTHCアナログの同定

薬学雑誌. 2024;144:823-837. doi: 10.1248/yakushi.24-00029.

Since around 2021, products claiming to contain a Δ^9-THC analog with different lengths of alkyl chain at C-3 position have been sold on the internet in Japan. Δ^9-THC has a pentyl group derived from the precursor olivetol at the C-3 position. These products include liquid cartridges for electronic cigarettes, herbal products, and gummy products.

This study analyzed and determined the ingredients in five oil products distributed on the internet from 2022 to 2023 that claim to contain THC analogs. Samples of each product were used for GC-MS and LC-MS measurements. After isolating and purifying the unknown components from the products, structural analysis was performed by measuring ^{1}H, ^{13}C-NMR and various two-dimensional NMR［HH correlation spectroscopy（H-H COSY）, heteronuclear multiple quantum coherence（HMQC）, heteronuclear multiple-bond correlation（HMBC）, and nuclear Overhauser effect spectroscopy（NOESY）］.

The analysis identified Δ^8-tetrahydrocannabivarin（THCV）, Δ^9-THCV, Δ^8-tetrahydrocannabutol（THCB）, Δ^9-THCB, Δ^8-tetrahydrocannabihexol（THCH）, Δ^9-THCH, Δ^8-3-octyl-THC（THCjd）and Δ^9-THCjd. These compounds were Δ^8-THC or Δ^9-THC analogs with different lengths of alkyl chain at C-3 position. Meanwhile, Δ$^{4(8)}$-*iso*-THCV and Δ^{11}-THCB were identified as minor components of the product, and were considered to be the reaction byproducts of the synthesis of the Δ^8-THC or Δ^9-THC analogs.

In the future, there are concerns about the distribution of products containing new THC analogs. Therefore, continuous provision monitoring of newly detected in the products is important.

Keywords: *Cannabis sativa* L., Δ^9-tetrahydrocannabinol（Δ^9-THC）analog, Δ^8-tetrahydrocannabinol（Δ^8-THC）analog

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J Jap Bot. 2024;99:221-241 doi: 10.51033/jjapbot.ID0197

To clarify species-level diversity in the botanical origin of Asparagi radix distributed in markets in China and Japan, we developed an identification method using DNA barcoding markers for *Asparagus cochinchinensis* and its allied species, and performed marker investigations. Nucleotide sequences of the ITS regions were determined in 107 accessions of 21 *Asparagus* species, along with partial *trnL-trnF*

intergenic spacer regions from a limited number of species. The examined species were discriminated from each other, except *A. meioclados* and *A. trichoclados*. Among 238 accessions of Asparagi radix distributed in markets, the most common origin was *A. cochinchinensis* (56%), followed by *A. subscandens* (25%), *A. taliensis* (16%), *A. lycopodineus* (2%), and *A. meioclados* or *A. trichoclados* (0.4%). All species were found in Chinese markets, whereas only three species, *A. cochinchinensis*, *A. taliensis*, and *A. subscandens*, were found in Japanese market. While four species were found to consist of only wild origin, *A. cochinchinensis* and *A. taliensis* were found to have both wild and cultivated origins. More than half of the *A. subscandens*-derived crude drugs in the Chinese markets was estimated to be sourced from Myanmar based on the intraspecific barcoding variation, even though 55 out of 60 samples were sold as products originating from China.

Keywords: Asparagi radix, botanical origin, DNA barcoding

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J Jap Bot. 2024;99:281-295. doi:10.51033/jjapbot.ID0152

In the previous DNA barcoding studies, four or five species, in addition to *Asparagus cochinchinensis*, were found in the crude drug 'Asparagi radix' from Chinese and Japanese markets. Among these, the proportion of cultivated samples among the identified crude drug samples was 78% for *A. taliensis* and 35% for *A. cochinchinensis* but cultivated *A. subscandens* was not observed. The increase of cultivated Asparagi radix in response to the decrease of wild resources will likely lead to potential changes in the species composition of the botanical origin of the Asparagi radix in the market in future, particularly with an expected increase in *A. taliensis*. Therefore, this study aimed to elucidate the reasons behind the diversity of the source plant species of Asparagi radix in China, considering the cultivation of *A. cochinchinensis* and *A. taliensis*. A literature review and on-site investigations were conducted to uncover the history and status of the production areas. In wild-harvested areas in Hubei, Guizhou, and Sichuan, only *A. cochinchinensis* was harvested. In contrast, in cultivated areas, *A. taliensis* was cultivated in Yunnan and Guizhou, whereas *A. cochinchinensis* was cultivated in Guangxi and Sichuan. This situation was probably caused by the cultivation history in the three regions where native species and strains were independently used. Among the cultivated crude drugs, those derived from *A. cochinchinensis* in Guangxi, the most prevalent in the market during the survey, were small and thin. In contrast, those derived from *A. taliensis* from Yunnan and Guizhou were large, well-filled, and light-colored, matching the characteristics of high-quality products in the crude drug market. As Yunnan and Guizhou are geo-authentic herb (Di Dao, geo-authentic "best quality") -producing areas of Asparagi radix, traditionally, Asparagi radix originated from *A. taliensis* will likely increase more in the future.

Keywords: Asparagi radix, botanical origin, field investigations

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Negishi H., Ito M.: Comparison of Menthae Herba

written with the same kanji characters（薄荷）in Japan and China.

J Nat Med. 2024;78(4):1071-1076 doi: 10.1007/s11418-024-01822-1

Menthae Herba is an herbal medicine whose name is written with the same *kanji* characters（薄荷）in both the *Japanese Pharmacopoeia*, 18th Edition（JP）and in the *Pharmacopoeia of the People's Republic of China*（CP）. However, the original plants are *Mentha arvensis* Linn. var. *piperascens* Malinvaud in JP and *Mentha haplocalyx* Briq. in CP. To clarify the similarities and differences between Menthae Herba in Japan and that in China, morphological observations, essential oil component analysis, and DNA analysis were performed on marketed products of Menthae Herba in Japan and in China. The morphological observations based on the description of JP Menthae Herba showed that most of the samples matched the items listed in the description. Essential oil component analysis by gas chromatography-mass spectrometry showed that the amount of menthol varied among samples and that menthol was not always the principal compound in the oil. The original plant species was confirmed by DNA analysis of the *rpl16* intron region in chloroplast DNA and all samples matched the sequence of *M. canadensis*. The results showed that Menthae Herba products distributed in both Japan and China contained *M. canadensis*, but they had different compositions of essential oil, with menthol-rich Menthae Herba being dominant in the Japanese market.

Keywords: *Mentha arvensis* var. *piperascens*, *Mentha haplocalyx* Briq., *Mentha canadensis*

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Huang X*, Hyuga S*, Ito M, Goda Y, Kobayashi Y*: Preventive and therapeutic effects of ephedrine alkaloids-free Ephedra Herb extract on paclitaxel-induced neuropathic pain.

J Nat Med. 2025;79:107-121. doi: 10.1007/s11418-024-01853-8

Currently, there are no effective prophylactic or therapeutic drugs for the treatment of paclitaxel（PTX）-induced peripheral neuropathic pain（PTX-PNP）, highlighting the urgent need for the development of effective prophylactic and therapeutic drugs. In this study, we initially compared the efficacy of Ephedra Herb extract（EHE）with that of ephedrine alkaloids-free Ephedra Herb extract（EFE）, which lacked ephedrine alkaloids（EAs）-associated side effects, against the onset of PTX-induced mechanical allodynia, thermal hyperalgesia, and cold allodynia in mice. EHE and EFE demonstrated comparable preventive effects on the PTX-PNP in a dose-dependent manner. These results indicated that the preventive properties of EHE were independent of the EAs. Since elderly people are overwhelmingly more susceptible to developing cancer, we considered that EFE has greater benefits than EHE, so we conducted a study focused on the effects of EFE. EFE showed dose-dependent preventive effects on the onset of PTX-PNP. As a result of detailed investigation, coadministration of PTX and EFE（Co-EFE）was more effective than preadministration of EFE alone（Pre-EFE）. And the effects of Co-EFE was same with the effect of preadministration of EFE and then coadministration of PTX and EFE（P&C-EFE）. Additionally, Co-EFE after the onset of PTX-PNP improved PTX-induced mechanical allodynia, thermal hyperalgesia, and cold allodynia, confirming the therapeutic efficacy of EFE on PTX-PNP. In contrast, goshajinkigan, a Kampo medicine, and diclofenac, a non-steroidal anti-inflammatory drug, showed minimal therapeutic effects on PTX-PNP. These findings demonstrate the significant potential of EFE as a novel, safe prophylactic and therapeutic agent against PTX-PNP.

Keywords: Paclitaxel, EFE, Neuropathic pain

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Tokumoto H, Uchiyama N, Ito M: High-resolution X-ray computed tomography for identifying herbal medicines was as effective as microscopic examination.

J Nat Med. 2025;79:278-287. doi: 10.1007/s11418-024-01840-z

Microscopic examination is one of the important identification methods for crude drug test described in the 18th Japanese Pharmacopoeia. This method is useful for identification because it can be used for

small amounts of samples regardless of their storage conditions; however, this method requires a lot of technical skill in sectioning intricate and/or small samples and is time-consuming. High-resolution X-ray computed tomography (HRXCT) is a novel method for observing the internal morphology of materials. Previously, we used HRXCT to visualize the internal morphology of the Ephedra Herb, obtaining observations that closely match those obtained via microscopic examination. HRXCT employs a low-energy X-ray source and the permeation distance of the X-rays is very short. Therefore, HRXCT can be used for elucidating the morphology of small herbal medicines. In this study, Artemisia Capillaris Flower (capitulum with a diameter of approximately 2 mm) and Plantago Seed (seeds with a length of approximately 2 mm) were examined. The results showed that HRXCT examination was sufficient to illustrate the internal independent organs of Artemisia Capillaris Flower and that their inflorescences remained intact. When observing Plantago Seed, the internal morphology of more than one seed can be depicted simultaneously. Therefore, observation using HRXCT was easy, simple, and effective to illustrate the internal morphology of herbal medicines, which is typically time-consuming and requires advanced microscopy skills.

Keywords: high-resolution X-ray computed tomography, *Artemisia capillaris*, *Plantago asiatica*

Sawada R, Kusakawa S, Kusuhara M, Tanaka K, Miura T, Yasuda S, Sato Y: Increasing robustness of *in vitro* assay for immunosuppressive effect of mesenchymal stromal/stem cells: The role of inflammatory cytokine production by peripheral blood mononuclear cells.

Regenerative Therapy. 2025;28:321-332. doi: 10.1016/j.reth.2024.12.016.

Introduction: The Quality by Design (QbD) approach for developing cell therapy products using mesenchymal stromal/stem cells (MSCs) is a promising method for designing manufacturing processes to improve the quality of MSC products. It is crucial to ensure the reproducibility and robustness of the test system for evaluating critical quality attributes (CQAs) in the QbD approach for manufacturing of pharmaceutical products. In this study, we explored the key factors involved in establishing a robust evaluation system for the immunosuppressive effect of MSCs, which can be an example of a CQA in developing and manufacturing therapeutic MSCs for treating graft-versus-host disease, etc, and we have identified method attributes to increase the robustness of a simple *in vitro* assay to assess the immunosuppressive effects of MSCs.

Methods: We evaluated the performance of an assay system to examine the proliferation of peripheral blood mononuclear cells (PBMCs) activated with the mitogen phytohemagglutinin (PHA) when co-cultured with MSCs, the so-called one-way mixed lymphocyte reaction (MLR) assay. The MLR assay was performed on the same MSCs using 10 PBMC lots from different donors. In addition, 13 cytokine production levels in PHA-stimulated PBMCs were assessed. Results: The PHA-stimulated proliferation response of PBMCs, the action of MSCs in the MLR test, and the cytokine release of the respective PBMCs significantly differed among the PBMC lots ($p < 0.05$). A correlation analysis between the amounts of cytokines released by PBMCs and the immunosuppressive potency of MSCs showed that IFNγ, TNFα, CXCL10, PD-L1, HGF, and CCL5 production in PBMCs was significantly correlated with the MSC-mediated inhibition of PBMC proliferation ($p < 0.05$). Therefore, we selected two PBMC lots with high PBMC proliferation and PHA-stimulated cytokine (such as IFNγ and TNFα) release for the subsequent one-way MLR assay. The robustness of the established test system was confirmed by repeating the assay several times on different days for the same MSCs (coefficient of variation <0.2). Conclusions: To make robust the MSC immunosuppressive potency assay system, controlling the quality of PBMCs used for the assay is essential. Evaluating the inflammatory cytokine production capacity of PBMCs is effective in assessing the quality of the MLR assay system.

Keywords: CQA, Inflammatory cytokines, MLR assay

Fujita E[*1], Yamamoto S[*2], Hanada T[*3], Jogasaki S[*4], Koga Y[*5], Yatsuda Y[*6], Kakizaki Y[*7], Jo Y[*8], Asano Y[*9], Yonezawa K[*1], Moriya Y[*2], Nakayama M[*2], Arimura Y[*4], Okawa Y[*4], Komatsu H[*7], Ito M[*8], Suzuki S[*9], Kuroda T, Yasuda S, Kamiyama Y[*1], Sato Y: Using qPCR and ddPCR to study

biodistribution of cell therapy products: a multi-site evaluation.

Cytotherapy. 2025;27:51-65. doi: 10.1016/j.jcyt.2024.09.003

BACKGROUD AIMS: Regenerative therapies employing cell therapy products (CTPs) have attracted considerable attention. Biodistribution (BD) evaluation of CTPs is mainly performed to clarify the cell survival time, engraftment, and distribution site. This evaluation is crucial for predicting the efficacy and safety profiles of clinical studies based on non-clinical BD study outcomes. However, no internationally unified method has been established for assessing cell BD after administration. Here, we aimed to standardize the BD assay method used for CTPs, conducting the following evaluations using the same protocol across multiple study facilities: (1) *in vitro* validation of quantitative polymerase chain reaction (qPCR) and droplet digital PCR (ddPCR) analyses using the primate-specific Alu gene, and (2) *in vivo* BD studies after the intravenous administration of human mesenchymal stem cells (hMSCs) to immunodeficient mice, commonly used in non-clinical tumorigenicity studies. METHODS: Quality control samples were prepared and analyzed by adding a fixed number of human-derived cells to several mouse tissues. The respective quantitative performances of the qPCR and ddPCR methods were compared for accuracy and precision. hMSCs were intravenously administered to immunodeficient mice, and tissues were collected at 1, 4, and 24 h after administration. RESULTS: Both methods demonstrated an accuracy (relative error) generally within ±50% and a precision (coefficient of variation) generally less than 50%. While differences in calibration curve ranges were observed between qPCR and ddPCR, no significant differences in quantification were found among the assay facilities. The BD of hMSCs in mice was evaluated at seven facilities (qPCR at three facilities; ddPCR at four facilities), revealing similar tissue distribution profiles in all facilities, with the lungs showing the highest cell distribution among the tissues tested. CONCLUSIONS: Quantitative evaluation of qPCR and ddPCR using Alu sequences was conducted, demonstrating that the test method can be adapted for BD evaluation.

Keywords: biodistribution, qPCR, ddPCR

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Yasuda S, Bando K[*1], Henry MP[*2], Libertini S[*3], Watanabe T[*4], Bando H[*5], Chen C[*6], Fujimori K[*1], Harada K[*4], Kuroda T, Lemmens M[*3], Marginean D[*2], Moss D[*2], Pereira Mouriès L[*6], Nicholas NS[*2], Smart MJK[*2], Terai O[*1], Sato Y: Detection of residual pluripotent stem cells in cell therapy products utilizing droplet digital PCR: an international multisite evaluation study.

Stem Cells Transl Med. 2024;13:1001-1014. doi: 10.1093/stcltm/szae058

The presence of residual undifferentiated pluripotent stem cells (PSCs) in PSC-derived cell therapy products (CTPs) is a major safety issue for their clinical application, due to the potential risk of PSC-derived tumor formation. An international multidisciplinary multisite study to evaluate a droplet digital PCR (ddPCR) approach to detect residual undifferentiated PSCs in PSC-derived CTPs was conducted as part of the Health and Environmental Sciences Institute Cell Therapy-TRAcking, Circulation & Safety Technical Committee. To evaluate the use of ddPCR in quantifying residual iPSCs in a cell sample, different quantities of induced pluripotent stem cells (iPSCs) were spiked into a background of iPSC-derived cardiomyocytes (CMs) to mimic different concentrations of residual iPSCs. A one step reverse transcription ddPCR (RT-ddPCR) was performed to measure mRNA levels of several iPSC-specific markers and to evaluate the assay performance (precision, sensitivity, and specificity) between and within laboratories. The RT-ddPCR assay variability was initially assessed by measuring the same RNA samples across all participating facilities. Subsequently, each facility independently conducted the entire process, incorporating the spiking step, to discern the parameters influencing potential variability. Our results show that a RT-ddPCR assay targeting ESRG,

LINC00678, and LIN28A genes offers a highly sensitive and robust detection of impurities of iPSC-derived CMs and that the main contribution to variability between laboratories is the iPSC-spiking procedure, and not the RT-ddPCR. The RT-ddPCR assay would be generally applicable for tumorigenicity evaluation of PSC-derived CTPs with appropriate marker genes suitable for each CTP.

Keywords: detection sensitivity, droplet digital PCR, multisite experiments

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Nishimura H, Hashii N, Yamamoto T, Sun Y, Miura T, Sato Y, Ishii-Watabe A: Usefulness of Size-Exclusion Chromatography-Multi-Angle Light Scattering to Assess Particle Composition and Protein Impurities for Quality Control of Therapeutic Exosome Preparations.

Pharmaceutics. 2024;16:1526. doi: 10.3390/pharmaceutics16121526

Background: Extracellular vesicles (EVs), including exosomes, are promising pharmaceutical modalities. They are purified from cell culture supernatant; however, the preparation may contain EVs with the desired therapeutic effects and different types of EVs, lipoproteins, and soluble proteins. Evaluating the composition of particulate impurities and the levels of protein impurities in final preparations is critical for quality control. However, few analytical methods can detect these impurities. Methods: We established and evaluated an analytical method using size-exclusion chromatography-multi-angle light scattering (SEC-MALS) for particle and protein impurity analyses of EV samples. Results: In the particle size distribution analysis of EV samples, SEC-MALS showed higher resolution compared with nanoparticle tracking analysis (NTA) and dynamic light scattering (DLS). MALS showed comparable accuracy and precision to that of other methods for particle size evaluation using polystyrene standard beads with 60, 100, or 200 nm diameter. Coupling SEC-MALS with UV detection quantitatively evaluated soluble protein impurities. Proteomic analysis on the SEC-MALS-fractionated samples identified different EV and lipoprotein marker proteins in different fractions. Conclusions: SEC-MALS can characterize EV preparations obtained from human adipose-derived mesenchymal stem cells, suggesting that it can evaluate the particle component composition in various EV samples and therapeutic exosome preparations.

Keywords: extracellular vesicles, multi-angle light scattering, size-exclusion chromatography

Bando K*1,2, Kusakawa S, Adachi H*1,2, Yamamoto M*2,3, Iwata M*2,4, Kitanaka A*2,4, Ogimura E*1,2, Osada T*2,5, Tamura M*2,6, Terai O*2,4, Watanabe A*2,7, Yoda T*1,2, Yotsumoto T*2,8, Zaizen K*2,5, Sato Y: Protocol improvement and multisite validation of a digital soft agar colony formation assay for tumorigenic transformed cells intermingled in cell therapy products.

Cytotherapy. 2024;26:769-777. doi: 10.1016/j.jcyt.2024.03.005.

Background aims: The administration of human cell-processed therapeutic products (hCTPs) is associated with a risk of tumorigenesis due to the transformed cellular contaminants. To mitigate this risk, these impurities should be detected using sensitive and validated assays. The digital soft agar colony formation (D-SAC) assay is an ultrasensitive *in vitro* test for detecting tumorigenic transformed cells in hCTPs. Methods: In this study, we first evaluated the colony formation efficiency (CFE) precision of tumorigenic reference cells in positive control samples according to a previously reported D-SAC assay protocol (Protocol I) from multiple laboratories. However, the CFE varied widely among laboratories. Thus, we improved and optimized the test protocol as Protocol II to reduce variability in the CFE of tumorigenic reference cells. Subsequently, the improved protocol was validated at multiple sites. Human mesenchymal stromal cells (hMSCs) were used as model cells, and positive control samples were prepared by spiking them with HeLa cells. Results: Based on the previously reported protocol, the CFE was estimated using an ultra-low concentration (0.0001%) of positive control samples in multiple plates. Next, we improved the protocol to

reduce the CFE variability. Based on the CFE results, we estimated the sample size as the number of wells (Protocol II) and assessed the detectability of 0.0001% HeLa cells in hMSCs to validate the protocol at multiple sites. Using Protocol I yielded low CFEs (mean: 30%) and high variability between laboratories (reproducibility coefficient of variance [CV]: 72%). In contrast, Protocol II, which incorporated a relatively high concentration (0.002%) of HeLa cells in the positive control samples, resulted in higher CFE values (mean: 63%) and lower variability (reproducibility CV: 18%). Moreover, the sample sizes for testing were estimated as the number of wells per laboratory (314-570 wells) based on the laboratory-specific CFE (42-76%). Under these conditions, all laboratories achieved a detection limit of 0.0001% HeLa cells in hMSCs in a predetermined number of wells. Moreover, colony formation was not observed in the wells seeded with hMSCs alone. Conclusions: The D-SAC assay is a highly sensitive and robust test for detecting malignant cells as impurities in hCTPs. In addition, optimal assay conditions were established to test tumorigenic impurities in hCTPs with high sensitivity and an arbitrary false negative rate.

Keywords: cell therapy products, *in vitro* assay, transformed cells, tumorigenicity

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Watanabe T*1, Yasuda S, Kusakawa S, Kuroda T, Furukawa H*2, Futamura M*3, Shimizu S*4, Morishita A*5, Hata S*5, Koeda A*6, Komatsu K*6, Sato Y: Multisite studies for optimization of a highly efficient culture assay used for *in vitro* detection of residual undifferentiated human pluripotent stem cells intermingled in cell therapy products.
Regen Ther. 2024;26:315-323. doi: 10.1016/j.reth.2024.06.007

Introduction: MEASURE2 (Multisite Evaluation Study on Analytical Methods for Non-clinical Safety Assessment of HUman-derived REgenerative Medical Products 2) is a Japanese experimental public-private partnership initiative that aims to standardize testing methods for tumorigenicity evaluation of human pluripotent stem cell (hPSC)-derived cell therapy products (CTPs). MEASURE2 organized multisite studies to optimize the methodology of the highly efficient culture (HEC) assay, a sensitive culture-based *in vitro* assay for detecting residual undifferentiated hPSCs in CTPs. Methods: In these multisite studies, 1) the efficiency of colony formation by human induced pluripotent stem cells (hiPSCs) under two different culture conditions and 2) the sorting efficiency of microbeads conjugated to various anti-hPSC markers during hiPSC enrichment were evaluated using samples in which hiPSCs were spiked into hiPSC-derived mesenchymal stem cells. Results: The efficiency of colony formation was significantly higher under culture conditions with the combination of Chroman 1, Emricasan, Polyamines, and Trans-ISRIB (CEPT) than with Y-27632, which is widely used for the survival of hPSCs. Between-laboratory variance was also smaller under the condition with CEPT than with Y-27632. The sorting efficiency of microbeads conjugated with the anti-Tra-1-60 antibody was sufficiently higher (>80%) than those of the other various microbeads investigated. Conclusions: Results of these multisite studies are expected to contribute to improvements in the sensitivity and robustness of the HEC assay, as well as to the future standardization of the tumorigenicity risk assessment of hPSC-derived CTPs.

Keywords: *In vitro* tumorigenicity assay, Multisite study, Residual pluripotent stem cell

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TLR9 activation.

Sci. Rep. 2024;14:11540. doi: 10.1038/s41598-024-61666-3

Antisense oligonucleotides (ASOs) are synthetic single-stranded oligonucleotides that bind to RNAs through Watson-Crick base pairings. They are actively being developed as therapeutics for various human diseases. ASOs containing unmethylated deoxycytidylyl-deoxyguanosine dinucleotide (CpG) motifs are known to trigger innate immune responses via interaction with toll-like receptor 9 (TLR9). However, the TLR9-stimulatory properties of ASOs, specifically those with lengths equal to or less than 20 nucleotides, phosphorothioate linkages, and the presence and arrangement of sugar-modified nucleotides—crucial elements for ASO therapeutics under development—have not been thoroughly investigated. In this study, we first established SY-ODN18, an 18-nucleotide phosphorothioate oligodeoxynucleotide with sufficient TLR9-stimulatory activity. We demonstrated that an unmethylated CpG motif near its 5′-end was indispensable for TLR9 activation. Moreover, by utilizing various sugar-modified nucleotides, we systematically generated model ASOs, including gapmer, mixmer, and fully modified designs, in accordance with the structures of ASO therapeutics. Our results illustrated that introducing sugar-modified nucleotides in such designs significantly reduces TLR9-stimulatory activity, even without methylation of CpG motifs. These findings would be useful for drug designs on several types of ASOs.

Keywords: antisense oligonucleotide, sugar-modified nucleotide, innate immunity

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今井峻司*1,2，深野泰史*1,3，庭山裕孝*1,4，田村直美*1,5，三好美佳*1,6，福原慶*1,7，小平浩史*1,8，岩崎紀彦*1,9，山中陽介*1,10，宮澤憲浩*1,11，高草英生*1,12，角辻賢太*1,13，後藤昭彦*1,14，島田俊介*1,15，吉田徳幸，小比賀聡*16，西川元也*17，井上貴雄：既承認核酸医薬品の組織分布及び血漿/血清タンパク結合評価に関する調査と考察.

医薬品医療機器レギュラトリーサイエンス. 2024;55:208-238. doi: 10.51018/pmdrs.55.3_208

In recent years, the clinical development of oligonucleotide therapeutics, such as antisense oligonucleotide (ASO) and small interfering RNA (siRNA), has been active. Applications for regulatory approval require a series of assessments of the absorption, distribution, metabolism, excretion, and drug-drug interaction characteristics of these oligonucleotide therapeutics using appropriate methods. It is particularly important to understand the tissue distribution and plasma/serum protein binding properties of oligonucleotide therapeutics in assessing their efficacy and safety. However, no comprehensive studies have been conducted to investigate how tissue distribution and protein binding are evaluated and what properties are determined as a result. In this study, we examined the review reports for approved oligonucleotide therapeutics released by the regulatory authorities, as well as related papers to investigate the evaluation methods and the tissue distribution and plasma/serum protein binding properties of the currently approved ASO and siRNA therapeutics. First, quantitative whole-body autoradiography (QWBA) studies using radiolabeled compounds were in principle conducted for the evaluation of tissue distribution throughout the whole body, as is the case with small-molecule drugs. In many cases, distribution to tissues of particular interest, such as organs with a high distribution rate, was evaluated by a combination of methods, including liquid chromatography-mass spectrometry (LC-MS), capillary electrophoresis (CE), high-performance liquid chromatography (HPLC), hybridization enzyme-linked immunosorbent assay (ELISA), and hybridization electrochemiluminescence (ECL) after administration of unlabeled compounds. The results of these tissue distribution evaluations showed that systemically administered ASO therapeutics consisting solely of oligonucleotides were rapidly distributed throughout the body and were highly concentrated in the kidneys in all cases, regardless of animal species. In contrast, all the siRNA therapeutics were highly directed to the liver, and GalNAc-siRNA, in particular, tended to accumulate predominantly in the liver, the therapeutic target tissue. The plasma/serum protein binding of ASO therapeutics was evaluated by methods commonly used for small-molecule drugs such as ultrafiltration

and ultracentrifugation, while gel-shift assay was also used for siRNA therapeutics as a new evaluation method. As regards the protein binding properties of the ASO therapeutics, the plasma/serum protein binding rate of morpholino ASOs was generally low (40% or less), whereas the plasma protein binding rate of phosphorothioate ASOs was 85% or more. In contrast, for siRNA therapeutics, LNP-siRNA showed a low serum protein binding rate of approximately 2% or less, whereas the plasma protein binding rate in GalNAc-siRNAs at concentrations around the clinical exposure level was 76% or higher in human. The tissue distribution and protein binding of oligonucleotide therapeutics are particularly sensitive to the molecular structure of oligonucleotides and the drug delivery system (DDS) technology employed, so an accurate understanding of these properties is important for the development of oligonucleotide therapeutics. This survey revealed that new evaluation methods for tissue distribution and protein binding were employed in addition to conventional evaluation methods, and indicated that these methods provided an improved understanding of the tissue distribution and protein binding properties.

Keywords: oligonucleotide therapeutics, tissue distribution, protein binding

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共通ウイルスゲノムRNAを用いたCOVID-19診断用核酸増幅検査薬の一斉性能評価試験.
医薬品医療機器レギュラトリーサイエンス. 2024;55:295-310. doi: 10.51018/pmdrs.55.4_295

At the beginning of the coronavirus disease 2019 (COVID-19) pandemic in early 2020, nucleic acid amplification test (NAT) kits to identify severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection were urgently developed, and within a short period of time (2-6 months), dozens of NAT kits were permitted for clinical diagnostic use in Japan. These urgently developed NAT kits were subjected to an emergent review process based on performance evaluation using a limited number of clinical specimens. To verify the performance of these NAT kits, we conducted a performance evaluation study in September 2020. For this purpose, genomic RNA isolated from the SARS-CoV-2 Wuhan strain with a defined copy number was used as a standard material to evaluate nine NAT kits permitted for emergency use by the end of May 2020. A series of diluted solutions of viral RNA (5 to 500 copies per reaction) were used, and the positive detection rate at each concentration was calculated based on the criteria for each NAT kit. The results showed that eight of the nine NAT kits were capable of detecting samples containing more than 50 copies/reaction with a probability of 100%. The “50 copies/reaction” is a reference value used by the National Institute of Infectious Diseases in the pathogen detection manual 2019-nCoV in 2020. These eight NAT kits also correctly judged samples containing no viral RNA as “negative”. Thus, eight of the nine NAT kits were considered to be reliable in terms of sensitivity and specificity. On the other hand, one NAT kit judged samples containing no viral RNA as “positive” with a probability of 33%. Based on this result, the company that developed this NAT conducted additional experiments and found that these false-positive results occur when a specific combination of measurement plates and nucleic acid amplification devices was used. In addition, our study showed that there were differences in the positive detection rate and nucleic acid amplification efficiency even between NAT kits with the same primers, probably due to differences in the composition and reaction conditions of the reagents used. Also, the positive detection rate and the

efficiency of nucleic acid amplification differed among the primer sets, which are constituents of the NAT kits.

Keywords: COVID-19, SARS-CoV-2, Nucleic acid amplification test

Omuro S[*1], Yamaguchi T[*1], Kawase T[*2], Hirose K[*2], Yoshida T, Inoue T, Obika S[*1]: Separation and characterization of therapeutic oligonucleotide isomer impurities by cyclic ion mobility mass spectrometry.

J. Am. Soc. Mass Spectrom. 2024;35:2156-2164. doi: 10.1021/jasms.4c00197

Therapeutic oligonucleotides such as antisense oligonucleotide (ASO) and small interfering RNA (siRNA) are among the most remarkable modalities in modern medicine. ASOs and siRNA are composed of single- or double-stranded 15-25 mer synthesized oligonucleotides, which can be used to modulate gene expression. Liquid chromatography-mass spectrometry (LC/MS) is a necessary technique for the quality control of therapeutic oligonucleotides; it is used to evaluate the quantities of target oligonucleotides and their impurities. The widely applied oligonucleotide therapeutic quantitation method uses both ultraviolet (UV) absorbance and the MS signal intensity. Peaks separated from the main peak, which contains full-length product, are generally quantitated by UV. However, coeluting impurities, such as *n* - 1 shortmers, abasic oligonucleotides, and PS → PO (phosphorothiate to phosphodiester) oligonucleotides, are quantitated by MS. These coeluting impurities can also be comprised of various isomers with the same modification, thus increasing the difficulty in their separation and relative quantitation by LC/MS. It is possible that a specific isomer with a certain structural form induces toxicities. Therefore, characterization of each isomer separation is in high demand. In this study, we separated and characterized oligonucleotide isomers by employing a cyclic ion mobility mass spectrometry (cyclic IMS) system, which allows the separation of ions with the same *m/z* ratio based on their structural differences. Patisiran antisense and sense strands and their *n* - 1 and abasic isomers were used as sample sequences, and their ratio characterization was achieved by cyclic IMS. In addition, we evaluated the PS → PO conversion isomers of the antisense strand of givosiran, which originally contained four PS modification sites. The PS → PO isomers exhibited specific and distinguishable mobiligram patterns. We believe that cyclic IMS is a promising method for evaluating therapeutic oligonucleotide isomers.

Keywords: oligonucleotide therapeutics, impurity, quality evaluation

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Kusumoto K[*1], Sasaki K, Uchida Y, Utsumi A[*2], Yoshida T, Obika S[*3], Inoue T, Okuhira K[*1]: Multispanning membrane protein SIDT2 increases knockdown activity of gapmer antisense oligonucleotides.

Sci. Rep. 2025;15:586. doi: 10.1038/s41598-024-84310-6

Recent advances in the clinical development of oligonucleotide therapeutics, such as antisense oligonucleotides (ASOs) and small interfering RNAs, have attracted attention as promising therapeutic modalities for genetic and intractable diseases. These oligonucleotide therapeutics exert their efficacy by binding to target RNAs present within cells; however, the mechanisms underlying their cellular uptake, especially their passage through membranes, remain largely unclear. In the nematode, *Caenorhabditis elegans*, the multi-pass transmembrane protein, SID-1, is involved in the cellular uptake of double-stranded RNAs. In mammals, SIDT1 and SIDT2 (SID-1 transmembrane family, members 1 and 2, respectively) are homologs of SID-1, yet their functional differences are not fully understood. In this study, we conducted a comparative analysis of the amino acid sequences of mammalian SIDT1 and SIDT2 to identify regions characteristic to each. By inducing SIDT1 or SIDT2 expression in human cell lines, we demonstrated that SIDT2 enhanced the knockdown activity of gapmer ASOs and potentially promoted their endosomal escape into the cytosol. Furthermore, by analyzing chimeric proteins of SIDT2 and SIDT1, we identified a region in SIDT2 that might be crucial for the enhancement of gapmer ASO activity. These findings elucidate the novel role of SIDT2 in the transport mechanism of gapmer ASOs and are expected to contribute to further development of oligonucleotide

therapeutics.
Keywords: oligonucleotide therapeutics, cellular uptake, endosomal escape

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Suzuki H*1, 2, Tsuboko Y, Tamura M*1, 3, Masamune K*1, 3, Iwasaki K*1, 4: Synthesis of the clinical utilities and issues of intraoperative imaging devices in clinical reports: a systematic review and thematic synthesis.

BMC Med. Inform. Decis. Mak. 2025;25:70. doi: 10.1186/s12911-025-02915-x

Background: Intraoperative imaging devices (i-ID), such as intraoperative optical coherence tomography (iOCT), offer surgeons critical insights previously unobservable, enhancing surgical precision and safety. Despite their benefits, i-IDs present challenges that necessitate early identification and synthesis of clinical issues to promote safer surgical implementation. This study aims to explore the potential of Qualitative Evidence Synthesis (QES) for synthesising qualitative evidence from clinical reports regarding the clinical utility and issues associated with iOCT devices. Methods: In June 2022, we conducted a systematic literature search using PubMed, Web of Science, Embase, and the Cochrane Library for articles on iOCT for retinal surgery. Criteria included articles in English, with at least ten cases, and providing qualitative insights into iOCT's utilities and issues. We performed thematic synthesis from the identified articles using qualitative data analysis software, beginning with initial coding of the 'Results' and 'Discussion' sections to create themes reflecting iOCT's utilities and issues. The created themes were further refined through axial coding and were used to construct a model illustrating iOCT's potential influence on patient outcomes. The reliability and validity of the themes were ensured through independent coding, expert consultations, and iterative revisions to achieve consensus among reviewers. Results: The QES approach enabled systematic data extraction and synthesis, providing a comprehensive view of both the utilities and issues associated with iOCT. Our findings emphasise the significant role of iOCT in enhancing decision-making, specifically in membrane peeling tasks and in detecting preoperatively undetected conditions such as full-thickness macular holes. This study also revealed critical insights into the technical challenges associated with iOCT, including device malfunctions and procedural interruptions, which are vital for improving device safety and integration into surgical practice. Conclusion: The application of QES facilitated a thorough investigation into the clinical utilities and issues of iOCT, encouraging the application of this method in the ongoing evaluation of i-ID technologies. This initial experience with QES confirms its potential in synthesising qualitative clinical data and suggests its applicability to other i-ID modalities. This approach enhances the reliability of findings and provides a solid foundation for assessing clinical utilities and issues for policymakers and medical specialists.
Keywords: qualitative evidence synthesis, thematic analysis, intraoperative imaging devices

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Yamamoto E, Nikko M*, Miyatsuji M, Ando D, Miyazaki T, Koide T, Sato Y: Physicochemical profiling of nanomedicines using centrifugal field flow fractionation.

Int. J. Pharm. 2024;663:124571. doi: 10.1016/j.ijpharm.2024.124571.

Nanomedicines comprise multiple components, and particle density is considered an important property that regulates the biodistribution of administered nanomedicines. The density of nanoparticles is characterized by centrifugal methods, such as analytical ultracentrifugation. Particle size and distribution are key physicochemical and quality attributes of nanomedicines. In this study, we developed a novel profiling method applicable to liposomes and lipid nanoparticles (LNPs), based on particle size and density, using centrifugal field-flow fractionation (CF3). We evaluated the elution profiles

of PEGylated liposomes of different sizes with various doxorubicin (DOX)-loading amounts using CF3. This method was applied to evaluate the drug release of DOX-loaded liposomes, intra- and inter-batch variability, reconstitution reproducibility of AmBisome®, and elution characteristics of LNPs in COVID-19 vaccines (Comirnaty® and Spikevax™). The data obtained in the present study underscore the significance of the proposed methodology and highlight the importance of profiling and characterizing liposomes and LNPs using CF3 fractograms and a multi-angle light-scattering detector.

Keywords: nanoparticle density, profiling, field flow fractionation

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Kato M[*1,2], Shirakawa Y[*3], Kanai Y[*3], Ota S[*4], Murayama N[*2], Miyazaki S[*4], Yamamoto E, Takaki T[*5]: Separation of 100 nm-sized nanoparticles using a poly-Lys-modified monolith column.

RSC Adv. 2025;15(5):3147-53. doi: 10.1039/D4RA07906J

Nanoparticles (approximately 100 nm in diameter) composed of lipid layers containing drugs or biologically active substances are attracting increasing attention in various fields, including medicine, as well as for signal transduction between cells. However, the separation of such nanoparticles *via* conventional HPLC is challenging, often resulting in the clogging and collapse of nanoparticles, as well as a low separation efficiency. Thus far, no HPLC column capable of efficiently separating two types of 100 nm-sized nanoparticles in a short time has been reported. In this study, a poly-Lys-modified monolithic column was prepared for nanoparticle analysis *via* HPLC using anticancer drug-encapsulated nanoparticles (Doxil®) and small extracellular vesicles (sEVs) to examine their elution behaviors. The zeta potentials of Doxil® and the sEVs were −24.4 and −45.5 V, respectively. A column with a low surface coverage (0.96 mg mL^{-1}) of poly-Lys adsorbed the nanoparticles but did not elute them, whereas a column with a high surface coverage (2.06 mg mL^{-1}) of poly-Lys retained these nanoparticles owing to the ion-exchange effect; sEVs with highly negative charges were strongly retained in the column. Using gradient elution with different 2-amino-2-hydroxymethyl-1,3-propanediol concentrations in the mobile phase, the two types of nanoparticles (Doxil® and sEVs) were eluted and successfully separated within 10 min. Thus, the developed column is a valuable tool for evaluating the safety and performance of larger-sized nanoparticles.

Keywords: nanoparticle, monolith column, Poly-Lys

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Sakoda H, Tamazawa K[*1], Shoyama Y[*1], Osaka Y[*2], Uetsuki K[*2], Okamoto Y, Yamamoto E: Sensitivity, robustness, and reproducibility of U-shaped delamination test for evaluation of candidate ultra-high molecular weight polyethylene materials for joint replacements.

Proc. Inst. Mech. Eng. Part H-J. Eng. Med. 2024;238:764-73. doi: 10.1177/09544119241253322

The delamination of ultra-high molecular weight polyethylene (UHMWPE) in artificial joints is a major cause limiting the long-term clinical results of arthroplasty. However, the conventional test method using simple reciprocation to evaluate the delamination resistance of UHMWPE materials has insufficient detection sensitivity. To reproduce delamination, the unconformity contact must be maintained throughout the test so that the maximum stress is generated below the surface. Therefore, a test method that applies a U-shaped motion comprising two long-linear and one short linear sliding motion was developed. The sensitivity, robustness, and reproducibility of the U-shaped delamination test were investigated and compared with the traditional test method. The traditional test method could reproduce delamination only in materials that had degraded considerably, whereas the U-shaped delamination test could reproduce delamination in a wide range of materials, demonstrating its superior sensitivity. Additionally, using a higher load helped accelerate the test without affecting the test results. The optimal length of the short linear sliding motion was confirmed to be 1 mm. Finally, the inter-laboratory reproducibility of the U-shaped delamination test was confirmed using the

round-robin test. The U-shaped delamination test demonstrates high sensitivity, robustness, and reproducibility and contributes to the selection and development of UHMWPE materials and artificial joints with a lower risk of delamination.

Keywords: ball-on-flat test, fatigue, unconformity contact

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Tsuboko Y†, Sakoda H†, Okamoto Y, Nomura Y, Yamamoto Y: Mechanical characterization of individual needles in microneedle arrays: factors affecting compression test results.
Pharmaceutics. 2024;16:1480. doi: 10.3390/pharmaceutics16111480

Background: This study aims to investigate the impact of test conditions on the results of the compression testing of microneedle arrays (MNAs). Methods: Uniaxial compression tests were conducted on polyglycolic acid-fabricated biodegradable MNAs. Load-displacement curves were obtained for varying conditions, including the number of microneedles (MNs) compressed simultaneously, compression speeds, and compression angles. Subsequently, the buckling load and stiffness were calculated, and the MN deformation during compression was observed. Results: The buckling load and stiffness per MN decreased significantly with a simultaneous increase in compressed MNs. The mean buckling load and stiffness of 52 MNs in single-needle compression tests were 0.211 ± 0.008N and 13.9 ± 1.3N/mm, respectively, with no variation among the three MNAs. However, a significant difference in buckling load and stiffness was observed among the MNs within the MNAs. Additionally, buckling loads and stiffnesses were significantly lower in certain MNs at the same location in different MNAs. Buckling load and stiffness decreased significantly during inclined compression compared to during vertical compression. While the tests evaluate the mechanical properties of MNAs, test results may vary depending on test conditions. Conclusions: Compression testing of the individual MNs comprising an MNA helps evaluate the mechanical properties of MNs and ensure the quality of MNAs.

Keywords: buckling load, single-needle compression, quality control

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Yahagi K*1,2, Nishimura G*3, Kuramoto K*3, Tsuboko Y, Iwasaki K*1,3: Hemodynamics with mechanical circulatory support devices using a cardiogenic shock model.
Sci. Rep. 2024;14:14125. doi: 10.1038/s41598-024-64721-1

Mechanical circulatory support (MCS) devices, including veno-arterial extracorporeal membrane oxygenation (VA-ECMO) and Impella, have been widely used for patients with cardiogenic shock (CS). However, hemodynamics with each device and combination therapy is not thoroughly understood. We aimed to elucidate the hemodynamics with MCS using a pulsatile fow model. Hemodynamics with Impella CP, VA-ECMO, and a combination of Impella CP and VA-ECMO were assessed based on the pressure and fow under support with each device and the pressure-volume loop of the ventricle model. The Impella CP device with CS status resulted in an increase in aortic pressure and a decrease in end-diastolic volume and end-diastolic pressure (EDP). VA-ECMO support resulted in increased afterload, leading to a significant increase in aortic pressure with an increase in end-systolic volume and EDP and decreasing venous reservoir pressure. The combination of Impella CP and VA-ECMO led to left ventricular unloading, regardless of increase in afterload. Hemodynamic support with Impella and VA-ECMO should be a promising combination for patients with severe CS.

Keywords: cardiogenic shock, mechanical circulatory support, veno-arterial extracorporeal membrane oxygenation

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Uematsu M, Miyamoto Y, Shimizu M*1, Kajiura T*1, Saito A*2, Takashina M*2, Fujita S*3, Nakano Y*3, Shimizu T*4, Nagahara Y*4, Kosaka H*4, Muramatsu H*5, Mori M*5, Suzuki T*5, Nakamura T*6,

Tanemura A[*6], Hosaka J[*7], Mori T[*7], Kato S[*8], Itagaki A[*8], Inoue T[*8], Matsumoto S[*9], Naito T[*10], Fujii S[*10], Nakaoka R, Yamamoto E: Design and validation of a method for evaluating medical device cleanliness by recovering and quantifying residual proteins on stainless plates.

Sci. Rep. 2024;14:21982. doi: 10.1038/s41598-024-72473-1

We recently reported a method for recovering and quantifying residual proteins bound to surfaces of various medical instruments via thermal coagulation under neutral pH and room temperature. The method effectively recovered and solubilised coagulated proteins at high temperatures in dry and humid conditions, with a protein recovery rate of > 90%. This study validated the previous method by comparing residual protein recovery from test samples using a conventional extraction solution (1% SDS, [pH 11.0]) and proposed solution (1% SDS, 10 mM TCEP, and 10 mM HEPES [pH 7.0]). To mimic soiled medical equipment, pseudo-blood-contaminated stainless steel plates were prepared. Residual protein was recovered using conventional and proposed solutions under varying temperature and humidity conditions. Quantitative protein recovery limits were determined at nine facilities. Compared with the conventional solution, the proposed solution recovered proteins more effectively from samples processed at temperatures > 60℃. However, low recovery rates were observed for samples processed at 95℃, possibly owing to differences in protein adhesion due to sample and plate-surface properties. Our findings present a method for quantifying residual proteins on medical instruments exposed to high temperatures during use or disinfection. Further studies should standardise test soiling conditions, materials, and solutions to evaluate cleaning methods.

Keywords: medical device cleanliness, protein residue quantification, stainless steel surface analysis

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Nomura Y[*1,2], Hanaoka S[*3,4], Hayashi N[*2], Yoshikawa T[*2], Koshino S[*3], Sato C[*5], Tatsuta M[*6], Tanaka Y[*4], Kano S[*3], Nakaya M[*4], Inui S[*3], Kusakabe M[*7], Nakao T[*2], Miki S[*2], Watadani T[*3,4], Nakaoka R, Shimizu A[*8], Abe O[*3,4]: Performance changes due to differences among annotating radiologists for training data in computerized lesion detection.

Int. J. Comput. Assist. Radiol. Surg. 2024;19(8):1527-36. doi: 10.1007/s11548-024-03136-9

Purpose: The quality and bias of annotations by annotators (e.g., radiologists) affect the performance changes in computer-aided detection (CAD) software using machine learning. We hypothesized that the difference in the years of experience in image interpretation among radiologists contributes to annotation variability. In this study, we focused on how the performance of CAD software changes with retraining by incorporating cases annotated by radiologists with varying experience. Methods: We used two types of CAD software for lung nodule detection in chest computed tomography images and cerebral aneurysm detection in magnetic resonance angiography images. Twelve radiologists with different years of experience independently annotated the lesions, and the performance changes were investigated by repeating the retraining of the CAD software twice, with the addition of cases annotated by each radiologist. Additionally, we investigated the effects of retraining using integrated annotations from multiple radiologists. Results: The performance of the CAD software after retraining differed among annotating radiologists. In some cases, the performance was degraded compared to that of the initial software. Retraining using integrated annotations showed different performance trends depending on the target

CAD software, notably in cerebral aneurysm detection, where the performance decreased compared to using annotations from a single radiologist. Conclusions: Although the performance of the CAD software after retraining varied among the annotating radiologists, no direct correlation with their experience was found. The performance trends differed according to the type of CAD software used when integrated annotations from multiple radiologists were used.

Keywords: machine learning, retraining, annotation

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Mabuchi K*1, Iwashita H*2, Sakai R*1, Nakaoka R: Accuracy of Stanton's formula used for the data processing of a pendulum friction test.
Tribol. Online. 2024;19(6):486-9. doi: 10.2474/trol.19.486

Pendulum friction-tester is specifically useful for experiments in biotribological field because that is good at the measure on circular sliding surfaces, like as synovial joints, joint prostheses, eye balls, or contact lenses. Stanton's formula has been generally used for the data processing as the calculation of friction coefficient from the amplitude decay during a pendulum libration. Because Stanton used the balance of moment to analyze the pendulum motion, his analysis must inevitably include an approximation of $\sin\theta$ as angle θ. However, the error in the formula has not been evaluated because the strict analysis was thought to be impossible. In the present study, the strict analysis was clarified by energy balance in a pendulum. As the result, error in Stanton's formula was able to be evaluated.

Keywords: friction test, Stanton's formula, rigorous solution

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Nakaoka R, Iwashita H*1, Hori Y*1, Mabuchi K*2, Matsunaga T*3, Haishima Y, Yamamoto E: Improvement of a pendulum-type apparatus for friction test of a contact lens to simulate the conditions of its actual usage.
Biosurf. Biotribol. 2024;10(4):167-75. doi: 10.1049/bsb2.12086

Friction between the contact lens (CL) and the corneal or conjunctival surfaces is considered one of key factors in triggering CL-associated adverse effects. However, the relationship between friction properties and these effects remains unclear. Traditional measurement methods often fail to replicate real-life conditions, thereby highlighting the need for more effective apparatus. In this study, the authors developed an optimised pendulum apparatus integrated with an inclinometer to enhance the measurement of CL friction coefficients, thereby improving its precision and relevance to clinical settings. This new design allows for faster and easier calculation of the friction coefficient based on the amplitude decay per libration cycle, surpassing the accuracy of previous video-based methods. The pendulum's hemisphere component was made from ethylene-propylene-diene monomer rubber (EPDM) 30, which has an elastic modulus similar to that of a human eyeball, creating a measurement environment that closely mimics real-world usage. The authors optimised the apparatus by evaluating the effects of hemisphere stiffness and saline volume on the friction coefficient. Measurements of multiple lenses recorded by the authors, particularly Lens A, made of narafilcon A, revealed significant consistency across different hemisphere materials with an optimal saline volume of 150 μL yielding a friction coefficient of 0.026 ± 0.003. No statistically significant differences in the friction coefficients were found across variations in the lens base

curve, diameter, centre thickness, or power. This improved apparatus demonstrates the capability of effectively measuring friction coefficients under conditions that simulate clinical usage, providing rapid and reliable results. The findings validate the apparatus and suggest its potential for broader applications in assessing CL properties, thereby facilitating future research on the material characteristics and safety of various CLs, including decorative lenses.

Keywords: friction coefficient, sliding friction, surface

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原守男*，島田夏帆*，中岡竜介，清水昭伸*：骨シンチグラムの陽性高集積検出支援システムのための深層学習を利用した逆フィルターの設計と評価.
Med. Imaging Technol. 2024;42(1):29-38. doi: 10.11409/mit.42.29

本論文では，骨シンチグラムに適用されている仕様の詳細が公表されていない画像処理フィルターを対象に，深層学習を利用してそのフィルター効果を無効化（以下，逆フィルター）する方法を提案する．骨シンチグラムに対するフィルターはノイズを削減するなど，人にとって見やすい画像を生成するが，フィルターなしの画像で学習した陽性高集積検出システムの性能を低下させることがある．そこで，フィルター適用後の画像からフィルター適用前の画像を予測する逆フィルターを，深層モデルを用いて設計する．本論文では実際にGE HealthCare社とSiemens Healthineers社の２種類のフィルターで処理した画像に本手法を適用し，フィルター適用前の画像を予測する．また，その予測画像を利用することで，フィルター適用前の画像のみで学習をした陽性高集積検出支援システムの精度が，フィルター適用後の画像を入力する場合よりも向上することを示す.

Keywords: 骨シンチグラム，深層学習，逆フィルター

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迫田秀行，坪子侑佑，岡本吉弘，山本栄一：コンタクトレンズ型ウェアラブルセンサーの目擦りを想定した性能劣化と安全性に関する*in vitro* 評価法の開発.
国立医薬品食品衛生研究所報告. 2024;142:42-8

A variety of contact lens-type wearable sensor devices are currently under development. These devices present an additional risk to traditional contact lenses, including performance degradation due to sensor malfunction and eye damage due to exposure to embedded sensor components. The objective of this study was to develop an *in vitro* test to evaluate the performance degradation and the risk of component exposure due to the physical load associated with eye rubbing. A contact lens-type wearable sensor product with embedded metal components in the polymer body was employed as the test specimen. The device was activated, and a cyclic compressive load simulating eye rubbing was applied in synchronization with the timing of its periodic measurements. Consequently, the number of cycles before the sensor malfunctioned could be evaluated.

Furthermore, the potential for X-ray computed tomography imaging to detect the exposure of metal components following the physical failure of the sensor was also investigated. The use of a copper filter served to mitigate the impact of metal artifacts introduced by the metal components, yet concurrently resulted in a reduction in contrast. However, the contrast between the polymer body and its surroundings improved when the sensor was imaged while immersed in a potassium iodide solution. Consequently, the outline of the sensor became discernible, thereby enabling the determination of whether the embedded components were exposed.

Keywords: wearable sensor, fatigue test, X-ray CT imaging

Hirasawa Y*1, Kakizoe Y*1, Tougan T*2, Uchiyama N, Horii T*3, Morita H*1: Vincarostine A, A Novel Anti-Malarial Trimeric Monoterpenoid Indole Alkaloid from *Catharanthus roseus*.
J. Nat. Med. 2024;78(3):768-73. doi: 10.1007/s11418-024-01795-1

A novel trimeric monoterpenoid indole alkaloid, vincarostine A (1) consisting of an aspidosperma-iboga-aspidosperma type skeleton, was isolated from the whole plant of *Catharanthus roseus*. The structure including absolute stereochemistry was elucidated on the basis of 2D NMR data and CD spectrum. Vincarostine A (1) showed anti-malarial activity.

Keywords: *Catharanthus roseus*, anti-malarial activity,

trimeric monoterpenoid indole alkaloid

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Uchiyama N, Hosoe J, Kiyota K*1, Komatsu T*2, Sugimoto N, Ishizuki K, Koide T, Murabayashi M*3, Shinozaki T*4, Fujimine Y*5, Ofuji K*6, Shimizu H*6, Fujita K*6, Hasebe T*7, Asai Y*7, Ena E*7, Makino Y*8, Miura T*9, Muto Y*9, Asakura K*2, Suematsu T*2, Abe H*2, Kohama A*10, Goto T*11, Yasuda M*11, Ueda T*12, Goda Y: Absolute purity determination of an organic fluorine pharmaceutical voriconazole via quantitative ^{19}F-NMR and method validation.

J. Pharm. Biomed. Anal. 2024;4:100039. doi: 10.1016/j.jpbao.2024.100039

The ^{19}F-NMR spectrum has a smaller number of signals than ^{1}H-NMR, therefore, quantitative ^{19}F-NMR (^{19}F-qNMR) can replace quantitative ^{1}H-NMR (^{1}H-qNMR) for the absolute quantitation of organic fluorine-bearing compounds. In this study, we determined the purity of voriconazole (VCZ), an organic fluorine pharmaceutical, using ^{19}F-qNMR, validated the results in multiple laboratories, and compared them with those obtained using an established ^{1}H-qNMR method. 3,5-Bis(trifluoromethyl) benzoic acid (3,5-BTFMBA) was selected as the reference standard for both ^{19}F-qNMR and ^{1}H-qNMR owing to its solubility characteristics and ^{19}F-qNMR and ^{1}H-qNMR chemical shifts. Since VCZ contains three fluorines, the ^{19}F-qNMR spectrum of VCZ showed three major fluorine signals (FA, FB, and FC). ^{19}F-qNMR measurements were performed under three conditions with each ^{19}F signal of VCZ (FA, FB, and FC) and the ^{19}F signal of RS as the center (offset) of each observation. The quantitative values of the three target signals FA, FB, and FC were calculated and were comparable; 99.40%, 99.66%, and 99.49%, respectively. Owing to a difference of up to 8.8% between the quantitative values of the two signals other than the targeted quantitative signal, the average FA, FB, and FC quantitative values were considered for purity (%). The purity of VCZ determined by ^{1}H- and ^{19}F-qNMR was comparable (99.65 ± 0.29% and 99.52 ± 0.44%, respectively), exhibiting variations within an acceptable range. Compared to conventional ^{1}H-qNMR methods, the developed ^{19}F-qNMR method has been proven efficient and highly promising for determining the accurate purity of organic fluorine pharmaceuticals.

Keywords: quantitative ^{19}F-NMR, voriconazole, absolute purity

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Hirasawa Y*1, Kasagi C*1, Koyama E*1, Myojin H*1, Tougan T*2, Horii T*3, Uchiyama N, Kaneda T*1, Morita H*1: Cathagines A-D, new bisindole alkaloids from *Catharanthus roseus*.

J. Nat. Med. 2025;79(1):134-42. doi: 10.1007/s11418-024-01857-4

Four new bisindole alkaloids, cathagines A (1)-D (4) consisting of an aspidosperma and the fused tetracyclic 3-spirooxindole derived from an iboga type skeleton were isolated from the whole plant of Catharanthus roseus. The structures including absolute stereochemistry were elucidated on the basis of 2D NMR data and CD spectra. Cathagine B (2) showed moderate anti-malarial activity against Plasmodium falciparum 3D7.

Keywords: *Catharanthus roseus*, 3-spirooxindole, antiplasmodial activity

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Oshima N, Tahara M, Kawakami T, Yagami A*, Akiyama T, Uchiyama N, Ikarashi Y: Estimation for raw material plants of a henna product using LC-high resolution MS and multivariate analysis.

Chem. Pharm. Bull. 2024;72:664-8. doi: 10.1248/cpb.c24-00278

Henna is a plant-based dye obtained from the powdered leaf of the pigmented plant Lawsonia inermis, and has often been used for grey hair dyeing, treatment, and body painting. As a henna product, the leaves of Indigofera tinctoria and Cassia auriculata can be blended to produce different colour variations. Although allergy from henna products attributed to p-phenylenediamine, which is added to enhance the dye, is reported occasionally, raw material plants of henna products could also contribute to the allergy. In this study, we reported that raw material plants of commercial henna products distributed in Japan can be estimated by LC-high resolution MS (LC-HRMS) and multivariate analysis. Principal Component Analysis (PCA) score plot clearly separated 17 samples into three groups [I; henna, II; blended henna primarily comprising Indigofera tinctoria, III; Cassia auriculata]. This grouping was consistent with the ingredient lists of products except that one sample listed as henna was classified as Group III, indicating that its ingredient label may differ from the actual formulation. The ingredients characteristic to Groups I, II, and III by PCA were lawsone (1), indirubin (2), and rutin (3), respectively, which were reported to be contained in each plant as ingredients. Therefore, henna products can be considered to have been manufactured from these plants. This study is the first to estimate raw material plants used in commercial plant-based dye by LC-HRMS and multivariate analysis.

Keywords: henna, raw material plant, LC-high resolution MS

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Yoshitomi T*, Nishi I*, Uemura H*, Tahara M, Sakai S: Simultaneous analysis of insecticides and phthalates in residential buildings based on Japan's indoor air quality guidelines.

BPB Reports. 2024;7:85-9. doi: 10.1248/bpbreports.7.3_85

The Ministry of Health, Labour and Welfare, Japan, had set guidelines for concentrations of indoor air pollutants such as di-n-butyl phthalate (DBP) and di-2-ethylhexyl phthalate (DEHP) and the insecticides fenobucarb, diazinon, and chlorpyrifos, which are semi-volatile pollutants in indoor air. The Committee on Indoor Air Pollution, Japan, is reviewing the 20-year old indoor air quality guidelines. Therefore, the current levels of semi-volatile pollutants in indoor air must be established. Insecticides and phthalates are estimated separately, necessitating more efficient analytical methods. We developed a gas chromatography-mass spectrometry for simultaneous analysis of insecticides and phthalates using a cartridge composed of a quartz filter and a styrene divinylbenzene copolymer and applied it to a field survey. The recovery and relative standard deviations (RSD) for the insecticides' were 97.4-103% and 3.58-9.65%, and those for phthalates were 87.4-102% and 1.35-8.22%, respectively. The limits of quantitation (LOQ) for chlorpyrifos, diazinon, and fenobucarb were less than 1/10 of guideline values at 0.0128, 0.0201, and 0.00667 μg/m^3, respectively. The LOQs of phthalates were 0.0882 μg/m^3 for DBP and 0.107 μg/m^3 for DEHP, each less than 1/193 and 1/935 of guideline values, respectively. The simultaneous analysis method was used to survey residential houses. Insecticides were not detected in the indoor air of residential houses. In contrast, phthalates, diethyl phthalate (DEP), DBP, and DEHP were detected, and their concentration distributions decreased from those found in the 2000s.

Keywords: indoor air, semi-volatile organic compound, insecticide

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千葉真弘*，兼俊明夫*，大泉詩織*，田原麻衣子，酒井信夫：除湿管を使用した室内空気中揮発性有機化合物分析を想定した添加回収試験.

室内環境. 2024;27:107-17. doi: 10.7879/siej.27.107

本研究では，室内濃度指針値策定物質であるトルエン，キシレン，エチルベンゼン，スチレン，パラジクロロベンゼンおよびテトラデカンに室内濃度指針値策定が

検討されている２-エチル-１-ヘキサノール（２EH），2,2,4-トリメチルペンタン-1,3-ジオールモノイソブチラート（TPMI）および2,2,4-トリメチルペンタン-1,3-ジオールジイソブチラート（TPDI）を加えた９物質を対象とした室内空気中の揮発性有機化合物（VOCs）の分析において，高湿度条件下での除湿管接続による定量値への影響について検討した．その結果，除湿管を接続しない場合は，加熱脱着法（TD法）および溶媒抽出法（SE法）ともに概ね良好な回収率が得られた．一方除湿管を接続した場合においては，TDおよびSE法ともに一部の物質（特にテトラデカン，２EH，TPMIおよびTPDI）の回収率が低下した．これらの物質は，除湿管への物理的な吸着が疑われたことから，VOCs測定では除湿管の接続が定量値に影響を及ぼす可能性が明らかとなった．

Keywords: dehumidification tube, volatile organic compound, indoor air

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久保田領志，秋山卓美，五十嵐良明：市販まつ毛美容液におけるプロスタグランジン Ｆ２ α類縁物質の含有実態調査．

日本香粧品学会誌. 2024;48(4):211-8.

Cosmetic products promoting eyelash growth have been marketed in European countries and the United States. These products contain prostaglandin F2-alpha (PGF2α) analogues, which enhance eyelash growth. Previous studies have identified the presence of not only the PGF2α analogues listed on the product labels but also other related compounds not mentioned. In this study, we developed an analytical method using high performance liquid chromatography (HPLC) to investigate the presence of PGF2α analogues in commercially available eyelash serums in Japan. The target substances measured were bimatoprost, isopropyl cloprostenate, and ethyl tafluprostamide. Several HPLC methods were evaluated based on column separation and mobile phase conditions. The limit of quantification for the target substances ranged from 0.0001-0.0003% under the analytical conditions, corresponding to 1/100-1/300 of the concentration found in ophthalmic and cutaneous solutions containing bimatoprost. This level of sensitivity was sufficient for the measurements. Method validation through additive recovery tests yielded satisfactory results, with recovery/precision ranges of 101-103%, repeatability (RSD_r %) ranging from 0.21-1.8%, and intermediate precision (RSD_R %) ranging from 1.1-1.4%. The HPLC analysis showed that bimatoprost was not detectable in any of the eyelash serum samples. Ethyl tafluprostamide was detected in two samples labeled with this compound. Isopropyl cloprostenate was observed in two products, although it was below the limit of quantitation in one and detected above the lower limit of quantitation in the other. These concentrations were similar to or slightly lower than those previously reported. In contrast, isopropyl cloprostenate was only detected in one unlabeled sample. High performance liquid chromatography-tandem mass spectrometry (LC/MS/MS) analysis using the same and another lot of this product also detected isopropyl cloprostenate, indicating a labeling omission of this compound in these products.

Keywords: isopropyl cloprostenate, eyelash serum, HPLC

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水道協会雑誌. 2024;93(6):13-21. doi: 10.34566/jwwa.93.6_13

水道水質検査の告示法においてガラス製採水容器が規定されている水質基準項目を対象に，採水に高密度ポリエチレン容器が使用可能か検討を行った．水道事業体を対象としたアンケートでは，吸着や耐久性等への懸念があるものの約８割の事業体が樹脂容器の使用を希望しており，ニーズは高かった．水道水添加試料を用いた試験では，TOC，ホルムアルデヒド，フェノール類及びハロ酢酸類について，ガラス容器と遜色ないことが分かった．但し，ホルムアルデヒド及びフェノール類を対象としたキャリーオーバー試験では，フェノール類にのみキャリーオーバーが認められた．これらの結果から，TOC，ホルムアルデヒド及びハロ酢酸類の検査には樹脂容器が使用可能であることが示された．

Keywords: water quality testing, analytical method, organic compound

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YAKUGAKU ZASSHI. 2024;144:463-71. doi: org/10.1248/yakushi.23-00188

In Japan, the use of frame retardants [tris(2,3-dibromopropyl)phosphate: TDBPP and bis(2,3-dibromopropyl)phosphate: BDBPP] in several household textile products is banned under the "Act on the Control of Household Products Containing Harmful Substances." As the official analytical methods for testing these substances have not been revised for over 42 years, several issues such as the using of harmful reagents, have been pointed out. Therefore, we developed a new method to revise the official method in our previous study. In this study, the validity of the developed test method is evaluated at six laboratories using two types of textile samples spiked with TDBPP and BDBPP at three concentrations (4, 8, and 20 μg/g). TDBPP and BDBPP are extracted under reflux using methanol containing hydrochloric acid. TDBPP is analyzed using GC-MS, and BDBPP is also analyzed using GC-MS after methylation with trimethylsilyl diazomethane. Although the accuracy (70-120%), repeatability (<10%), and reproducibility (<15%) of a few samples, mainly low concentration samples, are out of range, overall, the concentration level of detection limits of TDBPP and BDBPP (8 and 10 μg/g) in official analytical methods are quantifiable with sufficient precision using the proposed method. Furthermore, harmful reagents are not used in this method. Thus, the method validated in this study is effective as a revised method for the testing of TDBPP and BDBPP in household textile products.

Keywords: textile, tris(2,3-dibromopropyl)phosphate, bis(2,3-dibromopropyl)phosphate

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Miyazawa H*1, Kawakami T, Sugiyama M*2,3: Allergic contact dermatitis caused by menthoxypropanediol in a skin care lotion.
Contact Dermatitis. 2024;94:264-6. doi: org/10.1111/cod.14591

A 45-year-old woman with itchy, infiltrative erythema and papules on her left wrist and elbow was referred to us and mentioned that she used a skin care lotion to relieve itching. She had recently applied it to her left wrist and covered it with a dressing tape. A few days later, she experienced pruritic symptoms. Patch testing was carried out using a Japanese baseline series, the lotion as is, and the dressing tape. On day 3 (D3), a + positive reaction to the lotion was observed. The manufacture provided the ingredients of the lotion unlabeled, pre-diluted in petrolatum for patch testing. The second patch test showed a + reaction on D3 to only one of the ingredients. The manufacture provided the name of the ingredient, menthoxypropanediol (MPD). We patch tested the patient to investigating the optimal MPD (CAS RN: 87061-04-9) concentration with a dilution series in pet. Based on the results of patch test, we concluded that MPD was the causative allergen in this case. MPD has several stereoisomers due to its asymmetrical carbons, and there are two CAS RNs. MPD used for patch testing is a non-specific stereoisomer. Since accurate information on the MPD isomer used in the product could not be obtained from the manufacturer, identifying the culprit allergen is challenging.

Keywords: allergic contact dermatitis, skin care product, menthoxypropanediol

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Terashima A[*1], Iwasaki Y[*1], Taguchi T, Fukiwake T[*1], Tsutsumi T, Imamura T[*2], Akiyama H[*1]: Monitoring of cyanogenic compounds behavior during the manufacturing process of sweetened bean paste.

Heliyon. 2024;10(19):e338862. doi: 10.1016/j.heliyon.2024.e38862

To ensure food safety, food business operators must eliminate or reduce hazardous factors in manufacturing processes by implementing effective process controls. Since some beans are known to contain cyanogenic compounds, their distribution and use are permitted only as a raw bean paste material. Therefore, from the perspective of Hazard Analysis and Critical Control Points (HACCP), the purpose of this study is to demonstrate the validity of establishing CCPs and to determine the cyanogenic compounds in intermediate products for effectively managing hazardous substances in the manufacturing process. The previously reported method, post-column HPLC with fluorescence detection, was used for determine cyanogenic compounds in CCPs.

While free cyanide ions were only detected at CCP#1, cyanoglycoside analysis was crucial throughout the manufacturing process. Results indicated a decrease in cyanoglycoside concentration as manufacturing progressed, with levels below 10 ppm in the final product. Notably, cyanoglycosides decreased significantly during the shibukiri process (soaking, boiling, and discarding water). The concentration of cyanogenic compounds in raw beans were below the regulated 500 ppm, and the concentrations in the final product were below regulated 10 ppm. In conclusion, it was found that proposed method is very useful for HACCP management to monitor the decrease of cyanide compounds in the manufacturing process.

Keywords: critical control point (CCP), sweetened bean paste, cyanogenic compounds

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Sasano R[*1,2], Sekizawa J[*1], Saito I[*2], Harano A[*1], Katsumoto K[*1], Ito R[*1], Iwasaki Y[*1], Taguchi T, Tsutsumi T, Akiyama H[*1]: Simultaneous Determination of Glyphosate, Glufosinate and their Metabolites in Soybeans using Solid-phase Derivatization and LC-MS/MS Determination.

Food Chemistry: X. 2024;24:101806. doi: https://doi.org/10.1016/j.fochx.2024.101806

Glyphosate and glufosinate are the most widely used herbicides worldwide. We developed a simple and rapid analytical method for detecting glyphosate, glufosinate, and their metabolites (*N*-acetyl glyphosate: Gly-A, *N*-acetyl glufosinate: Glu-A, and 3-(hydroxymethylphosphinyl)propanoic acid: MPPA) in soybeans. The method involved extraction with water, trapping in a mini-column containing polymer-based resin with strong anion exchange groups, dehydration with acetonitrile, and solid-phase analytical derivatization at ambient temperature for 1 min using *N*-(*tert*-butyldimethylsilyl)-*N*-methyl trifluoroacetamide (MTBSTFA), followed by Liquid chromatography-tandem mass spectrometry (LC-MS/MS) determination. This method offers a straightforward and rapid analysis, using on-solid phase dehydration and rapid derivatization at an ambient temperature with MTBSTFA, yielding reliable results for glyphosate, glufosinate, and their metabolites. The method was applied to both domestic and imported soybean samples. Glyphosate, glufosinate, and Glu-A were detected in imported feed soybeans and processed soybean meal for feed use, reflecting the current conditions of GM soybean cultivation.

Keywords: glyphosate, *N*-(*tert*-butyldimethylsilyl)-*N*-methyl trifluoroacetamide, solid-phase analytical derivatization

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日本食品化学学会誌. 2025;32(1):10-14.

When human health is affected by food terrorism or other incidents involving cyanide-containing foods, public institutions are required to test human biological samples. However, conventional gas chromatograph (GC)-based analytical methods are not suitable for public testing due to the complex pretreatment

process and the recent shortage of helium gas. In this study, we developed an analytical method for cyanide and thiocyanate ions in human blood using post-column HPLC with fluorescence detection and evaluated the method ions using human blood. The recovery test for cyanide ions in human blood showed an accuracy of 88.4%, a repeatability of 1.8%, and an intra-laboratory precision of 7.0%. The recovery test for thiocyanate ions in the same human blood showed an accuracy of 95.5%, a repeatability of 5.9%, and an intra-laboratory precision of 11.5%. The study suggests that this analytical method is useful for measuring cyanide and thiocyanate ions in blood when health damage occurs during food terrorism or other intentional contamination incidents.

Keywords: シアン化合物，食品テロ，ヒト血液

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Hashimoto M*1, Ishikawa K*2, Fukushima Y*1, Shimazu S*1, Yabuzaki M*1, Kamezawa Y*1, Taguchi T, Ichinose K*1: Characterization of ActVI-ORF3 and ActVI-ORF4 as Lactonizing and Delactonizing Enzymes in Relation to Metabolic Flux in Actinorhodin Biosynthesis.
ChemBioChem. 2025;e202500049. doi: https://doi.org/10.1002/cbic.202500049

Actinorhodin (ACT) from Streptomyces coelicolor A3(2) is an aromatic polyketide antibiotic with a benzoisochromanequinone (BIQ) skeleton. Although actVI-ORF3 and actVI-ORF4 are not essential for ACT biosynthesis, homologous genes to these are present in the biosynthetic gene clusters of BIQ lactones. In this study, ActVI-ORF3 was identified as a cofactor-independent enzyme with lactonization activity, using ACT as a substrate. ActVI-ORF3 recognized dihydrokalafungin and 8-hydroxykalafafungin, which share the same pyran-ring configuration as ACT, but not nanaomycin A, which has an opposite configuration. In contrast, ActVI-ORF4 functioned as an NAD(P)-dependent oxidoreductase, catalyzing the delactonization of BIQ lactones. Conversion experiments using isotopically labeled compounds revealed that both lactonization and delactonization reactions of these enzymes yielded products in which the carboxyl oxygen at the C1 position was retained. Subsequently, we reexamined the accumulation of ACT-related compounds in the actVI-ORF3 and actVI-ORF-4 disruptants. The results suggested that ACT intermediates are predominantly pooled in the bacteria as (*S*)-DNPA rather than in lactone-form. The contribution of ActVI-ORF4 to metabolic flux is not significant, and endogenous reductases can convert these intermediates to the dihydro form, which subsequently re-enters the ACT biosynthetic pathway.

Keywords: actinorhodin, lactonization, metabolic flux

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Nabeshi H, Adachi R, Akiyama H*, Tsutsumi T: Suppression effect on acrylamide formation in french fries by soaking of potato in various solutions.
Japanese Journal of Food Chemistry and Safety. 2024; 31(1):10-20. doi: 10.18891/jjfcs.31.1_10

Herein, we examined the suppression effect of potato soaking in various solutions on acrylamide (AA) formation in french fries. Thin strips of potatoes soaked for 20-180 min in various solutions such as tap water, vinegar water, and salt water were used to cook french fries by using either a conventional deep fryer or air fryer. The AA concentrations in the french fries were determined by liquid chromatography-tandem mass spectrometry analysis. Compared to unsoaked controls, solution soaking suppressed AA formation when using both fryers. This suppression effect tended to increase with prolonged soaking times. Under the same soaking time, the use of vinegar water was tended to be more effectively suppressed AA formation in french fries compared to tap water (20℃) treatment. Changing the water was also tended to be effectively suppressed AA formation. On the other hand, the AA concentrations in the french fries cooked with an air fryer were higher than those observed when cooking by using a conventional deep fryer. This may be attributed to higher heating temperatures, longer heating times, and larger heating irregularities in the case of the air fryer compared to the conventional deep fryer. Furthermore, the AA concentrations in french fries cooked by an air fryer without oil were markedly higher than those determined when cooking in the presence of oil. Thus, it may be necessary to pay attention to the possibility

of increased AA formation in french fries cooked by air fryers (particularly non-oil cooking).
Keywords: acrylamide formation, suppression effect, french fries

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日本食品化学学会誌. 2024;31(3):111-115. doi: 10.18891/jjfcs.31.3_111

In response to food contamination by radioactive materials arising from the Fukushima Daiichi Nuclear Power Station Accident, new standard limits for radioactive materials in food were established in 2012 in Japan. As it is possible that infants have a higher sensitivity to radiation than adults, the standard limit for infant food (50 Bq/kg) was set at half the limit for food in general (100 Bq/kg). As infant foods are not as rigorously monitored by municipalities as fresh foods, it is crucial that infants' exposure to radiation be assessed by measuring and understanding the concentration of radioactive materials in infant foods. In this study, we investigated the concentration of radioactive cesium (Cs-134 and Cs-137) in 906 samples of infant foods available on the Japanese market from 2012 to 2023 using a germanium semiconductor detector. The sum of the limit of detection for Cs-134 and Cs-137 was set as less than 5 Bq/kg for infant foods and less than 1 Bq/kg for drinking water for infants. Totals of 238 samples of infant formula, 471 samples of infant foods, and 197 samples of snacks and beverages were analyzed, with none of the samples exceeding the new standard limits and the limit of detections for radioactive cesium. These results suggest that manufacturers have implemented strict production management procedures since the accident so that the intake of radioactive cesium from infant foods produced in Japan is negligibly low.
Keywords: radioactive cesium, infant food, Fukushima Daiichi Nuclear Power Station Accident

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鍋師裕美，前田朋美，五十嵐敦子，川又香予，堤智昭：乾燥コウタケの飲食に供される状態での重量変化に関する検討．
食品衛生学雑誌. 2024;65(6):239-247. doi: 10.3358/shokueishi.65.167

乾燥食品の放射性セシウム検査では，乾燥状態での分析結果を厚生労働省が示す重量変化率を用いて飲食に供される状態の濃度に換算し，検査結果とすることが認められているが，重量変化率が示されている乾燥食品はごく一部に限られている．乾燥食品の基準値への適合判定を適切に行うためには，科学的知見に基づいて設定された個々の乾燥食品の重量変化率を用いることが理想的である．そこで，比較的高濃度の放射性セシウムが検出されやすく，重量変化率が基準値への適合判定に及ぼす影響が大きい乾燥コウタケの重量変化率を検討した．乾燥コウタケの重量変化率は4.2～6.9（平均値5.7）であり，平均値は現在適用されている重量変化率4.0より1.4倍大きい値となった．一方，重量変化率の最小値は4.2であったことから，本検討結果から安全側に配慮して保守的に設定する場合，現在適用されている重量変化率は乾燥コウタケの重量変化率として妥当であると考えられた．しかし，乾燥コウタケ個別の重量変化率の設定や現在適用されている重量変化率の妥当性評価のためには，さらに多くのデータを収集する必要があると考えられた．
Keywords: 放射性セシウム検査，乾燥コウタケ，重量変化率

Saito-Shida S, Saito M, Tsutsumi T: Comparison of nitrogen and helium as carrier gases for determination of pesticide residues in foods via gas chromatography-tandem mass spectrometry with atmospheric pressure chemical ionization.
J. Food Compos. Anal., 2024;134: 106492. doi: https://doi.org/10.1016/j.jfca.2024.106492

The applicability of nitrogen as an alternative carrier gas to helium for the multiresidue analysis of pesticides using gas chromatography-tandem mass spectrometry with atmospheric pressure chemical ionization (GC-(APCI)MS/MS) was examined. The methods using both carrier gases were validated for 151 pesticides in foods at a spiking level of 0.01 ppm, maintaining identical conditions for both gases, including the GC column and carrier gas flow rate. The use of nitrogen resulted in slightly broader peaks and lower peak intensities that those observed upon using helium. However, sufficient sensitivity was achieved for all target compounds because of the high

sensitivity of the GC-(APCI)MS/MS method. Further, target-compound separation was not significantly affected by the carrier gas type, and no interfering peaks were observed, demonstrating the high selectivity of the nitrogen-based method, even beyond the optimum linear velocity. Finally, the analytical performance of the nitrogen-based method was comparable to those of the helium-based method. Considering cost-effectiveness of nitrogen, this method is highly valuable in the event of helium shortages and for routine pesticide residue monitoring.

Keywords: pesticide, atmospheric pressure chemical ionization, carrier gas

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食品衛生学雑誌. 2024;65(6):178-184. doi: https://doi.org/10.3358/shokueishi.65.178

Thiouracil (2-thiouracil) is a thyrostat used to promote weight gain in cattle. However, its use is prohibited within the European Union (EU), necessitating the monitoring of its presence in bovine urine for beef exports to the EU. In this study, we present the development and validation of a quantitative method for the determination of 2-thiouracil, 4-thiouracil, and 6-methyl-2-thiouracil in bovine urine using liquid chromatography-tandem mass spectrometry (LC-MS/MS). This method involves stabilizing the analytes by adding hydrochloric acid and ethylenediaminetetraacetic acid to the sample, followed by derivatization with 3-iodobenzyl bromide, cleanup with a divinylbenzene-*N*-vinylpyrrolidone copolymer cartridge, and subsequent LC-MS/MS analysis. The developed method was validated for determination of 2-thiouracil, 4-thiouracil, and 6-methyl-2-thiouracil in bovine urine at a concentration of 10 μg/L. The trueness ranged from 94 to 97%, with intra-day precisions below 5% and inter-day precisions below 8%. No chromatographic interference was observed near the analytes' retention times. This analytical method is particularly valuable because it can determine whether 2-thiouracil was illicitly administered or ingested via feed containing plants of the Brassicaceae family, by confirming the presence of 6-methyl-2-thiouracil or 4-thiouracil alongside 2-thiouracil in bovine urine.

Keywords: 2-thiouracil, 4-thiouracil, 6-methyl-2-thiouracil

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Akiyama H[*1], Suzuki Y, Adachi R, Kadokura M[*1], Takei A[*1], Tomiki M[*1,2], Nakamura K, Ito R[*1], Iwasaki Y[*1], Mills C[*3], Ohya Y[*4], Fukuie T[*4]: Egg protein exposure estimation in risk assessment for Japanese food allergy labeling.

Heliyon. 2024;10(13):e33545, doi: 10.1016/j.heliyon.2024.e33545.

To assess the risk of food allergies in foods processed under the Japanese food labeling system, estimating exposure to hidden allergens is necessary. We assessed exposure to egg protein in foods processed according to the Japanese food labeling system. First, we estimated the concentration distribution of egg protein by Bayesian methods using data from the literature and the measurement of food products with precautional declarations in the labeling margin. We then estimated the food-intake portion-size distribution under two scenarios: soft drink consumption as an example of single, high-intake consumption, and confections, which are frequently consumed by children, as a realistic example of low-intake consumption. Finally, we estimated the distribution of unexpected intake of egg proteins in the form of single consumption. The mean exposure to egg protein under the high-intake scenario was estimated to be 0.0164 mg for 1-15-year-olds, 0.0171 mg for 4-15-year-olds, 0.0181 mg for 7-15-year-olds, and ≥ 0.0188 mg for 16-year-olds. The mean exposure to egg protein under the low-intake scenario was estimated to be 0.0018 mg for 1-15-year-olds, 0.0019 mg for 4-15-year-olds, 0.0020 mg for 7-15-year-olds, and ≥0.0022 mg for 16-year-olds. Compared to the reference dose of 2.0 mg proposed by the Joint the Food and Agriculture Organization (FAO)/World Health Organization (WHO) Expert Committee, the risk of onset of food allergies due to egg protein contamination from foods without egg labeling is considered to be extremely low under the current Japanese food labeling system.

Keywords: labeling, food allergy, risk assessment, Bayesian estimation, egg protein

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Takahashi M, Suzuki Y, Aoyagi M*1, Toda E*2, Ito K*2, Fukumitsu T*3, Hagio M*3, Hayashi T*3, Shintaku S*4, Ihara S*5, Nakashima A*5, Sato T*6, Okamoto F*6, Hori T*6, Akiyama H*7, Tsutsumi T: Estimated daily intake of residual agricultural chemicals across general Japanese people based on the total diet study from 2019 to 2021.

Japanese Journal of Food Chemistry and Safety. 2024; 31(2):65-75. doi: 10.18891/jjfcs.31.2_65.

Public perceptions are significantly more concerned about agricultural chemicals including pesticides, feed additives, and animal drugs than food safety experts. To address these perceptions, we estimated the mean daily intake of 28 agricultural chemicals across the entire Japanese population (≥ 1 year old) using the total diet samples based on the market basket method (14 food groups). The survey was conducted with the collaboration of six local government research institutes (Hokkaido, Tohoku, Kanto, Kansai, Chugoku, and Kyushu) from 2019 to 2021. The estimation of the mean daily intake of residual agricultural chemicals through the consumption of each food group was calculated by multiplying the concentration in the respective food group by the daily food consumption. The highest ratio of estimated daily intake over acceptable daily intake was observed for acephate (0.39%). The contribution rates from crops were higher than those from livestock and aquatic products for many agricultural chemicals. Our results show that all of agricultural chemicals evaluated in this study were far below the ADIs, and these findings considered to be useful to bridge the perception gap.

Keywords: residual agricultural chemicals, total diet study, estimated daily intake

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Nakamura K, Gondo T*1, Chiba S, Akimoto S, Narushima J, Kondo K, Tanaka H*1, Hashiguchi M*1, Shiwa Y*2, Akashi R*1: Global gene expression profile of food-production grade germinating soybean seeds using single-seed high-throughput RNA sequencing.

Agriculture. 2024;14:2287. doi: 10.3390/agriculture14122287

Gene expression in individual germinated seeds of four different soybean (*Glycine max* [L.] Merr.) cultivars, GL3494, OAC Kent, Williams 82, and Jackson, was examined using single seed high-throughput RNA sequencing (ssRNA-seq). The gene expression was similar between two individual seeds of the same cultivar, but different among individual seeds of the four cultivars. Notably, ssRNA-seq identified five genes that were not stably expressed in Williams 82, having either no detectable sequence reads or less than five sequence reads mapped to the exon regions of chromosomes in Williams 82. These findings were validated by reverse transcription polymerase chain reaction analysis. The study's results demonstrate that gene expression in germinated seeds is unique to an individual seed from each soybean cultivar. This uniqueness may affect composition and content of nutrients in germinated soybeans used as food ingredients.

Keywords: germinated soybean, single seed, ssRNA-seq

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Torii A*1, Seki Y*1, Sasano R*1, Ishida Y*1, Nakamura K, Ito R*2, Iwasaki Y*2, Iijima K*1, Akiyama H*2: Development of a rapid and reliable method to simultaneously detect seven food allergens in processed foods using LC-MS/MS.

Food Chemistry: X. 2024;23:101558. doi: 10.1016/

j.fochx.2024.101558

Rapid analysis of multiple food allergens is required to confirm the appropriateness of food allergen labelling in processed foods. This study aimed to develop a rapid and reliable method to simultaneously detect trace amounts of seven food allergenic proteins (wheat, buckwheat, milk, egg, crustacean, peanut, and walnut) in processed foods using LC-MS/MS. Suspension-trapping (S-Trap) columns and on-line automated solid-phase extraction were used to improve the complex and time-consuming pretreatment process previously required for allergen analysis using LC-MS/MS. The developed method enabled the simultaneous detection of selected marker peptides for specific proteins derived from seven food ingredients in five types of incurred samples amended with trace amounts of allergenic proteins. The limit of detection values of the method for each protein were estimated to be <1 mg/kg. The developed analytical approach is considered an effective screening method for confirming food allergen labelling on a wide range of processed foods.

Keywords: LC-MS/MS, peptide marker, solid-phase extraction

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Koyama T*1, Nakamura K, Kiuchi T, Chiba S, Akiyama H*2, Yoshiike N*1: Development of a reverse-yield factor database disaggregating Japanese composite foods into raw primary commodity ingredients based on the Standard Tables of Food Composition in Japan.

Foods. 2024;13:988. doi: 10.3390/foods13070988

The reverse-yield factor (RF) database was developed for qualitatively and quantitatively disaggregating Japanese composite foods into raw primary commodity (RPC) ingredients. Representative equations for four types (dried, salted, fermented and mixed foods) were developed to calculate RFs using the food content and composition data for composite foods listed in the Standard Tables of Food Composition in Japan—2020—(STFCJ), published by the Ministry of Education, Culture, Sports, Science and Technology of Japan. Out of 1150 composite foods identified in the STFCJ, RFs for 54 dried, 41 salted, 40 fermented and 818 mixed foods were obtained. RFs for 197 mixed foods could not be calculated because these foods were produced from ingredients with no specified information and/or through complex processing. The content and composition of Japanese composite foods would be interpreted representatively by RFs in the developed database.

Keywords: food ingredient, composite food, exposure assessment

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Yamasaki Y, Nakamura K, Kashiwabara N, Chiba S, Akiyama H*, Tsutumi T: Development of processing factor prediction model for pesticides in tomato processed foods using elastic net regularization.

Food Chemistry. 2024;447:138943. doi: 10.1016/j.foodchem.2024.138943

A novel regularized elastic net regression model was developed to predict processing factor (PF) for pesticide residues, which represents a change in the residue levels during food processing. The PF values for tomato juice, wet pomace and dry pomace in the evaluations and reports published by the Joint FAO/WHO Meeting on Pesticide Residues significantly correlated with the physicochemical properties of pesticides, and subsequently the correlation was observed in the present tomato processing study. The elastic net regression model predicted the PF values using the physicochemical properties as predictor variables for both training and test data within a 2-fold range for 80-100% of the pesticides tested in the tomato processing study while overcoming multicollinearity. These results suggest that the PF values are predictable at a certain degree of accuracy from the unique sets of physicochemical properties of pesticides using the developed model based on a processing study with representative pesticides.

Keywords: processing factor, physicochemical property, pesticide

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Nishizaki Y*1, Sugimoto N, Miura T*2, Asakura K*3, Suematsu T*3, Korhonen S-P*4, Lehtivarjo J*4, Niemitz M*4, Pauli GF*5: Quantum Mechanical

Quantitative Nuclear Magnetic Resonance Enables Digital Reference Standards at All Magnetic Fields and Enhances qNMR Sustainability.

Anal. Chem. 2024;96(24):9790-9798. doi:10.1021/acs.analchem.3c05267

Quantum mechanics (QM)-driven ^{1}H iterative functionalized spin analysis produces HifSA profiles, which encode the complete ^{1}H spin parameters ("nuclear genotype") of analytes of interest. HifSA profiles enable the establishment of digital reference standards (dRS) that are portable, FAIR (findable - accessible - interoperable - reusable), and fit for the purpose of quantitative ^{1}H NMR (qHNMR) analysis at any magnetic field. This approach enhances the sustainability of analytical standards. Moreover, the analyte-specific complete chemical shift and *J*-coupling information in HifSA-based dRS enable computational quantitation of substances in mixtures via QM-total-line-shape fitting (QM-qHNMR). We present the proof of concept for HifSA-based dRS by resolving the highly overlapping NMR resonances in the experimental spectra ("nuclear phenotypes") of the diastereomeric mixture of (2*RS*, 4*RS*)- and (2*RS*, 4*SR*)-difenoconazole (DFZ), a widely used antifouling food additive. The underlying ^{1}H spin parameters are highly conserved in various solvents, are robust against variation in measurement temperature, and work across a wide range of magnetic fields. QM-qHNMR analysis of DFZ samples at 80, 400, 600, and 800 MHz showed high congruence with metrological reference values. Furthermore, this study introduces QM-qHNMR combined with chiral shift reagents for the analysis of all four DFZ stereoisomers: (2*R*, 4*R*)-, (2*S*, 4*S*)-, (2*R*, 4*S*)-, and (2*S*, 4*R*)-DFZ to perform chiral qHNMR measurements.

Keywords: qNMR, quantum mechanics, HifSA

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Kawasue S, Kuniyoshi K, Uema M, Oshiro N: Tetrodotoxin Derivatization with a Newly Designed Boron Reagent Leads to Conventional Reversed-Phase Liquid Chromatography.

Toxins. 2024;16:260. doi:10.3390/toxins16060260

Tetrodotoxin (TTX) is a representative natural toxin causing pufferfish food poisoning, which is especially prominent in East and Southeast Asia, including Japan. TTX has been analyzed through post-column derivatization high-performance liquid chromatography (HPLC), ion-pair LC-MS(/MS), and hydrophilic interaction liquid chromatography (HILIC)-MS(/MS) as alternatives to the mouse bioassay method. However, post-column derivatization requires a system for online derivatization reactions, and with the ion-pair LC-MS approach, it is difficult to remove residual ion-pair reagents remaining in the equipment. Moreover, HILIC-MS provides poor separation compared to reversed-phase (RP) HPLC and requires a long time to reach equilibration. Therefore, we decided to develop a TTX analytical method using pre-column derivatization and RP HPLC for the rapid assessment of outbreak samples, including food remnants. In this study, we focused on the vic-diol moiety of TTX and designed a new derivatization reagent coded as NBD-H-DAB. This NBD-H-DAB was synthesized from 4-hydrazino-7-nitro-2,1,3-benzoxadiazole (NBD-H) and 3-fluoro-2-formylphenylboronic acid (FFPBA) with a simple reaction system and rapidly converted to its boronate form, coded NBD-H-PBA, in an aqueous reaction solution. The NBD-H-PBA demonstrated appropriate hydrophobicity to be retained on the RP analytical column and successfully detected with a UV spectrometer. It was easily reacted with the vic-diol moiety of TTX (C6 and C11) to synthesized a boronic ester. The derivatized TTX could be detected using the RP HPLC-UV, and the limit of detection in the fish flesh samples was 0.06 mg/kg. This novel pre-column derivatization of TTX with NBD-H-PBA proves capable for the analysis of TTX.

Keywords: tetrodotoxin, derivatization, reversed-phase HPLC-UV, boronic acid, pufferfish

Abe Y, Yamaguchi M, Fujihara K, Kataoka Y, Mutsuga M, Sugimoto N: Application of high-performance liquid chromatography to caprolactam migration testing of food utensils, containers, and packaging.

Food Hyg. Saf. Sci. 2024;65:107-112. doi:10.3358/shokueishi.65.107

We assessed the applicability of high-performance liquid chromatography (HPLC) to the official testing method for the migration of caprolactam (CPL) from food utensils, containers, and packaging made from polyamide (PA). Hydrophilic interaction chromatography (HILIC) columns coated with unmodified silica, carbamoyl, and aminopropyl were used. Water and acetonitrile (ACN) were used as the mobile phase, and the analytical conditions were optimized. The test solution was diluted 10-fold with ACN, and standard solutions were prepared using 98% ACN. We validated using HPLC as limit testing and quantitative testing methods. Accuracy parameters corresponding to trueness, repeatability, and reproducibility (as intermediate precision) satisfied the target values in both cases, indicating that this method demonstrates good performance as a testing method.
Keywords: Food utensils, containers and packaging, caprolactam, polyamide, migration testing, validation study, HPLC

Kurohara T, Tatebe C, Fujiwara Y, Hioki F, Takada S, Atsuko Tada, Sugiomoto N: Ruhemann's Purple Monitoring by UHPLC/MS/MS for Ninhydrin Test.
Food Hyg. Saf. Sci. 2025;66:12-18. doi:10.3358/shokueishi.66.12

For amino acids used as food additives, Japan's Specifications and Standards for Food Additives stipulate the ninhydrin test as an identification test. The ninhydrin test is a simple method that involves the visual determination of purple color from the formation of Ruhemann's purple (RP) and does not require special equipment, facilitating its widespread use in society. However, because of this background, objective and molecular selective observation methods for monitoring RP itself as an analyte have not been fully investigated. Therefore, in this study, a UHPLC/MS/MS method was developed to specifically monitor RP and support visual judgment. This method identified RP-derived fragment ions at *m/z* 170 (ESI (-)) and 133 (ESI (+)), which can be monitored in the multiple reaction monitoring mode and were shown to correlate with the intensity of the purple color. In addition, computational chemistry was applied to scientifically estimate the molecular structures of the fragment ions. In this study, we established a useful analytical method that complements the objectivity of the ninhydrin test. This method is also expected to be utilized for further optimization of test reaction conditions.
Keywords: Ruhemann's purple, UHPLC/MS/MS, amino acid, food additive, ninhydrin

Colman K[*1], Funk KA[*2], Boyle M[*3], Brennan S[*4], Cain G[*5], Colleton C[*6], Morton LD[*7], Giusti AM[*8], Jacquinet E[*9], LaFranco-Scheuch L[*10], McKinney L[*11], Neyens E[*12], Romeike A[*13], Hayashi SM, Vahle JL[*14], Tomlinson L[*15]: Scientific and Regulatory Policy Committee Points to Consider for Determining and Reporting Cause of Death/Moribundity in Non-Rodent Species in Toxicity Studies.
Toxicol. Pathol. 2025;20:1926233251321781. doi:10.1177/01926233251321781

The Cause of Death in Non-Rodents (CODN) Working Group is an initiative under the Scientific and Regulatory Policy Committee (SRPC) of the Society of Toxicologic Pathology (STP), focused on understanding existing practices and expectations among pharmaceutical companies, academic entities, and contract research organizations (CROs) when it comes to identifying and reporting the "Cause of Death" (COD) or moribundity for early or unplanned necropsies in non-rodent animal species (mainly non-human primates [NHP] and dogs) within both GLP (Good Laboratory Practice) and non-GLP toxicity studies. A survey was sent out to STP members to collect data on industry practices for determining COD in animals that underwent unscheduled euthanasia or were found deceased. Other non-rodent animals (such as pigs and rabbits) were also included to evaluate different approaches taken with various species. The insights obtained led to the development of "Points to Consider" for establishing and documenting the COD in large animal toxicity studies. Four key considerations include utilizing information from both control and treated animals in the study, consideration of COD for cohabiting or co-shipped non-study animals, including additional evaluations to help rule-in or rule-out specific causes, and recording the COD consistently in pathology databases or reports as a standard practice.
Keywords: cause of death, dog, histopathology, non-rodent, nonhuman primate, toxicity

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Fennell TR*1, Black SR*1, Elkins P*1, Snyder R*1, Ishibashi R*2, Koyanagi M*2, Hayashi SM: Limited uptake of [^{14}C]Gardenia Blue administered orally in male and female rats and mice.
Food Chem. Toxicol. 2025;195:115107. doi:10.1016/j.fct.2024.115107.

Gardenia blue (GB), a widely used plant-derived food color is prepared by reaction of genipin, the aglycone of geniposide, with protein hydrolysate. Recent animal studies investigating GB toxicity have indicated blue coloration in the gastrointestinal tract, kidneys and mesenteric lymph nodes in rodents following dietary administration. This study investigated the uptake and disposition of [^{14}C]GB in male and female rats and mice administered 100 or 1000 mg/kg by gavage. [^{14}C]GB was prepared by reaction of [2-^{14}C]genipin with soy protein hydrolysate. Following administration in rats, ^{14}C was eliminated primarily in feces (89-97% of administered dose), exhaled volatile organic chemical (VOC) and CO_2 traps contained no radioactivity, and urine contained 0.2-0.4%. In bile-duct-cannulated rats (100 mg/kg [^{14}C]GB), 0.25% of dose was recovered in bile, and in urine, 0.5%. The percent of the dose absorbed was 0.9%, based on radioactivity in urine, bile, and carcass minus digestive tract contents. The highest level of radioactivity in tissues was in kidney; however renal recovery was low, with only 0.02-0.04% of the dose recovered in kidney. Repeated dosing indicated that ^{14}C accumulated in kidney, and was slowly removed following cessation of dosing, consistent with previous studies, in the absence of any functional or histopathological changes.
Keywords: Absorption, Accumulation, Gardenia blue, Kidney

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Liu H*1, Inoue R*2, Koyanagi M*3, Hayashi SM, Watanabe G*1, Nagaoka K*1: Comparison of the fecal bacterial microbiota in mice, rats, and pigs after oral administration of alpha-glycosyl isoquercitrin.
J. Toxicol. Sci. 2024;49:151-161. doi:10.2131/jts.49.151.

Alpha-glycosyl isoquercitrin (AGIQ) is composed of isoquercitrin and its glucosylated derivatives and has many biological activities, including anti-inflammatory, antioxidant, and anti-cancer properties. However, the effect of AGIQ administered orally on gut microbiota composition remains unclear. The objective of this study was to evaluate the effect of AGIQ on the gut microbiota of animals in different dose groups. Male rats and mice received different doses of AGIQ (1.5%, 3%, or 5% w/v) in diet for carcinogenic or chronic toxicity studies (rasH2 mice: 6 months; Sprague-Dawley rats: 12 months). Male minipigs received 100, 300, or 1000 mg/kg/day for 28 days. Fecal samples were collected from the different animal species and analyzed using 16S-rRNA gene sequencing. No significant changes were observed in alpha and beta diversity of the gut microbiota. Characteristic bacteria that responded to AGIQ were identified in each animal species, and, interestingly, Kineothrix alysoides, a butyrate-producing bacterium, was commonly detected in all three species, suggesting that it may be related to the biological activities of AGIQ. AGIQ selectively modulated the number of beneficial butyrate-producing commensal bacterium beneficial bacteria without changing the diversity of gut microbiota, which further supports the safe use of AGIQ in food products.
Keywords: 16S rRNA, Alpha-glycosyl isoquercitrin, Gut microbiota, Kineothrix alysoides

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Sasaki Y[*1], Furuya Y[*2], Suzuki S[*1], Momose Y, Uema M, Kayano M[*3], Aikawa C[*1], Sasaki M[*1], Okamura M[*1], Ohya K.: Geographical variation in antimicrobial resistant *Salmonella Schwarzengrund* from chicken meat in Japan.

J Vet Med Sci. 2025;87(3):315-319. doi: 10.1292/jvms.24-0279

Chicken meat is a major source of foodborne salmonellosis. In Japan, fluoroquinolones and third-generation cephalosporins are the first- and second-choice treatments for Salmonella gastroenteritis, respectively. We investigated the prevalence and antimicrobial resistance of Salmonella in 154 chicken meat products from Hokkaido (42), Tohoku (45), Kanto (5), and Kyushu (62), Japan. Salmonella was isolated from 133 products (86.4%). High resistance rates were observed for streptomycin (56.5%), tetracycline (50.7%), and kanamycin (47.8%), while all isolates were susceptible to cefazolin, cefotaxime, gentamicin, ciprofloxacin, colistin, and chloramphenicol. The most common serovar, Salmonella Schwarzengrund (83.3%), showed clear regional differences in multidrug resistance: 100% in Kyushu, 41.5% in Tohoku, and 0% in Hokkaido. These findings highlight significant geographical variation in antimicrobial resistance among Salmonella Schwarzengrund isolates.

Keywords: *Salmonella* prevalence, antimicrobial resistance, chicken meat

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Sasaki Y[*1], Ikeda T[*2], Momose Y, Yonemitsu K[*3], Uema M, Asai T[*4]: Geographical Variation of Antimicrobial Resistance of *Salmonella* in Japanese Chicken.

Food Safety (Tokyo). 2024;12(3):59-66. doi: 10.14252/foodsafetyfscj.D-24-00002

Chicken is a potent source of *Salmonella* infection in humans. Occasionally, patients with severe *Salmonella* enteritis require antimicrobial therapy. Antimicrobials are used to prevent and treat bacterial infections in broiler and breeder farms. Herein, we investigated the prevalence and antimicrobial resistance of *Salmonella* in 337 vacuum-packed chicken breast products manufactured in Japan between June and December 2021. *Salmonella* was isolated from 287 samples (85.2%). Among the products from Eastern Japan, the lowest *Salmonella* prevalence was observed in those processed in September (65.6%), which was significantly ($p < 0.05$) lower than that in November or December. Among the products from Western Japan, the lowest *Salmonella* prevalence was observed in those processed in August (61.9%), which was significantly ($p < 0.05$) lower than that in June, November, and December. The most frequent serovar was *Salmonella* Schwarzengrund (223 isolates), followed by *S.* Infantis (53 isolates), *S.* Manhattan (9 isolates), and *S.* Enteritidis (1 isolate). High rates of antimicrobial resistance were observed for streptomycin (64.5%), kanamycin (50.2%), tetracycline (65.2%), nalidixic acid (11.5%), and trimethoprim (35.9%). Resistance rates against these five antimicrobials in *S.* Schwarzengrund isolates were markedly higher in the isolates from Western Japan than in those from Eastern Japan. All 287 *Salmonella* isolates were susceptible to ciprofloxacin which belongs to fluoroquinolones and cefotaxime which belongs to third-generation cephalosporins. *Salmonella* prevalence in chicken products in Japan was found to be extremely high; therefore, chicken meat should be thoroughly heated before consumption. In Japan, fluoroquinolones and third-generation cephalosporins are recommended as the first- and second-choice antimicrobials for patients with severe *Salmonella* enteritis, respectively. The results of this study show that administering fluoroquinolones or third-generation cephalosporins is an effective option for patients with *Salmonella* enteritis caused by consuming chicken meat, and efficient strategies for *Salmonella* management on broiler farms and chicken-processing plants need to be developed.

Keywords: Antimicrobial resistance, Chicken meat, *Salmonella*

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Momose Y, Sasaki Y*1, Yonemitsu K*2, Kuroda M*3, Ikeda T*4, Uema M, Furuya Y*5, Toyofuku H*6, Igimi S*7, Asai T*8: Changes in the Phenotypes of *Salmonella spp.* in Japanese Broiler Flocks.
Food Safety (Tokyo). 2024;12(2):25-33. doi: 10.14252/foodsafetyfscj.D-24-00001

Salmonella infections represent a leading cause of foodborne illnesses; resistance to third-generation cephalosporins (TGCs), which are a first-choice antimicrobial for treating human *Salmonella* enteritis, has become a serious public health concern worldwide. Because the consumption of undercooked chicken meat products is a major cause of foodborne salmonellosis in Japan, we conducted three surveys at different periods between 2017 and 2022, with the cooperation of four abattoirs (two in Eastern and two in Western Japan). The first survey was conducted at abattoir A, which is located in Eastern Japan. *Salmonella* was detected in 84.4% of broiler flocks tested (27/32); among them, all the TGC-resistant isolates obtained from one farm (farm FA) were identified as *S.* Infantis. *Salmonella* was recovered from 62.5% of breast meat samples (20/32), with one case suggesting cross-contamination. The second survey was conducted at three other abattoirs to examine the prevalence of TGC-resistant *Salmonella*, in both Western (abattoirs B and C) and Eastern (abattoir D) Japan. *Salmonella* was detected in 90.6% of broiler flocks examined (29/32). TGC-resistant *S.* Infantis was isolated from 2 flocks until 2018 and not thereafter. Subsequently, isolates were identified as TGC-susceptible *S.* Schwarzengrund in both regions. The third survey was performed at abattoir A to elucidate whether there were changes in the phenotypes. Of the 11 broiler flocks introduced from farm FA, 10 were positive for *Salmonella* (90.9%); all the isolates were *S.* Schwarzengrund susceptible to TGC. This study shows that TGC-susceptible *S.* Schwarzengrund has replaced the resistant phenotypes among broiler flocks in both Eastern and Western Japan. Although chicken meat products could be cross-contaminated with *Salmonella* during the slaughtering process, reducing the prevalence of *Salmonella* in broiler flocks remains important to decrease *Salmonella* enteritis in humans.

Keywords: *Salmonella*, cross-contamination, Third-generation cephalosporin resistance

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Nishizaki N*1, Oshiro S*2, Tohya M, Watanabe S*3, Okazaki T*4, Takahashi K*1, Kirikae T*2,3, Shimizu T*5: *Propionimicrobium lymphophilum* in urine of children with monosymptomatic nocturnal enuresis.
Front Cell Infect Microbiol. 2024;14:1377992. doi: 10.3389/fcimb.2024.1377992

Despite a unique microbiome in urine, the relationship between nocturnal enuresis and the urobiome remains unclear. This study aimed to compare the presence of specific bacterial species in the urine of children with and without nocturnal enuresis. We used 16S ribosomal RNA gene sequencing to analyze the urobiome in urine samples obtained from the two groups of children. The presence of *Propionimicrobium lymphophilum* was examined using real-time PCR in the urine of 25 children diagnosed with monosymptomatic nocturnal enuresis (MNE), and 17 children without this condition. Children with MNE exhibited a significantly higher prevalence of *P. lymphophilum*: 16 out of 25 (64.0%) compared to 4 out of 17 (23.5%) in the control group. Among children with frequent bedwetting, there was a significantly higher prevalence of *P. lymphophilum*;15 out of 16 (93.8%) compared to 2 out

of 9 (22.2%) in those with infrequent bedwetting. Bacterial culture tests confirmed the anaerobic growth of *P. lymphophilum* isolates from urine samples of two PCR-positive patients with MNE. These isolates were found to be susceptible to ampicillin. These findings suggest that *P. lymphophilum* may be associated with chronic urinary tract infections and potentially contribute to the development of MNE in children.

Keywords: *Propionimicrobium lymphophilum*, nocturnal enuresis, urinary microbiome, urobiome

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Sakuma M*1, Tohya M, Hishinuma T*1, Sherchand JB*2, Kirikae T*3, Tada T*4: Molecular epidemiology of multidrug-resistant *Acinetobacter baumannii* isolates from a hospital in Nepal.
J Glob Antimicrob Resist. 2024:363-367. doi: 10.1016/j.jgar.2024.07.017

The emergence of multidrug-resistant (MDR) *Acinetobacter baumannii* has become a serious worldwide medical problem. This study was designed to clarify the genetic and epidemiological properties of MDR *A. baumannii* clinical isolates. A total of 66 MDR *A. baumannii* isolates were obtained from 66 inpatients between May 2019 and February 2020 in a university hospital in Nepal. Whole genomes of these isolates were sequenced using next-generation sequencing. Phylogenetic trees were constructed from single nucleotide polymorphism concatemers. Multilocus sequence typing (MLST) and clonal complex (CC) analysis were conducted, and drug-resistance genes were identified. Of the 66 isolates, 26 harboured a gene encoding NDM-type metallo-β-lactamase, and 55 harboured a gene encoding the 16S rRNA methyltransferase, ArmA. All isolates had point mutations in the quinolone-resistance-determining regions of *gyrA* and *parC*. Phylogenetic analysis showed that 55 isolates harboured armA, 26 harboured bla_{NDM-1}, and14 harboured bla_{PER-7}. Multilocus sequence typing and CC analysis revealed that 34 isolates belonged to CC2 (ST2), 10 to CC1 (nine ST1 and one ST623), and eight to CC149 (ST149). Compared to our previous study on MDR *A. baumannii* in Nepal in 2012, the isolation rate of CC2 increased, whereas that of CC149 decreased between 2012 and 2020. This study indicates that MDR A. baumannii producing carbapenemase and 16S rRNA methyltransferase, with high resistance to carbapenems and/or aminoglycosides, are spreading in medical settings in Nepal. The genetic backgrounds of MDR A. baumannii isolates have shifted to international clone 2 over several years.

Keywords: 16s rRNA methyltransferase, Carbapenemase, Molecular epidemiology, Multidrug-resistant Acinetobacter baumannii

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Shimojima Y*1, Ishikawa T*2, Noguchi E*2, Araki R*2, Gomyo K*1, Miyajima I, Akita Y*1, Ohara Y*1, Nakagawa R*1, Yumiko Okada, Morita Y*3: Bacteriological Survey of Insect Products in Japan.
Foodborne Pathogens and Disease. 2024; 21, 478-484. doi: 10.1089/fpd.2024.0004

A microbiological study was conducted on 41 insect product samples (29 raw frozen, 10 powdered, and 2 processed), which were commercially available in Japan. The average of total aerobic count for raw frozen insects was 5.61 log cfu/g, whereas that of the powdered insect was 2.89 log cfu/g, resulting significantly higher in raw frozen insects ($p < 0.05$). The coliform count for the raw frozen insects ranged from <1 to 6.90 log cfu/g, and that for the powdered insects ranged from <1 to 1.00 log cfu/g. The detection frequencies of aerobic spores (<1-4.63 log cfu/g), anaerobic spores (<0-4.40 log cfu/g), and *Bacillus cereus* (<1.7-3.83 log cfu/g) showed no sample type-related significant difference. *Listeria* spp. was isolated

from four samples of raw frozen insects, one of which was *Listeria monocytogenes*. We did not detect any of the following: *Salmonella* spp., STEC, *Campylobacter jejuni/coli*, or pathogenic *Yersinia*.

Keywords: edible insects, food safety, foodborne pathogens

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山田恭平*，設樂紘史*，神田典子*，近藤貴英*，西田道弘*，大城直雅：シガトキシン類分析法における適用性の高いLC-MS/MS条件の検討.

食品衛生学雑誌. 2024;65:72-7. doi: 10.3358/shokueishi.65.72

シガテラ食中毒は，原因毒素シガトキシン類（CTXs）に起因する魚類による食中毒で，近年全国的な発生リスクの増加が懸念されている．魚肉に含まれるCTXsは極めて少量であるためLC-MS/MSによる高感度検出法が求められるが，現在報告されている検出法は特定の装置にのみ適用可能で，多くの試験所で対応困難な状況にある．今回，CTXsに対して広く適用可能なLC-MS/MS分析法を検討した．ギ酸および水酸化リチウムを添加した水／アセトニトリル系移動相により開裂しづらいリチウムイオン付加分子：$[M+Li]^+$が特異的に形成された．CTXs 9物質を対象にmultiple reaction monitoring測定において $[M+Li]^+ > [M+Li]^+$ をモニターするLC-MS/MS分析を実施した結果，LOD 0.005～0.030 ng/mLおよびLOQ 0.010～0.061 ng/mLの検出が可能であった．魚肉5 gから試験溶液1 mLを調製した場合のLODとLOQはそれぞれ0.001～0.006 μg/kgおよび0.002～0.012 μg/kgと推算され，食用不適水準（0.175 μg/kg CTX 1B当量）の検査に対応可能であることが示唆された．本法は特定の装置に依存しない普遍的な手法であることが示唆され，全国の試験所のCTXs検査体制整備に資するものと考えられた.

Keywords: ciguatera poisoning, ciguatoxin, LC-MS/MS

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Ando M, Yamaguchi H, Iwashita N, Takagi Y, Yoshinari T, Fukuyama T: Oral exposure to low concentration of Fumonisin B2, but not Fumonisin B1, significantly exacerbates the pathophysiology of imiquimod-induced psoriasis in mice.

Int J Mol Sci. 2024;25:7852. doi: 10.3390/ijms25147852

This study aimed to determine whether oral fumonisin exposure contributes to the development of psoriasis. Oral administration of fumonisin B1 (FB1, 0.1 mg/kg) or fumonisin B2 (FB2, 0.1 mg/kg) was conducted for 10 days, in addition to the induction of psoriatic symptoms through topical application of 5% imiquimod cream from day 6 to day 10 (5 days) in female BALB/c mice. The results demonstrated that oral administration of FB2 significantly exacerbated psoriatic symptoms, including skin thickness, itching behavior, transepidermal water loss, immune cell infiltration in the dermis, and proinflammatory cytokine production. However, no changes were observed following exposure to FB1. Our results confirm that oral exposure to FB2 adversely affects the pathogenesis of psoriasis by increasing skin thickness and impairing barrier function.

Keywords: fumonisin, mouse, psoriasis

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Hayashi K, Ohya K, Kikuchi Y*1, Izutsu K*2, Hara-Kudo Y*3: Uniform Suspension of Heat-Killed *Staphylococcus aureus* for a Positive Control Used in the Monocyte-Activation Test.

Biol Pharm Bull. 2024;47:1321-1325. doi: 10.1248/bpb.b24-00139

Pyrogens, classified as bacterial endotoxins and non-endotoxin pyrogens (NEPs), induce fever or shock when released into the bloodstream or spinal fluid. Recently, a monocyte-activation test (MAT) involving human cell culture has been developed to detect pyrogens in injectable products. To evaluate the sensitivity of MAT, a reference standard endotoxin was used as a positive control; however, the reactivity differed between the endotoxins and NEPs, necessitating positive controls for NEPs. This study aimed to explore a preparation method for heat-killed *Staphylococcus aureus* (HKSA) as a positive control for NEPs in MAT. Because *S. aureus* forms grape-like clusters, nine types of glass filters with pore sizes of 0.5-2.7 μm were evaluated to obtain a uniform bacterial suspension. The suspension was then heat-treated to

kill the bacteria, resulting in HKSA samples. Serial dilutions of HKSA were tested by MAT using peripheral blood mononuclear cells. The interleukin-6 concentrations in the culture supernatant were measured by enzyme-linked immuno-sorbent assay to assess pyrogenic activities of HKSA. The pore sizes of the glass filters affected the uniformity of HKSA, and GF/C filter was selected for HKSA preparation. Repeated filtration improved uniformity, and a uniform suspension of HKSA was obtained through double filtration using a GF/C filter. Despite the decrease in HKSA activity as filtration frequency increased, the detection limit remained consistently unchanged. This suggests that repeated filtration can adjust the activity of HKSA to a baseline level and that a uniform suspension of HKSA exhibiting low variation is suitable as a positive control in MAT.

Keywords: glass filter, non-endotoxin pyrogen, monocyte-activation test, interleukin-6, heat-killed *Staphylococcus aureus*, positive control

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Hayashi K, Ohya K, Yoshinari T, Hirose S, Shimizu S*1, Morita Y*1, Ohnishi T, Watanabe M, Taharaguchi S*2, Mekata H*3, Taniguchi T*4, Hara-Kudo Y: MALDI-TOF MS analysis for detection of bovine coronavirus with tryptic peptides from viral proteins.

J Microorg Control. 2024;29:143-151. doi: 10.4265/jmc.29.4_143

Bovine coronavirus (BCoV), a significant cattle pathogen causing enteric and respiratory diseases, is primarily detected using reverse transcription-polymerase chain reaction. Our objective was to develop a novel detection method for BCoV by matrix-assisted laser desorption/ionization–time-of-flight mass spectrometry (MALDI-TOF MS). Peptide mass fingerprint analysis revealed that nucleocapsid (N), membrane (M), and hemagglutinin-esterase (HE) were three main BCoV proteins. Their tryptic peptides were used as target molecules for BCoV detection. When the tryptic digest of $10^{7.0}$ viral copies was analyzed by MALDI-TOF MS, five peptides with relatively strong peaks were detected. The detection limit was between $10^{5.0}$ and $10^{6.0}$ copies per test for BCoV alone. To detect BCoV in the swab eluate, ultrafiltration purification achieved a detection limit between $10^{6.0}$ and $10^{7.0}$ copies per test, sufficient to detect BCoV-infected calves. Our findings offer valuable insights for BCoV detection by MALDI-TOF MS.

Keywords: bovine coronavirus, detection limit, mass spectrometry, viral detection, viral respiratory infection

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Hayashi K, Sano M*1, Kanayasu-Toyoda T*2, Morita Y*1, Yamaguchi T*2,3, Ohya K, Kikuchi Y*4, Izutsu K*5, Hara-Kudo Y: Evaluation of the Effect of Cell Freshness on Pyrogen Detection using a Serum-free Monocyte-activation Test.

PLoS ONE. 2024;19:e0316203. doi: 10.1371/journal.pone.0316203

Pyrogens cause shock symptoms when released into the bloodstream. They are classified into two main categories: endotoxins (lipopolysaccharides [LPS]) and non-endotoxin pyrogens. The monocyte activation test (MAT) is an *in vitro* assay to detect pyrogens in human monocytes. Cells were incubated in the culture medium, and the cellular response, specifically the production of the inflammatory cytokine interleukin-6 in the culture supernatant, was analyzed using enzyme-linked immunosorbent assay (ELISA). Technical improvements, such as cell acquisition and culture media selection, will be beneficial for the popularization of MAT. The cell freshness was strictly controlled to achieve high MAT sensitivity. However, it is necessary to investigate the usability of older and stored blood samples in the MAT. This study evaluated the effect of cell freshness on MAT using peripheral blood mononuclear cells (PBMCs) isolated from 2- and 5-d-old donated whole blood samples. To mitigate the influence of serum in the culture medium, a serum-free MAT was developed using the LPS-binding protein (LBP) as an enhancer for LPS detection. PBMCs were incubated with a two-fold dilution series of LPS at 0.001-4.096 endotoxin units/

mL (EU/mL). Interleukin-6 levels in the culture supernatant were quantified by ELISA in the presence and absence of LBP. In the presence of LBP, the limit of detection (LOD) for LPS was 0.001-0.008 EU/mL. However, in the absence of LBP, the LOD was 0.512 EU/mL. Peripheral PBMCs were 38.6 times more sensitive in the presence of LBP than in its absence. When utilizing the developed serum-free MAT with LBP, 5-d-old PBMCs showed LODs of 0.016-0.064 EU/mL, indicating a 3.1-fold increase in sensitivity compared with 5- to 2-d-old PBMCs. These results suggest that the sensitivity of PBMCs decreased gradually rather than sharply. The study concluded that 2-d-old PBMCs were sufficiently fresh and could be used as serum-free MAT.

Keywords: endotoxin, non-endotoxin pyrogen, monocyte-activation test, peripheral blood mononuclear cells, interleukin-6, heat-killed *Staphylococcus aureus*

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Hirose S, Tomaru A, Akiyama H*, and Hara-Kudo Y*: Effective decontamination methods for Shiga toxin-producing *Escherichia coli* on beef surfaces for application in beef carcass hygiene.

J Food Prot. 2024;87:100366. doi: 10.1016/j.jfp.2024.100366

Effective methods for decontamination of Shiga toxin-producing *Escherichia coli* (STEC) on beef were evaluated by 48 mL spraying, 100 mL, and 500 mL flushing with ethanol, hydrogen peroxide, peracetic acid, acidified sodium chlorite, and sodium hypochlorite in this study. The flushing with 500 mL of 1,000 ppm peracetic acid was most effective, reducing pathogens by 2.8 log CFU/cm2, followed by 1,200 ppm acidified sodium chlorite. The spraying with 1,000 ppm peracetic acid reduced pathogens by 1.6 log CFU/cm2. The flushing with 500 mL of 200 and 500 ppm acidified sodium chlorite, and 50, 100, 200, and 500 ppm peracetic acid significantly reduced the STEC population compared with those treated with distilled water ($p < 0.05$), reducing pathogens by 2.1, 2.4, 1.6, 1.8, 2.1 and 2.4 log CFU/cm2, respectively. Additionally, the flushing with 500 mL of 200 and 500 ppm acidified sodium chlorite significantly changed the color of beef samples ($p < 0.05$), whereas 100-500 ppm peracetic acid did not significantly change the color ($p > 0.05$). The flushing with 500 mL of 200 and 500 ppm acidified sodium chlorite and 200 and 500 ppm peracetic acid significantly changed the odor of beef samples compared with those treated with distilled water ($p < 0.05$). There was no difference in the reduction of STEC population between peracetic acid treatment at 25℃ and 55℃ , with or without washing with sterilized distilled water after decontamination. Washing with distilled water after flushing with peracetic acid tended to reduce the odor of the samples. These results suggest that treatment with 100, 200, and 500 ppm peracetic acid, followed by washing with distilled water, might reduce the STEC population without retaining the odor of the sanitizer.

Keywords: Beef carcass, Decontamination, Discoloration, Odor, Peracetic acid, Washing

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Ikeuchi S*1, Hirose S, Shimada K*2, Koyama A*3, Ishida S*4, Katayama N*5, Suzuki T*6, Tokairin A*7, Tsukamoto M*8, Tsue Y*9, Yamaguchi K*10, Osako H*11, Hiwatashi S*12, Chiba Y, Akiyama H*13, Hayashidani H*1 and Hara-Kudo Y*1: Isolation of Shiga Toxin-Producing *Escherichia coli* from the Surfaces of Beef Carcasses in Slaughterhouses in Japan.

J Food Prot. 2024;87:100263. doi: 10.1016/j.jfp.2024.100263

Shiga toxin-producing *E. coli* (STEC) is an important foodborne pathogen worldwide. It is necessary to control and prevent STEC contamination on beef carcasses in slaughterhouses because STEC infection is associated with beef consumption. However, the frequencies of STEC contamination of beef carcasses in various slaughterhouses in Japan are not well known. Herein, we investigated the contamination of beef carcasses with STEC in slaughterhouses to assess the potential risks of STEC. In total, 524 gauze samples were collected from the surfaces of beef carcasses at 12 domestic slaughterhouses from November 2020 to February 2023. The samples were measured for aerobic plate counts and tested for

pathogenic genes (stx and eae) and major O-serogroups (O26, O45, O103, O111, O121, O145, and O157) by real-time PCR screening. Subsequently, immunomagnetic separation (IMS) was performed on samples positive for stx, eae, and at least one of the seven O-serogroups of STEC. Isolation process without IMS was performed on samples positive for stx, including those subjected to IMS. STEC O157:H7 and stx-positive *E. coli* other than serotype O157:H7 were isolated from 0.6% and 4.6% of beef carcass surfaces, respectively. Although the STEC O157:H7 isolation rate was low and stx-positive *E. coli* other than serotype O157:H7 belonged to minor O-serogroups, the results mean a risk of foodborne illness. Furthermore, a moderate correlation was observed between aerobic plate counts and detection rates of stx-positive samples by real-time PCR screening. The STEC O157:H7 isolated facilities showed higher values on aerobic plate counts and detection rates of stx-positive samples than the mean values of total samples. Therefore, these results suggest that it is important to evaluate hygiene treatments against beef carcasses for the reduction of STEC contamination risk, particularly in facilities with high aerobic plate counts.

Keywords: Aerobic plate count, Beef carcass, Contamination, Isolation, Slaughter house, STEC

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Ohnishi T, Watanabe M, Yodotani Y*1, Nishizato E*1, Araki S*1, Sasaki S*2, Hara-Kudo Y*3, Kojima Y*1, Misawa N*2, Okabe N*1: Contamination of Japanese retail foods with enterotoxigenic *Clostridium perfringens* spores.

J Food Prot. 2025;88:100429. doi: 10.1016/j.jfp.2024.100429

The contamination of Japanese retail foods and the intestinal contents of animals with the spores of enterotoxigenic *Clostridium perfringens* were investigated by analyzing clostridial toxin genes (*cpa* and *cpe*) using a culture method and PCR. Enterotoxigenic *C. perfringens* was detected in 12.3% (8/65 samples) of shell fishes, 8.4% (7/83 samples) of dried seafoods, 7.4% (15/204 samples) of curry mixes and spices, 2.6% (1/39 samples) of dried seaweeds, 2.5% (2/79 samples) of fishes and shrimp, 1.9% (2/105 samples) of chicken and 0.8% (1/121 samples) of root vegetables. Enterotoxigenic *C. perfringens* was not detected in beef (95 samples) and pork (110 samples). The ratio of enterotoxigenic *C. perfringens*-positive to all *C. perfringens*-positive samples was high for fish and shrimp (40.0%), curry mixes and spices (19.0%), shellfish (18.1%), dried seafood (16.7%), and dried seaweed (16.7%). Although *C. perfringens* was investigated in the intestinal contents of cattle (212 samples), pigs (207 samples) and chicken (159 samples), enterotoxigenic *C. perfringens* was not detected. These results indicate that beef and pork sold in Japan are unlikely to be contaminated with enterotoxigenic *C. perfringens*, and that other foods such as curry powder, shellfish and dried seafoods are more important as the sources of contamination in Japan. Dried seafoods are frequently used to make soup stock in Japanese and other Asian dishes. In cases of food-borne illness linked to *C. perfringens* contamination of Japanese and Asian dishes, dried seafood should be investigated, in addition to other ingredients such as meat.

Keywords: *Clostridium perfringens*, contamination, food

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Ohya K, Hirose S, Nishikaku K, Ohnishi T, Lee K*1, Iyoda S*1, Kubomura A*1, Akeda Y*1, Mizukami K*2, Suzuki T*2, Takinami K*2, Taquahashi Y, Kuwagata M, Kitajima S, Inoue T*3, Hara-Kudo Y: Genomic features and pathogenicity of atypical diarrheagenic Escherichia coli from a large foodborne outbreak.

Int J Food Microbiol. 2025;434:111134. doi: 10.1016/j.ijfoodmicro.2025.111134

An outbreak of diarrheal illness related to milk cartons served in school lunches, occurred in June 2021, involving more than 1800 cases from 25 schools. A strain of *Escherichia coli* OUT（OgGp9):H18 was implicated in the outbreak. This strain does not possess virulence factors typical of other E. coli pathotypes. In this study, we examined the pathogenicity of the *E. coli* OUT（OgGp9):H18 strain using genomic analysis and animal models. A core genome-based phylogenetic analysis revealed that this strain belongs to a clade comprising ST1380 strains and is distinct from enteroaggregative *E. coli* 042 and uropathogenic *E. coli* UMN026, which were previously considered to be phylogenetically related to this strain. In addition, the strain harbors a plasmid similar to that of atypical enterotoxigenic *E. coli*, encoding Coli Surface antigen CS8 and a type VI secretion system（T6SS). The strain caused mortality in mice following intraperitoneal inoculation. Marmosets inoculated orally, experienced diarrhea and long-term shedding. Curing the strain of the 103 Kbp plasmid it carries reduced mortality rates and colonization in the experimental animals, indicating that the plasmid encodes virulence factors. However, the mortality of mice treated with the plasmid-cured strain was higher than that of those treated with nonvirulent *E. coli* K-12, indicating that the chromosome also encodes virulence factors. Identified chromosomal virulence factors include a T6SS, the second type III secretion system in *E. coli*, ETT2, and the capsule gene cluster *kps*. These findings suggest that atypical diarrheagenic *E. coli*, such as the strain investigated in this study, may be the cause of foodborne illness in patients with diarrhea with an unknown cause.

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Ojiro R[*1], Zou X[*1], Yamagata H[*2], Ebizuka Y[*1], Kobayashi M[*1], Kigata T[*1], Tang Q[*3], Yoshida T[*1], Yoshinari T, Shibutani M[*1]: Emerging mycotoxin moniliformin induces renal tubular necrosis after oral exposure in mice.

Food Chem Toxicol. 2025;199:115336. doi: 10.1016/j.fct.2025.115336

Toxicological information on moniliformin（MON), an emerging mycotoxin, is limited. This study examined the acute and 28-day toxicity of orally administered MON in male ICR mice. Regarding the acute toxicity, among single oral doses of 0, 20, 40, and 80 mg/kg body weight（BW), MON caused proximal tubular necrosis in the kidneys at ≥ 40 mg/kg BW, and the lethal dose 50 value was estimated as 68.1 mg/kg BW. Regarding the 28-day toxicity, among oral doses of 0, 10, 20, and 40 mg/kg BW/day, MON increased absolute heart weight at 40 mg/kg BW, but histopathological changes were not evident in the heart. In contrast, 40 mg/kg BW MON induced centrilobular liver cell hypertrophy accompanied by increased absolute liver weight. Moreover, MON dose-dependently increased the absolute kidney weight at ≥ 20 mg/kg BW and increased the incidence of renal tubular regeneration at 40 mg/kg BW. RNA sequencing analysis in the renal cortex after a single dose of 40 mg/kg BW MON revealed upregulation of metabolic response-related genes, such as Cyp3a13, Cyp26b1, and Cyp4f15, and oxidative stress-related Gpx7. These results suggest that MON targets the kidneys in mice. Orally ingested MON may be metabolized in the kidneys as well as in the liver, and active intermediates or reactive oxygen species may induce renal tubular toxicity, causing proximal tubular necrosis. Based on kidney changes, the no-observed-adverse-effect-level of MON in the 28-day oral toxicity study of male mice was determined to be 10 mg/kg BW/day.

Keywords: moniliformin, mouse, kidney

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Oshikata C[*1,2], Watanabe M, Hashimoto K[*3], Yamazaki A[*4], Kobayashi N[*5], Konuma R[*6], Ishida M[*7], Kobayashi S[*7], Shimada T[*8], Kaneko T[*2], Kamata Y[*9], Kuriyama S[*10,11], Kure S[*12], Yanai M[*7], Tsurikisawa N[*12]: Mite allergen levels and fungal counts in children's bedding in four widely separated towns in Japan.

Rev Fr Allergol. 2024;64:104084. doi: 10.1016/

j.reval.2024.104084

We evaluated the relationships between children's housing, earthquake or tsunami damage affecting children, prevalence of allergic diseases, and levels of mite allergens and fungi on children's bedding after the Great East Japan Earthquake in four municipalities in Japan. We surveyed 464 children in Ishinomaki, 254 in Kami, 614 in Iwanuma, and 300 in Oiso whose parents or guardians had completed the International Study of Asthma and Allergies in Childhood questionnaire. We measured the levels of Dermatophagoides 1 allergens (*Der* 1) (*Dermatophagoides pteronyssinus* and *Dermatophagoides farinae*) on children's mattresses or futons and fungal counts (*Aspergillus* and yeast) between 2016 and 2018. Housing and allergen-avoidance strategies varied among the four municipalities. Mite allergen and fungal levels in these Japanese municipalities might have been affected by the earthquake and tsunami, and these changes might have affected the prevalence of allergic diseases in children.

Keywords: *Der* 1, fungal count, asthma

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Nakamura Y*, Nguyen NH*, Yoshinari T, Hachisu M*, Nguyen PT*, Shimizu K*: Identification of the oosporein biosynthesis gene cluster in an entomopathogenic fungus *Blackwellomyces cardinalis*.

Mycoscience. 2024;65:96-104. doi: 10.47371/mycosci.2024.02.005

Blackwellomyces cardinalis (≡ *Cordyceps cardinalis*) is an entomopathogenic fungus that hosts lepidopteran insect larvae. Oosporein, produced by *Bl. cardinalis*, is a red secondary metabolite that is also produced by other entomopathogens and is known to contribute to entomopathogenic activity. In this study, a homologous region of the oosporein biosynthesis gene cluster (*BcOpS* cluster) was found from the genome sequence of *Bl. cardinalis* strain NBRC 103832. Within the cluster, a putative transcription factor gene *BcOpS3* was deleted by homologous recombination. The deletion strain (*ΔBcOpS3*) did not produce oosporein. Real-time qPCR analysis showed that the expression of all genes was either lost or greatly reduced compared to the wild type strain (WT). Infection assay using silkworms showed that the virulence of the *ΔBcOpS3* strain was not different from that of the WT strain. We compared the expression levels of antimicrobial peptide genes in silkworm infected with these strains, and found that the increased expression of the *cecA* gene in WT was not observed in the *ΔBcOpS3* strain, suggesting that the immune response of the silkworm was altered.

Keywords: *Blackwellomyces cardinalis*, oosporein, biosynthesis gene

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Tsuboi K*1, Konuma R*2, Watanabe M, Okuyama H*1, Kobayashi N*1: Fungal resistance of thinly applied “modern lime plaster” used as interior wall finishing material.

Indoor Environ. 2024;27:175-186. doi: 10.7879/siej.27.175

Lime plaster is renowned for its hygroscopic capacity, ability to capture formaldehyde and other volatile organic compounds (VOCs), and antimicrobial properties. However, these properties are primarily documented in studies on thick plaster used in traditional buildings, with limited data on the thin applications used in modern housing. This study investigates the fungal resistance of thinly applied "modern lime plaster" and its varying effectiveness against different fungal species. Fungal resistance tests were conducted on lime plaster samples with different concentrations of slaked lime, using *Aspergillus niger* and *Cladosporium sphaerospermum* as test organisms.

The findings indicate that "modern lime plaster" exhibited high alkalinity and exerted strong fungal resistance despite its thinner application than traditional lime plaster. In particular, formulations containing over 30% slaked lime effectively inhibited fungal growth. Additionally, the antifungal efficacy was greater against *A. niger* than *C. sphaerospermum*, reflecting differences in alkaline tolerance among fungal species. Therefore, lime plaster is a highly effective wall material with inherent fungal resistance, and may contribute to residents' health through improve indoor air quality.

Keywords: lime plaster, fungal resistance, *Aspergillus*

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Yoshinari T, Watanabe T, Takeuchi T*, Ohnishi T: Determination of total aflatoxins in polished rice by liquid chromatography-fluorescence detection with multifunctional column cleanup and precolumn derivatization: single-laboratory and inter-laboratory validation studies.

J AOAC Int. 2024;107:953-959. doi: 10.1093/jaoacint/qsae066

In this study, an HPLC-fluorescence method coupled with multifunctional column cleanup and trifluoroacetic acid derivatization was developed for the determination of AF levels in polished rice. Our method was validated in a single-laboratory study using AF-spiked materials, followed by an inter-laboratory validation study. Twelve laboratories participated in the inter-laboratory validation study, and five polished rice test samples artificially contaminated with AFs were analyzed. In a single-laboratory study, the ranges of mean recoveries of AFB_1, B_2, G_1, G_2, and total AFs were 101, 100-103, 93-96, 95-98, and 97-99%, respectively. The RSDs for within-day and between-day variations were all ≤4.4%. In the inter-laboratory validation study, the RSDs for repeatability and reproducibility were from 0.7 to 2.7% and 3.3 to 8.9% for all analytes, respectively. In response to the Codex ML and method performance criteria for AFs in polished rice, an analytical method based on HPLC-fluorescence detection was developed. All method performance parameters estimated from the test results of the single-laboratory and inter-laboratory validation studies met the criteria required by the Codex.

Keywords: aflatoxin, polished rice, validation study

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日本食品微生物学会雑誌. 2024;41(2):65-76. doi: 10.5803/jsfm.41.65

Escherichia albertii is an emerging enteropathogen and its distribution in various foods and environmental samples has been reported in many regions around the world. In this study, we aimed to identify effective isolation and detection methods for *E. albertii* in various foods and environmental water samples. E. albertii-specific polymerase chain reaction (PCR) was positive in chicken, oyster, river water, and wastewater samples, and *E. albertii* was isolated from these PCR-positive samples except the wastewater sample. *E. albertii* was not isolated from any of the samples without screening PCR; therefore, PCR is useful for the detection and isolation of *E. albertii* in foods and environmental water samples. The effect of two-step enrichment with four kinds of selective enrichment broth was compared with cycle threshold (Ct) values of the *E. albertii*-specific real-time PCR assay and the isolation results. The Ct values in three out of five samples were lower in the second enriched culture than those of the first enriched culture, and *E. albertii* was isolated from enriched cultures showed Ct values <25. These results suggest that the population of *E. albertii* in these three samples increased in the second enriched culture compared with the first enriched culture, and isolating *E. albertii* from an enriched culture showing Ct values <25 is an efficient method. Genetic analysis was performed to *E. albertii* isolates from food, environmental water, and human fecal samples, and all the isolates possessed *eae*, and

isolates from chicken, pork, and river water samples showed the same EAOg type as *E. albertii* isolated from human fecal samples. Therefore, it was suggested that a continuous attention should be paid to *E. albertii* in food and environment.
Keywords: *Escherichia albertii*, food, environmental water, 2-step enrichment method, real-time PCR assay, biochemical analysis, genetical analysis

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林克彦，芦田龍太*，森田雄二*，大屋賢司，工藤由起子：日本薬局方無菌試験法に収載された培地の微生物検出能に関する研究.
医薬品医療機器レギュラトリーサイエンス. 2024;55:132-139. doi: 10.51018/pmdrs.55.2_132

日本薬局方（日局）一般試験法4.06 無菌試験法では，液状チオグリコール酸培地（FTM）とソイビーン・カゼイン・ダイジェスト培地（SCDM）の両方に医薬品を接種し，14日間以上培養することが定められている．本研究では，無菌試験法で指定されている5菌種（*Bacillus subtilis*，*Candida albicans*，*Clostridium sporogenes*，*Pseudomonas aeruginosa*，*Staphylococcus aureus*）に加え，医薬品回収事例で特定された3菌種（*Methylobacterium extorquens*，*Mycobacterium chelonae*，*Serratia marcescens* subsp. *marcescens*）の合計8菌種について，FTMとSCDMの検出能を検証した．FTMおよびSCDMに，日局無菌試験法の培地性能試験に従い100コロニー形成単位（CFU）以下で接種したところ，検討したすべての微生物種と培地の組み合わせで微生物の増殖が認められた．しかし，これより低い菌数レベルである1 $\log_{10}$ CFU接種レベル（10 CFUレベル）で接種したところ，SCDMでは*B. subtilis*および*C. albicans*，FTMでは*C. sporogenes*の増殖が確認されたものの，FTMでは*P. aeruginosa*および*S. aureus*の増殖が確認されない場合があった．これらの結果から，FTMは微生物の検出能が比較的低い可能性があり，検出能の高い他の培地の使用も検討することが必要と考えられる．さらに，医薬品回収事例の細菌のうち*M. extorquens*では，FTMに3 $\log_{10}$ CFUを接種し28日間培養しても検出されなかった一方で，SCDMでは2 $\log_{10}$ CFUを接種し14日間培養すると検出率は44.4％，28日間で100％に達した．したがって，日局無菌試験法で要求されている14日間以上の培養期間を設定することが重要であると考えられる．
Keywords：日本薬局方，無菌試験法，検出感度，ソイビーン・カゼイン・ダイジェスト培地，液状チオグリコール酸培地

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林克彦，上原由依加*，森田雄二*，大屋賢司，工藤由起子：医薬品等汚染原因として報告された細菌の無菌試験法用培地での検出の検証.
医薬品医療機器レギュラトリーサイエンス. 2025;56:38-45. doi: 10.51018/pmdrs.56.1_38

無菌製剤は，日本薬局方無菌試験法によって微生物汚染がないことが確認されている．この試験では，液状チオグリコール酸培地（FTM）およびソイビーン・カゼイン・ダイジェスト培地（SCDM）の両方で14日間以上培養し，微生物の増殖を検出する．以前の報告　では，無菌試験法に記載されている5菌種の微生物と，無菌製剤の汚染事例で特定された3菌種の細菌について，FTMおよびSCDMでの検出を検討した．しかし，10コロニー形成単位（CFU）レベルで接種した場合，すべての微生物が両培地で検出されたわけではなく，SCDMで*Methylobacterium extorquens*を検出するには28日間の培養が必要であった．さらに，無菌製剤の汚染事例で特定されている*Burkholderia cepacia*，*Pseudomonas fluorescens*，*Ralstonia pickettii*などの細菌については，検出能が未検証である．そこで本研究では，無菌製剤の汚染事例で検出された細菌3菌種（*B. cepacia*，*P. fluorescens*および*R. pickettii*）と，検出に長期間の培養が必要であった*M. extorquens*について，FTMおよびSCDMに10および100 CFUレベルで接種して検出可能性

を検討した．100 CFUを接種すると，FTMと*M. extorquens*の組み合わせを除き，すべての培地と細菌の組み合わせで検出された．10 CFUを接種すると，SCDMでは*B. cepacia*，*M. extorquens*，*P. fluorescens*および*R. pickettii*のすべてが検出されたものの，*M. extorquens*では検出に少なくとも28日間の培養が必要であった．FTMでは*B. cepacia*および*P. fluorescens*が検出された一方で，*R. pickettii*および*M. extorquens*は完全には検出されなかった．これらの結果から，*M. extorquens*を確実に検出するためには，日局無菌試験法に指定された培地とは異なる培地や代替法の検討が必要であることが示唆された．

Keywords：日本薬局方，無菌試験法，検出感度，ソイビーン・カゼイン・ダイジェスト培地，液状チオグリコール酸培地

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渡辺麻衣子，西角光平，大屋賢司，吉冨真理[*1]，工藤由起子[*2,3]：世界各地域の食中毒を含むサルモネラ症発生報告の解析による本菌血清型の流行性評価．

日本食品微生物学会雑誌. 2024;41(4):158-167. doi: 10.5803/jsfm.41.158

北米，ヨーロッパ，オセアニアおよびアジア（日本を除く）の４地域における食中毒を含むサルモネラ症事例のデータを収集し，患者数や患者由来菌株数に基づくサルモネラ属菌の血清型毎の流行度合について解析した．情報源は，公的機関の統計情報およびアメリカ国立医学図書館が運営するPubMedおよびGoogleが提供するGoogle Scholarを用い，可能な限り2016年から2019年までの情報の抽出を行った．その結果，全ての地域で共通して食中毒または感染症が発生しており流行性が高いと考えられる注意が最も必要な血清型はEnteritidisおよびTyphimuriumであることを確認した．またInfantisおよびI 4,[5],12:i:についても比較的流行性が高いと考えられたことから，これらの血清型についても今後の流行の動向に注意が必要である．

Keywords：サルモネラ症，Enteritidis，Typhimurium

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辻厳一郎，出水庸介：日本薬局方の国際調和に資する定量法の改正に関する研究

医薬品医療機器レギュラトリーサイエンス. 2025;56:172-182. doi: 10.51018/pmdrs.56.2_172

日本薬局方（JP）は，医薬品の性状及び品質の適正な確保に必要な規格・基準及び標準的試験法等のための，日本の医薬品の公的な規範書である．JPは科学技術の進展並びに国際調和に対応するため，５年ごとに改正が行われており，部分改正等が適宜行われている．次のJP改正に向けたJP19作成基本方針の５本の柱においても，「最新の学問・技術の積 極的導入による質的向上」，また「医薬品のグローバル化に対応した国際化の一層の推進」が引続き掲げられており，他局で規定されているより優れた試験方法について国内外の関係者が利用できるようJPでの採用の可能性を検証することは重要である．本研究ではアロプリノール及びナファゾリン塩酸塩について，試験法の設定を目的とした類縁物質の合成及び各化合物のHPLCクロマトグラム上における完全分離条件について検討した．

Keywords: Japanese pharmacopoeia, HPLC, international harmonization

Fujita M, Demizu Y: Advances in the development of Wnt/β-catenin signaling inhibitors

RSC Med. Chem. 2025;16:984-999. doi: 10.1039/D4MD00749B

The Wnt/β-catenin signaling pathway plays a critical role in various biological processes, including cell proliferation, differentiation, and tissue homeostasis. Aberrant activation of this pathway is strongly associated with the development of various cancers, including colorectal, pancreatic, and gastric cancers, making it a promising therapeutic target. In recent years, inhibitors targeting different components of the Wnt/β-catenin pathway, including small molecules, peptides, and nucleic acid-based therapies, have been developed to suppress cancer cell growth. These inhibitors work by disrupting key interactions within the pathway, thereby preventing tumor progression. Antibody-based therapies have also emerged as potential strategies to block ligand-receptor interactions within this pathway. Despite these advancements, challenges such as the complexity of the pathway and toxicity concerns remain. Innovative approaches, including allosteric inhibitors, proteolysis-targeting chimeras (PROTACs), and peptide-based inhibitors, offer new opportunities to address these challenges. This review provides an overview of the latest progress in the development of Wnt/β-catenin pathway inhibitors and explores future directions in cancer therapy.

Keywords: β-Catenin, Peptides, Antibody

Takano R, Ohoka N, Kurohara T, Arakawa N, Ohgane K*, Inoue T, Yokoo H, Demizu Y: Clozapine as an E3 ligand for PROTAC technology
ACS Med. Chem. Lett. 2025;16:258-262. doi: 10.1021/acsmedchemlett.4c00500

New ubiquitin ligase (E3) ligands are crucial for developing proteolysis-targeting chimeras (PROTACs) to induce the degradation of a target protein. In this study, we developed a PROTAC using the antipsychotic drug clozapine as a new E3 ligand. First, a clozapine PROTAC targeting a model target HaloTag protein (Halo-PEG-Clozapine) was synthesized, and the PROTAC induced degradation of the HaloTag-fused protein in a cell culture system. Another clozapine PROTAC targeting the cancer therapeutic target estrogen receptor α (ERα) (Tamoxifen-PEG-Clozapine) was synthesized and induced degradation of the ERαprotein in MCF-7 breast cancer cells. Experiments with inhibitors and siRNAs showed that Tamoxifen-PEG-Clozapine degraded ERα via a ubiquitin-proteasome system that uses the ubiquitin protein ligase E3 component N-recognin 5. These results indicate that clozapine is a promising E3 ligand that may expand the molecular design of PROTACs, contributing to the advancement of drug discovery by facilitating the degradation of disease-related proteins.
Keywords: ubiquitin-proteasome system, PROTAC, UBR protein

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Yokoo H, Osawa H, Saito K, Demizu Y: Correlation Analysis Between Membrane Permeability and Intracellular Degradation Activity of PROTACs
Chem. Pharm. Bull. 2024;72:961-965. doi: 10.1248/cpb.c24-00615

Proteolysis-targeting chimeras (PROTACs) have attracted attention as an innovative drug modality that induces the selective degradation of target proteins. This technology shows higher activity than conventional inhibitors and holds great potential in the field of drug discovery. Optimization of the linker is essential for PROTACs to achieve sufficient activity, particularly with regard to cell membrane permeability. However, the correlation between membrane permeability and the activity of PROTACs has not been fully explored. To address this, we established a new molecular design approach to remove the linker and optimize PROTAC structure. These PROTAC compound groups were used to analyze the correlation between membrane permeability and activity using LC-tandem mass spectrometry (LC-MS/MS). Results revealed that the degradation activity of PROTACs fluctuates with increasing membrane permeability and changes in response to linker optimization, while sufficient proteolytic activity can be retained. These findings demonstrate the importance of considering the balance between membrane permeability and activity in PROTAC design and provide a new strategy for developing more effective PROTACs.
Keywords: proteolysis-targeting chimera, membrane permeability

Ito T, Ohoka N, Aoyama M, Nishikaze T, Misawa T, Inoue T, Ishii-Watabe A, Demizu Y: Strategic design of GalNAc-helical peptide ligands for efficient liver targeting
Chem. Sci. 2024;15(45):18789-18795. doi: 10.1039/d4sc05606j

There is a growing need for liver-selective drug delivery systems (DDS) in the treatment and diagnosis of liver diseases. The asialoglycoprotein receptor, a trimeric protein specifically expressed in the liver, is a key target for DDS. We hypothesized that peptides with reduced main-chain flexibility and strategically positioned N-acetylgalactosamine (GalNAc) moieties could enhance liver selectivity and uptake efficiency. The helical peptides designed in this study demonstrated superior uptake efficiency and liver selectivity compared with the conventional triantennary GalNAc DDS. These peptides also showed potential in protein delivery. Furthermore, we explored their application in lysosome-targeting chimeras (LYTACs), gaining valuable insights into the requirements for effective LYTAC functionality. This study not only highlights the potential of helical peptides as liver-selective DDS ligands, but also opens avenues for their use in various therapeutic and diagnostic applications, making significant strides in the targeted treatment of liver diseases.

Keywords: GalNAc, Helical peptides, DDS

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Naganuma M, Ohoka N, Hirano M, Watanabe D, Tsuji G, Inoue T, Demizu Y: Hydrophobic CPP/HDO Conjugates: A New Frontier in Oligonucleotide-Warheaded PROTAC Delivery

RSC Med. Chem. 2024;15:3695-3703. doi: 10.1039/D4MD00546E

Proteolysis-targeting chimeras (PROTACs) have emerged as a potent strategy for inducing targeted degradation of proteins, offering promising therapeutic potential to treat diseases such as cancer. However, oligonucleotide-based PROTACs face significant delivery challenges because of their anionic nature and chemical instability. To address these issues, we developed a novel hydrophobic cell-penetrating peptide (CPP) and heteroduplex oligonucleotide (HDO)-conjugated PROTAC, CPP/HDO-PROTAC, to enhance intracellular delivery and degradation efficiency. CPP/HDO-PROTAC was designed to enter the cell through the activity of the conjugated hydrophobic CPP and release decoy oligonucleotide-based PROTACs by RNase H-mediated RNA strand breaks. Our findings demonstrated that CPP/HDO-PROTAC binds to the estrogen receptor α (ERα) with higher affinity than previous constructs, significantly degrades ERα in MCF-7 human breast cancer cells and inhibits cell proliferation at 10 μM. This research highlights the potential of CPP/HDO-PROTAC as a viable method for delivering and activating decoy oligonucleotide-based PROTACs within cells, overcoming the limitations of traditional transfection methods and paving the way for their clinical application.

Keywords: PROTACs, decoy, DNA/RNA heteroduplex

Horikoshi K, Miyamoto M, Tsuchiya K*, Yokoo H, Demizu Y: Dual-modified penetratin peptides: Enhancing nucleic acid delivery through stapling and endosomal escape domain

Bioorg. Med. Chem. 2024;111:117871. doi:10.1016/j.bmc.2024.117871

Cell-penetrating peptides (CPPs) are crucial for delivering macromolecules such as nucleic acids into cells. This study investigates the effectiveness of dual-modified penetratin peptides, focusing on the impact of stapling structures and an endosomal escape domain (EED) on enhancing intracellular uptake. Some CPPs were synthesized with an EED at either the N- or C-terminus and stapling structures, and then complexed with plasmid DNA (pDNA) to evaluate their cellular uptake. Results revealed that the combination of stapling and an EED significantly improved delivery efficiency, primarily via macropinocytosis and clathrin-mediated endocytosis. These findings underscore the importance of optimizing CPP sequences for effective nucleic acid delivery systems.

Keywords: Cell-penetrating peptides, Stapling structures, Endosomal escape domain

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Tsuji G, Yokoo H, Demizu Y, Abe Y, Masada S, Uchiyama N, Tsutsumi T, Yamamoto E: Nitrosamine contamination of pharmaceuticals: Cases in Japan, formation mechanisms, detection methods, regulatory perspectives, and insights

JPBA Open. 2024;4:100034. doi: 10.1016/j.jpbao.2024.100034

In recent years, mutagenic nitrosamines, such as *N*-nitrosodimethylamine, have been detected in medicine. This has led to global product recalls and long-term supply suspensions by pharmaceutical companies and consequent clinical impacts. Measures to control nitrosamines in medicine, including detection methods and clarification of contamination routes, are being implemented worldwide. In this review, we focus on case reports of nitrosamine contamination of drug products in Japan, nitrosamine formation mechanisms during manufacturing and storage, as well as detection methods. We also discuss the acceptable nitrosamine intake (ng/day) in chemically synthesized drug substances in human drugs (including drug products) in the US, EU, and Japan. Overall, nitrosamine contamination of medicines is expected to remain a global public health issue. Therefore, detection methods using new technologies and detailed analysis of the formation mechanisms are necessary. However, excessive regulation may cause essential drug

shortages owing to product recall; therefore, a realistic and prudent response based on regulatory science is needed.

Keywords: Regulatory science, Nitrosamine, Impurity analysis

Kamata K*, Kuriyama M*, Tahara H*, Nishikawa A*, Yamamoto K*, Demizu Y, Onomura O*: One-pot C(sp^3)-H difluoroalkylation of tetrahydroisoquinolines and isochromans via electrochemical oxidation and organozinc alkylation.

Chem. Commun. 2025;60:6395-6398. doi: 10.1039/D4CC02033B

The C(sp^3)-H difluoroalkylation for the introduction of carbonylated CF_2 groups into tetrahydroisoquinolines (THIQs) and isochromans has been achieved by using electrochemical oxidation and organozinc alkylation. This one-pot process proceeded smoothly under transition-metal catalyst- and chemical oxidant-free conditions, and the desired products were obtained in good to high yields with a broad scope, except for *N*-Boc-THIQ. In addition, the gram-scale experiment successfully demonstrated the promising scalability. This is the first example of an electrochemical method for C(sp^3)-H difluoroalkylation of amines and ethers.

Keywords: C(sp^3)-H difluoroalkylation, electrochemical oxidation, organozinc alkylation

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Tsuji G., Misawa T, Demizu Y: The application of microsampling disks in circular dichroism spectroscopy for peptide and nucleic acid drugs

Chem. Pharm. Bull. 2024;72:658-663. doi: 10.1248/cpb.c24-00244

In recent years, there has been a growing focus on the development of medium-sized drugs based on peptides or nucleic acids owing to their potential therapeutic benefits. As some of these medium-sized drugs exert their therapeutic effects by adopting specific secondary structures, evaluating their conformational states is crucial to ensure the efficacy, quality, and safety of the drug products. It is important to assess the structural integrity of biomolecular therapeutics to guarantee their intended pharmacological activity and maintain the required standards for drug development and manufacturing. One widely utilized technique for quality evaluation is secondary structural analysis using circular dichroism (CD) spectroscopy. Given the higher production and quality control costs associated with medium-sized drugs compared with small molecule drugs, developing analytical techniques that enable CD analysis with reduced sample volumes is highly desirable. Herein, we focused on a microsampling disk-type cell as a potential solution for reducing the required sample volume. We investigated whether CD spectral analysis using a microsampling disk could provide equivalent spectra compared with the standard cell (sample volume: approx. 300 μL). Our findings demonstrated that the microsampling disk (sample volume: 2-10 μL) could be successfully applied to CD spectral analysis of peptide and nucleic acid drugs, paving the way for more efficient and cost-effective quality evaluation processes.

Keywords: medium-sized drug, secondary structure

Yokoo H, Shibata N, Demizu Y: In silico design, synthesis and evaluation of PROTAC against hematopoietic prostaglandin D synthase

Methods Mol. Biol. 2024;2780:345-359. doi: 10.1007/978-1-0716-3985-6_18

Chemical protein knockdown technology using proteolysis-targeting chimeras (PROTACs) to hijack the endogenous ubiquitin-proteasome system is a powerful strategy to degrade disease-related proteins. This chapter describes *in silico* design of a hematopoietic prostaglandin D synthase (H-PGDS) degrader, PROTAC(H-PGDS), using a docking simulation of the ternary complex of H-PGDS/PROTAC/E3 ligase as well as the synthesis of the designed PROTAC(H-PGDS)s and evaluation of their H-PGDS degradation activity.

Keywords: In silico molecular design, Hematopoietic prostaglandin D synthase, PROTAC

Xu H, Ohoka N, Inoue T, Yokoo H, Demizu Y: Photo-regulated PROTACs: A novel tool for temporal control of targeted protein degradation

Bioorg. Med. Chem. Lett. 2024;107:129778. doi: 10.1016/j.bmcl.2024.129778

PROTACs (Proteolysis targeting chimeras) are

chimeric molecules designed to induce targeted protein degradation via the ubiquitin-proteasome system. These molecules catalytically degrade target proteins and sustainably inhibit their function. Therefore, PROTAC's unique mechanism of action is not only beneficial in medicine but also serves as a valuable tool for molecular biological analysis in fields like chemical biology, biochemistry, and drug discovery. This study presents a novel turn-off（ON-OFF）type PROTAC development strategy utilizing a photocleavable linker. The inclusion of this linker enables temporal control of the degradation activity targeting BRD4 protein upon UV light exposure. PROTAC-2 demonstrated the most potent degradation activity against BRD4 among the other synthesized PROTACs with varying linker lengths. The UV light-induced cleavage of PROTAC-2 was confirmed, leading to a reduction in its BRD4 degradation activity. Notably, this study introduces a novel linker capable of nullifying degradation activity of PROTACs which is activated by light irradiation. These findings offer a promising strategy for the development of turn-off type PROTACs, providing enhanced temporal control over protein degradation. The approach holds significant potential for applications in molecular function studies and drug discovery.

Keywords: PROTAC, Chemical tool, Photocleavable linker

辻厳一郎，伊藤貴仁，出水庸介：日本薬局方の国際化の一層の推進を目指した定量法改正に関する研究

医薬品医療機器レギュラトリーサイエンス. 2024;55:140-156. doi: 10.51018/pmdrs.55.2_140

日本薬局方（JP）は，医薬品の性状及び品質の適正な確保に必要な規格・基準及び標準的試験法等のための，日本の医薬品の公的な規範書である．JPは科学技術の進展並びに国際調和に対応するため，５年ごとに改正が行われており，部分改正等が適宜行われている．次のJP改正に向けたJP19作成基本方針の５本の柱においても，「最新の学問・技術の積 極的導入による質的向上」，また「医薬品のグローバル化に対応した国際化の一層の推進」が引続き掲げられており，他局で規定されているより優れた試験方法について国内外の関係者が利用できるようJPでの採用の可能性を検証することは重要である．本研究ではグリクラジドについて，試験法の設定を目的とした類縁物質の合成及び各化合物のHPLCクロマトグラム上における完全分離条件について検討した．

Keywords: Japanese pharmacopoeia, HPLC, international harmonization

Yokoo H, Tsuji G, Inoue T, Naito M, Demizu Y, Ohoka N: Expansion of Targeted Degradation by Gilteritinib-Warheaded PROTACs to ALK Fusion Proteins

Bioorganic Chemistry. 2024;145:107204. doi: 10.1016/j.bioorg.2024.107204

Proteolysis targeting chimeras（PROTACs）induce the ubiquitination and ubsequent proteasomal degradation of targeted proteins. Numerous PROTACs have emerged as promising drug candidates for various disease-related proteins. This study investigates PROTACs targeted to degrade anaplastic lymphoma kinase（ALK）fusion proteins, which are implicated in diseases such as anaplastic large cell lymphoma and non-small cell lung cancer. We recently reported the development of a gilteritinib-warheaded PROTAC to target and degrade the Fms-like tyrosine kinase 3（FLT3）protein. Gilteritinib is a tyrosine kinase inhibitor that targets FLT3, and recent studies have revealed that it also functions as an ALK inhibitor. We conducted a structure-activity relationship（SAR）study and expanded the range of target proteins for gilteritinib-warheaded PROTACs to include echinoderm microtubule-associated protein-like 4（EML4）-ALK and nucleophosmin（NPM）-ALK, in addition to FLT3. Our SAR study utilized three types of ligands for E3 ligase— inhibitor of apoptosis protein（IAP）, cereblon（CRBN）, and von Hippel-Lindau（VHL）— in the PROTAC designs and we observed varied efficacy in the degradation of target proteins. The CRBN-based PROTAC effectively reduced the protein expression of FLT3, EML4-ALK, and NPM-ALK. The IAP-based PROTAC reduced expression of both FLT3 and EML4-ALK proteins but not that of NPM-ALK, while the VHL-based PROTAC was ineffective against all target proteins. Several ALK-targeted PROTACs have already been developed using CRBN or VHL as E3 ligase, but this is the first report of an IAP-based ALK degrader. The length of the linker structure utilized in PROTAC also had a significant effect on their efficacy and activity. PROTACs formed with shorter linkers demonstrated an enhanced degradation activity to target proteins

compared with those formed with longer linkers. These findings provide valuable insight for the development of effective PROTACs to target and degrade ALK fusion proteins.

Keywords: Targeted protein degradation, PROTAC, Gilteritinib

Yokoo H, Aoyama Y[*1], Ogaeri Y, Matsumoto T[*2], Yamamoto E, Uchiyama N, Demizu Y: Rapid determination of the absolute configuration of ranitidine hydrochloride API in two crystal forms using microcrystal electron diffraction

Chem. Pharm. Bull. 2024;72:471. doi:10.1248/cpb.c23-00745

The solid-state properties of drug candidates play a crucial role in their selection. Quality control of active pharmaceutical ingredients (APIs) based on their structural information involves ensuring a consistent crystal form and controlling water and residual solvent contents. However, traditional crystallographic techniques have limitations and require high-quality single crystals for structural analysis. Microcrystal electron diffraction (microED) overcomes these challenges by analyzing difficult-to-crystallize or small-quantity samples, making it valuable for efficient drug development. In this study, microED analysis was able to rapidly determine the configuration of two crystal forms (Forms 1, 2) of the API ranitidine hydrochloride. The structures obtained with microED are consistent with previous structures determined by X-ray diffraction, indicating microED is a useful tool for rapidly analyzing molecular structures in drug development and materials science research.

Keywords: ranitidine hydrochloride, microcrystal electron diffraction, active pharmaceutical ingredient

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Shoda T, Tsuji G, Kawamura M, Kurohara T, Misawa T, Hanajiri-Kikura R, Demizu Y: Structural analysis of an lysergic acid diethylamide (LSD) analogue *N*-methyl-*N*-isopropyllysergamide (MiPLA): Insights from Rotamers in NMR spectra

Drug Test. Anal. 2024;16:588-594. doi: 10.1002/dta.3586

Lysergic acid diethylamide (LSD) is a hallucinogenic compound that binds to and activates the serotonin 2A receptor and is classified as a controlled narcotic in Japan. Recently, MiPLA, an *N*-methyl-*N*-isopropyl derivative of LSD, has been detected in paper-sheet products in several countries. This study focuses on the synthesis of MiPLA and includes a comprehensive analysis involving structural and liquid chromatography-mass spectrometry (LC-MS). Particularly, MiPLA was synthesized in three-steps starting from ergometrine maleate, which resulted in the formation of (8S)-isomer, iso-MiPLA, as a by-product. The LC-MS results showed that LSD, MiPLA, and iso-MiPLA exhibited different retention times. Their chemical structures were determined using nuclear magnetic resonance spectroscopy, which revealed the presence of rotamers involving the *N*-methyl-*N*-isopropyl groups of tertiary amides in MiPLA and iso-MiPLA.

Keywords: LSD, MiPLA, NMR

Fukuda N, Soga K, Taguchi C, Narushima J, Sakata K, Kato R, Yoshiba S, Shibata N, Kondo K*: Cell cycle arrest combined with CDK1 inhibition suppresses genome-wide mutations by activating alternative DNA repair genes during genome editing.

J Biol Chem. 2024;300:107695. doi: 10.1016/j.jbc.2024.107695

Cells regularly repair numerous mutations. However, the effect of CRISPR/Cas9-induced dsDNA breaks on the repair processes of naturally occurring genome-wide mutations is unclear. In this study, we used TSCE5 cells with the heterozygous thymidine kinase genotype (TK+/-) to examine these effects. We strategically inserted the target sites for guide RNA (gRNA)/Cas9 and I-SceI into the functional allele and designed the experiment such that deletions of > 81 bp or base substitutions within exon five disrupted the TK gene, resulting in a TK-/- genotype. TSCE5 cells in the resting state exhibited 16 genome-wide mutations that affected cellular functions. After gRNA/Cas9 editing, these cells produced 859 mutations, including 67 high-impact variants that severely affected cellular functions under standard culture conditions. Mutation profile analysis indicated a significant accumulation of C to A substitutions, underscoring the widespread induction of characteristic mutations by gRNA/Cas9.

In contrast, gRNA/Cas9-edited cells under conditions of S~G2/M arrest and cyclin-dependent kinase 1 inhibition showed only five mutations. Transcriptomic analysis revealed the downregulation of DNA replication genes and upregulation of alternative DNA repair genes, such as zinc finger protein 384 (ZNF384) and dual specificity phosphatase, under S~G2/M conditions. Additionally, activation of nucleotide and base excision repair gene, including O-6-methylguanine-DNA methyltransferase and xeroderma pigmentosum complementation group C, was observed. This study highlights the profound impact of CRISPR/Cas9 editing on genome-wide mutation processes and underscores the emergence of novel DNA repair pathways. Finally, our findings provide significant insights into the maintenance of genome integrity during genome editing.

Keywords: CRISPR/Cas, alternative DNA repair, genome-wide mutation

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Tanaka H*1,2, Nishimaki-Mogami T, Tamehiro N, Shibata N, Mandai H*2, Ito S*2, Wakamatsu K*2: Pterostilbene, a Dimethyl Derivative of Resveratrol, Exerts Cytotoxic Effects on Melanin-Producing Cells through Metabolic Activation by Tyrosinase.
Int. J. Mol. Sci. 2024;25:9990. doi: 10.3390/ijms25189990.

Pterostilbene (PTS), which is abundant in blueberries, is a dimethyl derivative of the natural polyphenol resveratrol (RES). Several plant species, including peanuts and grapes, also produce PTS. Although RES has a wide range of health benefits, including anti-cancer properties, PTS has a robust pharmacological profile that includes a better intestinal absorption and an increased hepatic stability compared to RES. Indeed, PTS has a higher bioavailability and a lower toxicity compared to other stilbenes, making it an attractive drug candidate for the treatment of various diseases, including diabetes, cancer, cardiovascular disease, neurodegenerative disorders, and aging. We previously reported that RES serves as a substrate for tyrosinase, producing an *o*-quinone metabolite that is highly cytotoxic to melanocytes. The present study investigated whether PTS may also be metabolized by tyrosinase, similarly to RES. PTS was oxidized as a substrate by tyrosinase to form an *o*-quinone, which reacted with thiols, such as *N*-acetyl-L-cysteine, to form di- and tri-adducts. We also confirmed that PTS was taken up and metabolized by human tyrosinase-expressing 293T cells in amounts several times greater than RES. In addition, PTS showed a tyrosinase-dependent cytotoxicity against B16BL6 melanoma cells that was stronger than RES and also inhibited the formation of melanin in B16BL6 melanoma cells and in the culture medium. These results suggest that the two methyl groups of PTS, which are lipophilic, increase its membrane permeability, making it easier to bind to intracellular proteins, and may therefore be more cytotoxic to melanin-producing cells.

Keywords: melanin-producing cells, ortho-quinone, pterostilbene

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江木智宏*1，高畠令王奈*2，岸根雅宏*2，曽我慶介，吉場聡子，柴田識人，近藤一成*3，髙嶋康晴*1：大豆およびとうもろこし加工食品の遺伝子組換えDNA検査におけるDNA抽出精製方法の同等性確認試験.
食品衛生学雑誌. 2024;65:25-30. doi: 10.3358/shokueishi.65.25.

わが国における食品に関する表示のうち，遺伝子組換え食品の表示については，大豆，とうもろこし等の農産物およびこれらを主な原材料とする加工食品が対象となっている．遺伝子組換え食品の表示が適正になされているかどうかを科学的に検証するために，リアルタイムPCRによるDNA検査法が公定法として定められている．大豆およびとうもろこし加工食品からのDNA抽出精製方法として，公定法には代表的なものが示されているが，これら以外の方法についても同等性を確認の上使用することが認められている．本研究では，大豆およびとうもろこし加工食品からのDNA抽出精製方法について，新たな方法と公定法に示されている既存の方法との同等性確認試験を行った．この結果，新たな方法は既存の方法と同等かそれ以上であると考えられた．

Keywords：DNA抽出精製，遺伝子組換え食品，リアルタイム PCR

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Shibata N, Nakasaka T*1, Narushima J, Taguchi C, Sugino M, Yoshiba S, Soga K, Kajiwara M*1, Watanabe T*1, Kondo K*2: Laboratory Performance Study of the Japanese Official Method to Detect Genetically Modified Papaya Line PRSV-YK.
Shokuhin Eiseigaku Zasshi. 2024;65:61-66. doi: 10.3358/shokueishi.65.61.

Since the establishment of procedures for the safety assessment of food products that use recombinant DNA technology, the manufacture, import, and sale of genetically modified (GM) foods that have not undergone safety assessment are prohibited under the Food Sanitation Act. Therefore, a performance study to confirm the GM food testing operations of each laboratory is very important to ensure the reliability of the GM food monitoring system. In 2022, GM papaya line PRSV-YK-which has not yet been authorized in Japan-was selected for testing, and a papaya paste and a DNA solution were used as the test samples. With these samples, a laboratory performance study of the DNA extraction and real-time PCR operations was conducted. This confirmed that the 18 participating laboratories were generally performing the DNA extraction and real-time PCR operations correctly. However, some laboratories using certain DNA amplification reagent with some real-time PCR instruments were not able to determine the PRSV-YK detection test. This suggests that the PRSV-YK detection test may not be able to correctly detect samples containing GM papaya when performed with these combinations of instruments and reagent. In order to ensure the reliability of the PRSV-YK detection test, it is necessary to examine in detail how the combination of DNA polymerase reagents and real-time PCR instruments affects the detection limit, and to implement an appropriate solution.

Keywords: genetically modified papaya, laboratory performance study, real-time PCR

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Taguchi C, Soga K, Sugano Y*, Hosokawa A*, Sugino M, Narushima J, Yoshiba S, Adachi R, Shibata N: Verification Study of the Detection Method for Unauthorized Genetically Modified Papaya by combining DNA Polymerases and Real-time PCR Instruments.
Shokuhin Eiseigaku Zasshi. 2024;65:67-71. doi: 10.3358/shokueishi.65.67.

In the Japanese official detection method for unauthorized genetically modified (GM) papayas, one of two types of real-time PCR reagents with DNA polymerase (TaqMan Gene Master Mix [TaqMan Gene] or FastGene QPCR Probe Mastermix w/ROX [FastGene]) is primarily used for measurement. In 2022, we conducted a laboratory performance study on the unauthorized GM papaya line PRSV-YK, and the results revealed that high threshold cycle (Cq) values for the PRSV-YK detection test were obtained using TaqMan Gene with the 7500 Fast & 7500 Real-Time PCR System (ABI7500) and QuantStudio 12K Flex (QS12K), indicating the possibility of false negatives. The possibility of similar problems with all unauthorized GM papaya lines detection tests needs to be evaluated. In this study, we performed detection tests on unauthorized GM papaya lines (PRSV-YK, PRSV-SC, and PRSV-HN), the cauliflower mosaic virus 35S promotor (CaM), and a papaya positive control (Chy), and examined how the limits of detection (LOD) for each test are affected by two types of DNA polymerases (TaqMan Gene and FastGene) and three types of real-time PCR instruments (ABI7500, QS12K, and LightCycler 480 Instrument II [LC480]). In the PRSV-YK and PRSV-SC detection tests using ABI7500 and QS12K, measurement with TaqMan Gene showed a higher LOD than FastGene. In this case, an exponential amplification curve was confirmed on the amplification plot; however, the amplification curve did not cross the ΔRn threshold line and the correct Cq value was not obtained with a threshold line=0.2. The other tests (PRSV-HN, CaM, and Chy with ABI7500 and QS12K, and all detection tests with LC480) showed no important differences in the LOD for each test using either DNA polymerase. Therefore, when performing PRSV-YK and PRSV-SC detection tests with the ABI7500 or QS12K, FastGene should be used to avoid false negatives for foods containing GM papaya lines PRSV-YK and PRSV-SC at low mixing levels.

Keywords: GM papaya, DNA polymerase, real-time PCR

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田口千恵，柴田識人，近藤一成*：ゲノム編集食品安全性確保の取り組み周知と現在の安全性確認手法に関する調査研究.
食品衛生学雑誌. 2024;65:89-94. doi: 10.3358/shokueishi.65.89

厚生労働省（現在は消費者庁）が運用するゲノム編集技術応用食品等（以下，ゲノム編集食品）の届出制度では事前相談において届出に該当するかの確認が行われ，加えて，食品として安全性の解析が十分に行われたかの確認が行われているが，今後さらなる技術の進歩が見込まれるゲノム編集食品に対して，現在の安全性確認手法は十分であると国民が受容するのかを検討しておく必要がある．そこで，ゲノム編集食品に関する安全性確保の取り組みに関する情報提供媒体を作成し，現在の安全性確保に対する認識や受容を調べるとともに，生命科学分野の研究者から現在の安全性確認手法についての意見を聞くことで課題や今後取り入れるべき新たな視点を見出すことを目的とした調査を実施した．その結果，一般消費者の62%，生命科学分野の研究者の68%は安全性が確保されている/必要な確認が行われていると認識し，安全性が確保されていると認識することが受容の向上につながることや，安全性の確認が不十分だと感じた生命科学分野の研究者は第三者の検証がないことを懸念していることが明らかとなった．したがって，ゲノム編集食品に関する国民理解を得るためには，事前相談で行われている安全性確保の取り組みをわかりやすく発信して国民に周知していくことが有用であることが示唆された.
Keywords：ゲノム編集食品，事前相談，安全性確認

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Soga K, Hashimoto Y*[1], Egi T*[2], Taguchi C, Yoshiba S, Shibata N, Kondo K*[3], Takabatake R*[1]: Distribution status of genetically modified soybeans from the United States and Canada in 2021 and 2022.
GM Crops and Food. 2025;16:116-125. doi: 10.1080/21645698.2024.2444048

The number of authorized genetically modified (GM) soybeans has increased worldwide. In Japan, 34 GM soybeans containing single events and their stacked varieties have been approved as food. However, not all approved GM events are commercially cultivated or distributed. In this study, we evaluated domestically distributed samples from the United States (US) and Canada using 17 event-specific detection methods for GM soybeans. Identity-preserved (IP) soybean samples imported from the US and Canada, and non-IP samples from the US in 2021 and 2022 were analyzed. Four GM soybean events consisting of MON89788, A5547-127, MON87708, and DAS-44406 were detected in all lots in the non-IP samples. Furthermore, a single-kernel-based analysis was conducted to determine whether the detected GM soybean events are stacked. The results suggest that DAS-44406 is rapidly increasing, particularly as a single event among GM soybeans.
Keywords: distribution, genetically modified, soybean

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Nishimaki-Mogami T, Ito S*, Wakamatsu K*, Akiyama T, Tamehiro N, Shibata N: A Cell-Based Evaluation of the Tyrosinase-Mediated Metabolic Activation of Leukoderma-Inducing Phenols, II: The Depletion of *Nrf2* Augments the Cytotoxic Effect Evoked by Tyrosinase in Melanogenic Cells.
Biomolecules. 2025;15:114. doi: 10.3390/biom15010114.

Chemical leukoderma is a disorder induced by chemicals such as rhododendrol and monobenzone. These compounds possess a *p*-substituted phenol moiety and undergo oxidation into highly reactive and toxic *o*-quinone metabolites by tyrosinase. This metabolic activation plays a critical role in the development of leukoderma through the production of damage to melanocytes and immunological responses. This study aimed to develop a simple method for assessing the metabolic activation of leukoderma-inducing phenols without analyzing the metabolite. Although B16BL6 melanoma cells showed insufficient sensitivity to the cytotoxicity assay, the siRNA-mediated knockdown of the transcription factor NRF2 (NFE2L2) repressed the expression of cytoprotective factors, thereby augmenting the cytotoxicity of all six leukoderma-inducing phenols tested in a tyrosinase-dependent manner, indicating enhanced sensitivity to *o*-quinone metabolites. Additionally, the knockdown of the NRF2-target *Slc7a11* elevated the cytotoxicity of three out of the six compounds, indicating the involvement of cystine transport in cellular protection. In contrast, the knockdown or inhibition of the NRF2-target *Nqo1* had minimal effects. The same response

was induced upon *Nrf2* and *Slc7a11* knockdown in B16-4A5 cells, albeit with low sensitivity owing to low tyrosinase expression. We conclude that the analysis of tyrosinase-dependent cytotoxicity in *Nrf2*-depleted B16BL6 cells may serve as a useful strategy for evaluating the metabolic activation of chemicals.

Keywords: chemical leukoderma, tyrosinase, *Nrf2*

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Zhao S*, Pineda García JC*, Ren-shi L*, Kikura-Hanajiri R, Demizu Y, Tanaka Y*, Ishii Y*: Enzymatic hydrolysis of delta8-THC-O, delta9-THC-O, 11-alpha-HHC-O, and 11-beta-HHC-O by pooled human liver microsomes to generate delta8-THC, delta9-THC, 11-alpha-HHC, and 11-beta-HHC.

Forensic Toxicol. 2025. doi: 10.1007/s11419-025-00719-2

In recent years, analogues of Δ^9-tetrahydrocannabinol (Δ^9-THC) have been widely distributed in Japan via the internet. Hexahydrocannabinol (HHC), synthesized by reducing THC, was controlled as a designated substance under the Pharmaceutical and Medical Device Act in Japan in 2022. However, other semi-synthetic cannabinoids, such as acetyl derivatives of THC and HHC, appeared soon. Herein, we examined whether the enzymatic hydrolysis of acetylated forms of Δ^9-THC, Δ^8-THC 11-α-HHC, and 11-β-HHC by human liver microsomes (HLM) occurs.

The hydrolysis reaction was accomplished with HLM. Liquid chromatography-tandem mass spectrometry (LC-MS/MS) was used to determine products. Recombinant enzymes carboxylesterase 1C (CES1c), carboxylesterase 2 (CES2), and carboxylesterase inhibitor bis-(4-nitrophenyl) phosphate (BNPP) were used to clarify the principal hydrolysis enzymes for acetylated cannabinoids.

The acetylated form underwent hydrolysis with HLM time-dependently, with almost no acetylated product remaining after 60 min. Furthermore, results from LC-MS showed that only the deacetylated form was present after hydrolysis. Although hydrolysis did not occur when HLM was pre-incubated with the carboxylesterase inhibitor BNPP, it was observed when CES1c or CES2 was used for *in vitro* experiments.

This is the first time that it is elucidated that Δ^9-THC-O, Δ^8-THC-O, 11-α-HHC-O, and 11-β-HHC-O are enzymatically hydrolyzed with HLM to produce Δ^9-THC, Δ^8-THC, 11-α-HHC, and 11-β-HHC, respectively. Our results also support that CES1c and CES2 were the main enzymes involved in the hydrolysis of the acetylated cannabinoids. This study provides scientific support for the metabolism of newly regulated acetylated cannabinoids to cause the parent compound *in vivo*.

Keywords: cannabinoids, acylation, liver microsomes

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Yamamichi G*[1], Kato T*[1], Arakawa N, Ino Y*[2], Ujike T*[1], Nakano K*[1], Koh Y*[1], Motoyama Y*[1], Outani H*[1], Myoba S*[3], Ishizuya Y*[1], Yamamoto Y*[1], Hatano K*[1], Kawashima A*[1], Fukuhara S*[1], Uemura H*[4], Okada S*[1], Morii E*[1], Nonomura N*[1], Uemura M*[1,5,6]: GDF15 propeptide promotes bone metastasis of castration-resistant prostate cancer by augmenting the bone microenvironment.

Biomarker Res. 2024, 25;12(1):147. doi: 10.1186/s40364-024-00695-6.

Background: Bone metastasis (BM) is a common and fatal condition in patients with castration-resistant prostate cancer (CRPC). However, there are no useful blood biomarkers for CRPC with BM, and the mechanism underlying BM is unclear. In this study, we investigated precise blood biomarkers for evaluating BM that can improve the prognosis of patients with CRPC.

Methods: We comprehensively examined culture supernatants from four prostate cancer (PCa) cell lines using Orbitrap mass spectrometry to identify specific proteins secreted abundantly by PCa cells. The effects of this protein to PCa cells, osteoblasts, osteoclasts were examined, and BM mouse model. In addition, we measured the plasma concentration of this protein in CRPC patients for whom bone scan index (BSI) by bone scintigraphy was performed.

Results: A total of 2,787 proteins were identified by secretome analysis. We focused on GDF15 propeptide (GDPP), which is secreted by osteoblasts, osteoclasts, and PCa cells. GDPP promoted the proliferation, invasion, and migration of PC3 and DU145 CRPC cells, and GDPP aggravated BM in a mouse model.

Importantly, GDPP accelerated bone formation and absorption in the bone microenvironment by enhancing the proliferation of osteoblasts and osteoclasts by upregulating individual transcription factors such as RUNX2, OSX, ATF4, NFATc1, and DC-STAMP. In clinical settings, including a total of 416 patients, GDPP was more diagnostic of BM than prostate-specific antigen (PSA) (AUC = 0.92 and 0.78) and the seven other blood biomarkers (alkaline phosphatase, lactate dehydrogenase, bone alkaline phosphatase, tartrate-resistant acid phosphatase 5b, osteocalcin, procollagen I N-terminal propeptide and mature GDF15) in patients with CRPC. The changes in BSI over time with systemic treatment were correlated with that of GDPP (r = 0.63) but not with that of PSA (r = -0.16).

Conclusions: GDPP augments the tumor microenvironment of BM and is a novel blood biomarker of BM in CRPC, which could lead to early treatment interventions in patients with CRPC.

Keywords: Biomarker, Bone metastasis, Castration-resistant prostate cancer

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Sakuma N*, Abe M*, Ishii D*, Kawasaki T*, Arakawa N, Matsuyama S, Saito Y, Suzuki T*, Tatsumi K*: Serum stratifin measurement is useful for evaluating disease severity and outcomes in patients with acute exacerbation of interstitial lung disease: a retrospective study.

BMC Pulm Med. 2024;24(1):364. doi: 10.1186/s12890-024-03184-6.

Background: Serum levels of stratifin (SFN), a member of the 14-3-3 protein family, increase in patients with drug-induced lung injury associated with diffuse alveolar damage. Therefore, we hypothesised that SFN levels would be higher in those experiencing acute exacerbation of interstitial lung disease (AE-ILD). A secondary analysis was also planned to determine whether SFN levels could discriminate survival in those with AE.

Methods: Thirty-two patients with clinically stable ILD (CS-ILD) and 22 patients with AE-ILD were examined to assess whether high serum SFN levels were associated with AE-ILD and whether SFN levels reflected disease severity or prognosis in patients with AE-ILD.

Results: Serum SFN levels were higher in the AE-ILD group than in the CS-ILD group (8.4 ± 7.6 vs. 1.3 ± 1.2 ng/mL, $p < 0.001$). The cut-off value of the serum SFN concentration for predicting 90-day and 1-year survival was 6.6 ng/mL. SFN levels were higher in patients who died within 90 days and 1 year than in patients who survived beyond these time points (13.5 ± 8.7 vs. 5.6 ± 5.3 ng/mL; $p = 0.011$ and 13.1 ± 7.5 vs. 3.1 ± 1.9 ng/mL; $p < 0.001$, respectively) in the AE-ILD group. When this cut-off value was used, the 90-day and 1-year survival rates were significantly better in the population below the cut-off value than in those above the cut-off value ($p = 0.0017$ vs. $p < 0.0001$).

Conclusions: High serum SFN levels are associated with AE-ILD and can discriminate survival in patients with AE-ILD.

Keywords: Acute exacerbations, Biomarker, Interstitial lung disease

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Ogawara Y*1, Yokota NR*1, Yamada Y*2, Arakawa N, Sakamaki K*3, Kobayashi H*2,4, Kubota K*5, Kimura F*2, Mizushima T*1, Yamazaki E*6, Miyagi E*1: Assessment of tissue factor pathway inhibitor 2 (TFPI2) as a novel serum marker for malignant tumors of the ovary before and after treatment: A case-control study.

J Obstet Gynaecol Res. 2025;51(2):e16241. doi: 10.1111/jog.16241.

Aim: Tissue factor pathway inhibitor 2 (TFPI2) is a preoperative biomarker that was developed to discriminate ovarian benign tumors from cancer and is covered by health insurance in Japan. The purpose of this study was to evaluate how the TFPI2 changes after treatment.

Methods: Serum levels of TFPI2 (cut off 191 pg/mL) and CA125 (cut off 35 U/mL) before and after primary debulking surgery in patients with ovarian malignant tumors were evaluated among recurrent and nonrecurrent cases, respectively.

Results: A total of 46 cases were analyzed, including

11 borderline tumors, 13 clear cell carcinomas, 15 serous carcinomas, 4 endometrioid carcinomas, and 3 mucinous carcinomas. Among 37 patients without recurrence, the preoperative mean levels of TFPI2 (235.3 pg/mL, range: 78.3-607.7) and CA125 (1125.5 U/mL, range: 6.2-6272.0) were higher than the cutoff values. The mean minimum level of TFPI2 decreased to below the cutoff (150.2 pg/mL, range 56.4-471.1) at 3 months or more after primary debulking surgeries. The postoperative TFPI2 level exceeded the cutoff in 11 out of 37 patients without recurrence (29.7%); however, the postoperative TFPI2 level decreased in 8 patients. The mean maximum levels of TFPI2 and CA125 in 9 patients after recurrence were 492.6 pg/mL and 727.4 U/mL, respectively. Moreover, the mean TFPI2 level was higher than the preoperative one (421.5 pg/mL), different from CA125 (2903.8 U/mL).

Conclusions: Our results suggest the clinical validity of TFPI2 as a serum tumor marker for postoperative recurrence screening among malignant ovarian tumors.

Keywords: TFPI2, borderline tumor, ovarian cancer, serum tumor marker

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Yoshida A, Hashimoto Y, Saito Y, Kikura-Hanajiri R, Arakawa N: Dataset for proteomic analysis of lung tissues from an oleic acid induced acute lung injury model rat.

Journal of Proteome Data and Methods. 2025;7;4;1-3. doi: 10.14889/jpdm.2025.0004

This dataset was utilized to investigate the temporal proteomic changes in an oleic acid-induced acute lung injury rat model. Tissue extracts were analyzed by mass spectrometry using a data-independent acquisition approach. The data described in this paper have been deposited to jPOST with the identifiers JPST003445.

Keywords: rat, data-independent acquisition, diffuse alveolar damage

Yoshida A, Hashimoto Y, Akane H, Matsuyama S, Toyoda T, Ogawa K, Saito Y, Kikura-Hanajiri R, Arakawa N: Analysis of Stratifin Expression and Proteome Variation in a Rat Model of Acute Lung Injury.

Journal of Proteome Research. 2025;24;4;1941-1955. doi: 10.1021/acs.jproteome.4c00980

Diffuse alveolar damage (DAD) is a pathological hallmark of severe interstitial lung diseases, such as acute respiratory distress syndrome (ARDS), and is linked to poor prognosis. Previously, we identified 14-3-3σ/stratifin (SFN) as a serum biomarker candidate for diagnosing DAD. To clarify the time-dependent relationship between SFN expression and DAD, we here investigated pathological and molecular changes in serum, bronchoalveolar lavage fluid (BALF), and lung tissue in an oleic acid (OA)-induced ARDS rat model. Acute alveolar edema was observed after OA administration, followed by alveolar epithelial cell proliferation and increased BALF and serum SFN levels. Proteomic analysis of lung tissue extracts revealed that proteins related to "inflammatory response" and "HIF-1 signaling," including plasminogen activator inhibitor-1, were markedly increased 3 h after acute lung injury, followed by a gradual decrease. Conversely, proteins associated with "cell cycle" and "p53 pathway," including SFN, showed a persistent increase starting at 3 h and peaking at 48 h. Western blotting and immunohistochemistry confirmed that SFN was expressed in a part of proliferated alveolar type-II cells, accompanied by p53 activation, an important event for differentiation into type-I cells. SFN may be a biomarker closely related to alveolar remodeling during the repair process after lung injury.

Keywords: diffuse alveolar damage, interstitial lung disease, acute lung injury

Ishikawa R, Misawa T, Demizu Y, Saito Y, Kikura-Hanajiri R, Saito K: Comprehensive invitro evaluation of the inhibitory effects of relatively high molecular weight peptides on drug-drug interaction-associated four liver transporters and its association with physicochemical properties.

Drug Metab Pharmacokinet. 2025;61:101055. doi: 10.1016/j.dmpk.2025.101055.

In recent years, advances in peptide synthesis have

enabled the construction of relatively high molecular weight (Mw; >1 kDa) peptides using various types of amino acids (AAs), including proteinogenic/natural and nonnatural AAs. This advancement helps in obtaining peptides with improved stability, cell membrane permeability, and/or target-binding affinity. However, drug-drug interaction (DDI) information for these peptides remains scarce. Therefore, we focused on relatively high Mw peptides to examine their potential in inhibiting liver transporters, organic anion transporting polypeptide (OATP) 1B1, OATP1B3, P-glycoprotein, and breast cancer resistant protein (BCRP) *in vitro*. We addressed the inhibitory effects of various types of cyclic peptides containing non-natural AAs and cell-penetrating peptides composed of proteinogenic/natural AAs. Our results demonstrated that several peptides inhibited transport activities, indicating that they can potentially cause DDI. We further evaluated the relationship between their inhibition potency and physicochemical properties (Mw and hydrophobicity or charge of the constituting AA) to characterize the specific physicochemical properties contributing to their inhibition potency. The hydrophobic AA contents of the peptides correlated with the inhibition potencies for all four transporters. Our findings demonstrate the transporter-mediated DDI potential of peptides and the necessity of their evaluation for drug development.

Keywords: Cell penetrating peptide, Cyclic peptide, DDI-Associated transporters

Ri M[*1], Iida S[*1], Saito K, Saito Y, Maruyama D[*2], Asano A[*1], Fukuhara S[*2], Tsujimura H[*3], Miyazaki K[*4], Ota S[*5], Fukuhara N[*6], Negoro E[*7], Kuroda J[*8], Yoshida S[*9], Ohtsuka E[*10], Norifumi T[*11], Tabayashi T[*12], Takayama N[*13], Saito T[*14], Suzuki Y[*15], Harada Y[*16], Mizuno I[*17], Yoshida I[*18], Maruta M[*19], Takamatsu Y[*20], Katsuya H[*21], Yoshimitsu M[*22], Minami Y[*23], Kanato K[*2], Munakata W[*2], Nagai H[*15]: Lipidomic profiling of plasma from patients with multiple myeloma receiving bortezomib: an exploratory biomarker study of JCOG1105 (JCOG1105A1).

Cancer Chemother Pharmacol. 2025;95(1):29. doi: 10.1007/s00280-025-04752-1.

Purpose: A comprehensive analysis of metabolites (metabolomics) has been proposed as a new strategy for analyzing liquid biopsies and has been applied to identify biomarkers predicting clinical responses or adverse events associated with specific treatments. Here, we aimed to identify metabolites associated with bortezomib (Btz)-related toxicities and response to treatment in newly diagnosed multiple myeloma (MM).

Methods: Fifty-four plasma samples from transplant-ineligible MM patients enrolled in a randomized phase II study comparing two less-intensive regimens of melphalan, prednisolone and Btz (MPB) were subjected to the lipidomic profiling analysis. The amount of each lipid metabolite in plasma obtained prior to MPB therapy was compared to toxicity grades and responses to MPB therapy.

Results: High levels of 7 phospholipids (4 lysophosphatidylcholines and 3 phosphatidylcholines) were observed in cases with Btz-induced ≥ grade 2 peripheral neuropathy (BiPN) (n = 11). In addition, low levels of 3 fatty acids (FAs)-FA (18:2), FA (18:1), and FA (22:6)-were observed in patients who developed severe skin disorders ≥ grade 2 (n = 10). No metabolite significantly associated with treatment response was identified.

Conclusion: We conclude that levels of specific plasma lipid metabolites are associated with the severity of BiPN and skin disorders in patients with MM. These metabolites may serve as candidate biomarkers to predict Btz-induced toxicity in patients with MM before initiating Btz-containing therapy.

Keywords: Bortezomib, Lipidomics, Multiple myeloma

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Hayashi Y*, Sun Y: Overcoming Challenges in Oligonucleotide Therapeutics Analysis: A Novel Nonion Pair Approach.

J Am Soc Mass Spectrom. 2024;35(9):2034-2037. doi: 10.1021/jasms.4c00270.

Oligonucleotide therapeutics (OT) have emerged as promising drug modality for various intractable diseases. Recently, liquid chromatography-mass spectrometry (LC-MS) has been commonly employed for characterizing and quantifying OT in biological samples. Traditionally, the ion pairing-reverse phase (IP-RP) LC-MS method has been utilized in OT bioanalyses; however, this approach is associated with several limitations, including the memory effect and ion suppression effect of IP reagents. Therefore, this study aimed to develop a new RP-LC-MS method that eliminates the need for IP reagents. Our investigation revealed that ammonium bicarbonate was essential for the successful implementation of this nonIP-RP-LC-MS-based bioanalysis of OT. Moreover, the developed method demonstrated high versatility, accommodating the analysis of various natural or chemically modified oligonucleotides. The sensitivity of the method was further assessed using reconstituted plasma samples (the lower limit of quantification in this experiment was 0.5-1 ng/mL). In summary, the developed nonIP-RP-LC-MS method offers an easy, reliable, and cost-effective approach to the bioanalysis of OT.

Keywords: oligonucleotide therapeutics, bioanalysis, LC-MS

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Adachi K*1, Ohyama K*2, Tanaka Y, Murayama N*1, Shimizu M*1, Saito Y, Yamazaki H*1: Modeled hepatic/plasma exposures of omeprazole prescribed alone in cytochrome P450 2C19 poor metabolizers are likely associated with hepatic toxicity reported in a Japanese adverse event database.

Biol Pharm Bull. 2024;47(5):1028-1032. doi: 10.1248/bpb.b24-00145

Omeprazole, a gastric acid pump inhibitor, is repeatedly administered and is oxidatively metabolized mainly by polymorphic cytochrome P450 2C19. The prescribed dosage of omeprazole was discontinued or reduced in 47 of the 135 patients who received omeprazole alone in this survey, as recorded in the Japanese Adverse Drug Event Report database. The days to onset of omeprazole-related disorders were 3-4 d (median) and 16 d for intravenous 20-40 mg and oral 20 mg daily doses, respectively, in 34 patients for whom relevant data were available. The maximum plasma concentration of omeprazole was pharmacokinetically modeled after a single oral 40-mg dose in P450 2C19-defective poor metabolizers and was 2.4-fold higher than that in extensive metabolizers. The modeled area under the hepatic concentration curves of omeprazole in P450 2C19 poor metabolizers after virtual daily 40-mg doses for 7 d was 5.2-fold higher than that in the extensive metabolizers. Omeprazole-induced P450 2C19 (approx. 2-fold), resulting in increased hepatic intrinsic clearance in repeated doses, was considered after the second day. Virtual plasma/hepatic exposure estimated using pharmacokinetic modeling in subjects with P450 2C19 poor metabolizers indicated that these exposure levels virtually estimated could be one of causal factors for unexpected hepatic disorders induced by prescribed omeprazole, such as those resulting from drug interactions with repeatedly co-administered medicines.

Keywords: CYP2C19, autoinduction, physiologically based pharmacokinetic modeling

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Hangai M*1,2, Kawaguchi T*3, Takagi M*4, MatsuoK*5, Jeon S*6, Chiang CWK*6,7, Dewan AT*8, de Smith AJ*6, Imamura T*9, Okamoto Y*10, Saito AM*11, Deguchi T*12, Kubo M*13, Tanaka Y, Ayukawa Y*1, Hori T*14, Ohki K*15, Kiyokawa N*15, Inukai T*16, Arakawa Y*17, Mori M*17, Hasegawa D*18, Tomizawa D*12, Fukushima H*19, Yuza Y*20,

Noguchi Y*21, Taneyama Y*22, Ota S*23, Goto H*24, Yanagimachi M*24, Keino D*24, Koike K*25, Toyama D*26, Nakazawa Y*27, Nakamura K*28, Moriwaki K*29, Sekinaka Y*30, Morita D*27, Hirabayashi S*31, Hosoya Y*18, Yoshimoto Y*32, Yoshihara H*19, Ozawa M*18, Kobayashi S*1, Morisaki N*1, Gyeltshen T*33, Takahashi O*33, Okada Y*34, 35, 36, Matsuda M*37, Tanaka T*37, Inazawa J*38, Takita J*39, Ishida Y*40, Ohara A*41, Metayer C*42, Wiemels JL*6, Ma X*8, Mizutani S*4, Koh K*17, Momozawa Y*13, Horibe K*11, Matsuda F*3, Kato*2, Manabe A*31, Urayama KY*1, 33: Genome-wide assessment of genetic risk loci for childhood acute lymphoblastic leukemia in Japanese patients.

Haematologica. 2024;109(4):1247-1251. doi: 10.3324/haematol.2023.282914

In this first case-control GWAS effort in Japanese, we confirmed the strong ALL risk associations with ARID5B and IKZF1 variation, and we report two putative ALL risk associations suggesting a role for the 1q24.1 region and SAMD3, but confirmation is necessary. Together with also characterizing the effects of known risk loci in Japanese, we expect this study to aid efforts in understanding the heritability of childhood ALL in this population, a key step for elucidating the causes of this devastating disease.

Keywords: acute lymphoblastic leukemia, genetic risk, Japanese children

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Shimizu M*1, Uehara S*2, Ohyama K*3, Nishimura H*1, Tanaka Y, Saito Y, Suemizu H*2, Yoshida S*4, Yamazaki H*1: (2024) Pharmacokinetic Models Scaled-up from Humanized-liver Mouse Data Can Account for Drug Monitoring Results of Atomoxetine and Its 4-Hydroxylated and N-Demethylated Metabolites in Pediatric Patients Genotyped for Cytochrome P450 2D6.

Drug Metab Dispos. 52:35-43. doi: 10.1124/dmd.123.001481.

Validated simple pharmacokinetic models are able to predict steady-state plasma concentrations of the approved medicine atomoxetine and its primary metabolites in the majority of pediatric patients. The package insert advises careful dose escalation, especially for poor metabolizers; however, no simple way exists to determine P450 2D6 phenotypes. A relatively narrow range ratio of 4-hydroxyatomoxetine and N-desmethylatomoxetine in spot urine/plasma samples could be a simple semi-quantitative determinant factor for P450 2D6 intermediate metabolizers to optimize or confirm the correct dosage.
Keywords: CYP2D6, pharmacogenetics, pharmacokinetic modeling

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Fukunaga K*1, Tsukagoshi E, Nakamura R, Matsunaga K*2, Ozeki T*1, Watanabe H*3, Hasegawa A*4, Hama N*4, Kurata M*5, Mizukawa Y*5, Watanabe Y*6, Yamaguchi Y*6, Niihara H*7, Morita E*7, Asada H*8, Abe R*4, Saito Y, Mushiroda M*1: Association of HLA-A*11:01, HLA-B*39:01 and HLA-B*56:03 with salazosulfapyridine-induced cutaneous adverse drug reactions.

J Allergy Clin Immunol. 2024;12(5): 1355-1358.e3. doi: 10.1016/j.jaip.2024.02.041

Salazosulfapyridine (SASP) is used for the treatment of ulcerative colitis and rheumatoid arthritis. As with all prescribed drugs, numerous adverse drug reactions (ADRs) have been induced by SASP. This study aims to delineate HLA-A, -B, -C, and -DRB1 alleles contributing to the risk prediction of SASP-induced cADRs in the Japanese population.

We conducted an association study involving 15 cases of cADR, comprising 10 DIHS/DRESS, 3 SJS/TEN, and 2 MPE patients, respectively, compared to 2,823 controls, using individual genotypes of HLA-A, -B, -C, and DRB1 genes. After genotyping with high accuracy, 8 HLA-A alleles, 20 HLA-B alleles, 10 HLA-C alleles, and 18 HLA-DRB1 alleles were identified in the 15 cases, and the significance threshold for Bonferroni correction for multiple testing was set at 8.93 × 10-4 (0.05/56). HLA-A*11:01, HLA-B*39:01, and HLA-B*56:03 alleles showed significant association with an increased risk of SASP-induced cADRs. Multiple logistic regression analysis showed independent associations among HLA-A*11:01, HLA-B*39:01, and HLA-B*56:03 alleles with adjusted ORs [95% CI] of 6.5 [2.1-20.2], 9.6 [3.1-29.8], and 38.7 [7.6-197.3], in contrast with crude ORs of 9.9 [3.4-29.1], 9.8 [3.5-28.0], and 70.3 [17.2-287.9]. Next, we performed an association study of HLA alleles with DIHS/DRESS, SJS/TEN or MPE induced by SASP. Similar to the results of the association study for cADRs, HLA-A*11:01, HLA-B*39:01, and HLA-B*56:03 alleles were associated with the DIHS/DRESS.

We have replicated the association of SASP-induced cADRs with the HLA-A*11:01 allele we previously published. Moreover, we have identified novel association of HLA-B*39:01 and HLA-B*56:03 alleles with SASP-induced cADRs. As these biomarkers were independent among each other, combining with three HLA alleles is best to predict the risk of SASP-induced

cADRs. The docking simulation showed that SASP exhibits stronger binding than the metabolites to HLA molecules.

Keywords: Case-control study, Cutaneous adverse drug reaction, Drug-induced hypersensitivity syndrome

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Miyaso H*1, Yokota S, Suga K, Hashimoto Y*2, Kouno C*2, Nagahori K*1, Itoh M*1, Kitajima S: Histological differences between the central and peripheral areas of the testes of busulfan-administrated mice.
J Toxicol Sci. 2024;49(4):139-149. doi: 10.2131/jts.49.139

Busulfan is an anticancer drug known to cause serious damage to seminiferous tubules in the testes and deplete germ cells in human and animal models. The testicular artery is anastomosed with deferential and cremasteric arteries and is divided into capsular arteries, which give rise to the centripetal arteries and then recurrent arteries. The arterial blood in the testicular tissue is supplied by such a consequent system of arterial vessels, in order from the peripheral to the central area. As anticancer drugs are generally distributed throughout the whole body via the bloodstream and the running and distribution of arteries differ among the testicular areas, we hypothesized that the efficacy of busulfan differs in different testicular areas, particularly between the central and peripheral areas. In this study, busulfan was intraperitoneally injected at 40 mg/kg body weight into C57BL/6J male mice. After 28 days, in busulfan-treated mice, the diameters of seminiferous tubules were significantly higher in the central than in the peripheral area of the testes. The seminiferous tubular areas also significantly decreased in the peripheral areas compared with the central areas. The number of germ cells per seminiferous tubule was significantly higher in the central than in the peripheral area. Sertoli cell nuclei were detached into the lumen in the peripheral area. The number of Leydig cells was significantly lower in the peripheral areas. These data suggest that the effects of busulfan differ between the central and peripheral areas of the testis at 4 weeks after busulfan administration.

Keywords: busulfan, central and peripheral area of testes, spermatogenesis disorder

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Tominaga S*1, Yoshioka H*2, Yokota S, Tsukiboshi Y*2, Suzui M*2, Nagai M*2, Hara H*2, Miura N*3, Maeda T*1: Copper-induced renal toxicity controlled by period1 through modulation of Atox1 in mice.
Biomedical Research (Tokyo). 2024;45(4):143-149. doi: 10.2220/biomedres.45.143

Copper (Cu) is known to induce oxidative stress and apoptosis in the liver, kidney, and brain. We previously demonstrated the molecular mechanism underlying the Cu-induced hepatic diurnal variation. However, the cellular molecule(s) involved in Cu-induced renal chronotoxicity remain unknown. In this study, we aimed to elucidate the molecular mechanisms underlying Cu-induced diurnal toxicity in the kidneys. We evaluated cell viability and clock gene expression levels in mouse renal cortex tubular cells (MuRTE61 cells) after Cu treatment. We also examined the Cu homeostasis- and apoptosis-related gene levels after period 1 (Per1) overexpression in MuRTE61 cells. Cu treatment decreased MuRTE61 cell viability in a dose-dependent manner. It increased the Per1 expression levels after 24 h. Notably, Per1 overexpression alleviated the Cu-induced inhibition of MuRTE61 cell viability. Moreover, Per1 overexpression downregulated the cleaved caspase-3 and reduced Cu levels by upregulating the antioxidant 1 copper chaperone (Atox1) levels. These results suggest that Cu-induced renal toxicity is associated with Per1 expression via the regulation of the copper chaperone, Atox1.

Keywords: copper, antioxidant 1 copper chaperone, period 1

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Yoshioka H*1,2, Tominaga S*1, Amano F*1, Wu S*1, Torimoto S*1, Moriishi T*1, Tsukiboshi Y*1, Yokota S, Miura N*3, Inagaki N*1, Matsushita Y*1, Maeda T*2: Juzen-taiho-to alleviates cisplatin-induced renal injury in mice.

Traditional and kanpo. 2024;11(2):147-155. doi: 10.1002/tkm2.1417

Cisplatin is a highly effective anti-cancer agent, but its clinical use is restricted due to severe renal toxicity. This study aimed to investigate the alleviative effects of juzentaihoto (JTT) in a mouse model of cisplatin-induced renal injury. Four groups of seven-week-old male C57BL/6J mice (control, JTT, cisplatin, and JTT + cisplatin groups) were used in the study. The JTT and JTT + cisplatin groups received oral JTT (500 mg/kg) once a day for three days. After 24 h, the cisplatin, and JTT + cisplatin groups were intraperitoneally injected with cisplatin (15 mg/kg). The mice in each group were euthanized 72 h after cisplatin administration, and blood and kidney samples were collected. Cisplatin injection decreased body weight and elevated plasma blood urea nitrogen and creatinine levels, while also increasing renal oxidative stress, inflammation, and cell death. These changes were alleviated by JTT administration. We also found that platinum accumulation in the kidneys following cisplatin injection was attenuated by JTT treatment. Furthermore, Mate1 expression levels (a cisplatin efflux transporter) were upregulated by JTT injection. Our results demonstrated that JTT mitigated cisplatin-induced renal injury in mice by alleviating oxidative stress, inflammation, and cell death, achieved through the upregulation of the cisplatin efflux transporter Mate1.

Keywords: juzentaihoto, cisplatin, renal injury

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Kuwagata M, Doi Y*1,2, Saito H, Tsurumoto M, Igarashi T, Nishimura T, Taquahashi Y, Kitajima S: A 90-day repeated oral dose toxicity study of p-cymene in rats.

Fundam Toxicol Sci. 2024;11(4):169-181. doi.org/10.2131/fts.11.169

p-Cymene, is a monocyclic monoterpene hydrocarbon, commonly used as a flavoring agent in food. A 90-day repeated oral toxicological study of p-cymene was conducted to examine the toxicological properties and determine the no-observed-adverse-effect level (NOAEL) of p-cymene in Crl:CD (SD) rats at the following doses: 0 (corn oil), 2.4, 12, and 60 mg/kg/day. No mortality or abnormal clinical signs were observed in the treatment groups. The body weight, food consumption, ophthalmoscopy, and gross pathology of the rats were also not affected by p-cymene treatment. However, in the 60 mg/kg group, certain parameters decreased in males, including hemoglobin and hematocrit, red blood cell count, triglyceride, total protein, and albumin. In females, urine volume and total potassium excretion increased, whereas specific gravity, and sodium, potassium, and chlorine concentrations decreased. Increased liver weight was observed in both males and females. Histopathological observations revealed centrilobular hepatocellular hypertrophy. In the 12 mg/kg group, no adverse effects of p-cymene treatment were observed in both sexes. In conclusion, the NOAEL of p-cymene was 12 mg/kg/day for both sexes under the present experimental conditions, considering the alterations in urinalysis, hematology, clinical biochemistry, and histopathology.

Keywords: p-Cymene, flavoring agent, toxicity

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Taquahashi Y, Aisaki K, Morita K, Suga K, Kitajima S: Application of the matrix profile algorithm for detecting abnormalities in rat electrocardiograms.

Fundam. Toxicol. Sci. 2024;11(6):289-296. doi:10.2131/fts.11.289

An electrocardiogram (ECG) is useful for diagnosing heart diseases, particularly arrhythmias; however, it requires time to detect rare abnormalities that occur infrequently in normal conditions. This study aimed to detect abnormalities in the ECG by evaluating the performance of the matrix profile (MP) algorithm,

which is used to detect outliers in data with repetitive patterns. Female hairless rats (HWY/Slc) were used, and all procedures were performed under isoflurane anesthesia. The tricyclic antidepressant amitriptyline hydrochloride (50 mg/kg) was administered intraperitoneally, and ECG measurements were obtained using carbon nanotube yarn as the surface electrode. The measurements were collected by an analog-to-digital converter at a sample rate of 2 kHz. Jupyter Lab 4.0.9 was used for the Python 3.9.1 script environment in the MP analysis, with the following related libraries: Numpy 1.19.5 and Pandas 1.2.1 for data processing, Matplotlib 3.3.4 for data visualization, and Matrixprofile 1.1.10 as the implementation library for the MP algorithm. A data size of 2.5 or 25 sec was applied without prior baseline adjustment, labeling, or normalization. The MP analysis did not detect abnormalities in cases with slow changes in the waveform, even when the waveforms were abnormal. However, it detected heart rate and amplitude fluctuations that rarely occurred in normal conditions. In particular, the MP analysis detected discord during a sudden change, such as a cardiac arrest. The MP algorithm showed excellent performance in terms of the time required for analysis and demonstrated the potential to detect ECG abnormalities in real-time during toxicity testing.

Keywords: electrocardiogram, vital signs, matrix profile algorithm

Fujioka T[*1], Shiura H[*1,2], Ishii M[*1], Ono R, Endo T[*1], Kiyonari H[*3], Hirate Y[*1], Ito H[*1,4], Kanai-Azuma M[*1], Kohda T[*2], Kaneko-Ishino T[*5], Ishino F[*1]:
Targeting of retrovirus-derived Rtl8a/ 8b causes late-onset obesity, reduced social response and increased apathy-like behaviour.
OPEN BIOLOGY. 2025;15(1):240279. doi: 10.1098/rsob.240279

Retrotransposon Gag-like (RTL) 8A, 8B and 8C are eutherian-specific genes derived from a certain retrovirus. They cluster as a triplet of genes on the X chromosome, but their function remains unknown. Here, we demonstrate that Rtl8a and Rtl8b play important roles in the brain: their double knockout (DKO) mice not only exhibit reduced social responses and increased apathy-like behaviour, but also become obese from young adulthood, similar to patients with late Prader-Willi syndrome (PWS), a neurodevelopmental genomic imprinting disorder. Mouse RTL8A/8B proteins are expressed in the prefrontal cortex and hypothalamus and localize to both the nucleus and cytoplasm of neurons, presumably due to the N-terminal nuclear localization signal-like sequence at the N-terminus. An RNAseq study in the cerebral cortex revealed reduced expression of several GABA type A receptor subunit genes in DKO, in particular Gabrb2, which encodes its β2 subunit. We confirmed the reduction of GABRB2 protein in the DKO cerebral cortex by western blotting. As GABRB2 has been implicated in the aetiology of several neurodevelopmental and neuropsychiatric disorders, it is likely that the reduction of GABRB2 is one of the major causes of the neuropsychiatric defects in the DKO mice.

Keywords: Prader-Willi syndrome, neuronal development, retrovirus-derived genes

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Yokota S, Hashimoto K*, Sato T*, Uemura K*, Makiyama K*, Nishimura T, Kitajima S, Ogawa T*:
A Long-term Mouse Testis Organ Culture System to Identify Germ Cell Damage Induced by Chemotherapy.
Curr Res Toxicol. 2025;8:100228. doi: 10.1016/j.crtox.2025.100228

We previously developed the acrosin-green fluorescent protein (GFP) transgenic neonatal mouse organ culture system for rapid and accurate assessment of testicular toxicity. This system effectively evaluates drug-induced toxicity in male germ cells before meiotic entry but cannot assess post-meiotic germ cell toxicity. For many chemicals, the specific stage of germ cell differentiation that is susceptible to toxicity remains unclear, highlighting the need for new methods. In this study, we incubated neonatal mouse testis organ cultures for 35 days to allow post-meiotic cells to develop. The tissue was then exposed to cisplatin to determine the cells that are targeted and to assess the reversibility of the toxicity.

We monitored changes in tissue volume and GFP fluorescence, which tracks the progression of spermatogenesis, and confirmed findings by histological analysis. Cisplatin inhibited tissue growth and reduced GFP fluorescence in a concentration-dependent manner. Higher concentrations targeted not only spermatogonia, but also spermatocytes and spermatids. Recovery from toxicity was observed at clinically relevant doses. This study demonstrates that long-term mouse testis organ culture can be used to assess testicular toxicity, enabling the identification of specific germ cell stages targeted by chemicals such as cisplatin.

Keywords: cisplatin, testicular toxicity, testis organ culture

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Takahashi Y, Igawa T*1, Nanba C*1, Ogino H*1, Uchiyama H*2, Kitajima S: Perichordal Vertebral Column Formation in *Rana kobai*.

Journal of Morphology. 2025;286(4):e70044 doi: 10.1002/jmor.70044

The vertebral column of anurans exhibits morphological diversity that is often used in phylogenetic studies. The family *Ranidae* is one of the ecologically most successful groups of anurans, with the genus *Rana* being distributed broadly in Eurasia. However, there are relatively sparse detailed studies on the development of the vertebral column in *Rana* species, and images of the entire axial skeleton have seldom been illustrated till date. Here, we provide an illustrated description on the development of the entire vertebral column in *Rana kobai*, a Japanese small frog from the Amami Islands. Our observation of double-stained skeletal specimens revealed that in *R. kobai*, the original atlas and the first dorsal are fused into one vertebra, and the ninth neural arch is fused with the tenth arch in half of the examined larvae. Anuran vertebral column development is classified into two modes, perichordal and epichordal. *Rana* species undergo the typical perichordal mode of centrum formation. Kemp and Hoyt (1969) described that centrum formation in *R. pipiens* starts from a saddle-shaped bone on the dorsal half of the notochord. Nevertheless, our detailed observations revealed that centrum ossification initially emerges at the base of the paired neural arches and then forms the saddle-shaped bone. In *Xenopus*, a species with epichordal centra, centrum formation starts from a pair of ovoid bone elements at the base of the neural arches. Overall, our results imply that centrum ossification starts from the base of neural arches in anurans, irrespective of whether it is perichordal or epichordal. Our observations also revealed the presence of the crescent-shaped cartilage domain in the intervertebral region in *R. kobai*. The location of the crescent-shaped domain in *R. kobai* is consistent with that of the intercentrum in *Ichthyostega* and several temnospondyls. Based on our observations, we propose a hypothesis on the difference between perichordal and epichordal modes in light of evolution.

Keywords: Anura, Rana, vertebral column

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Kimura S*1, Tagami S*2, Mano H, Kittaka A, Ida Y*2, Takagi Y*2, Nakagawa K*2, Arai T*2, Yokota S, Tsugawa N*1, Kamao M*1, Suhara Y*1, Sakaki T*3, Nakagawa K*1, Okano T*1, Hirota Y*2: Divergent Roles of 25-Hydroxyvitamin D3 and 1α,25-Dihydroxyvitamin D3 in Neural Fate Determination: A CYP27B1-Dependent Neuron Formation and VDR-Dependent Astrocyte Development.

Biochem Biophys Res Commun. 2025;755:151547. doi: 10.1016/j.bbrc.2025.151547

Vitamin D plays a crucial role in neural differentiation, yet its precise mechanisms remain unclear. In this study, we investigated the effects of vitamin D metabolites, 25-hydroxyvitamin D3 (25D3) and 1α,25-dihydroxyvitamin D3 (1α,25D3), on neural differentiation using Cyp27b1-/- and Vdr-/- knockout mice-derived neural stem cells. We found that 1α,25D3 promotes neuronal differentiation via vitamin D receptor (VDR), whereas some of its effects occur independently of VDR. Additionally, 25D3 requires conversion to 1α,25D3 by CYP27B1 to induce neuronal differentiation. In contrast, both 25D3 and 1α,25D3 promoted astrocyte differentiation regardless of CYP27B1 expression but required VDR. Furthermore, using vitamin D derivatives, we demonstrated that VDR binding affinity does not directly correlate with

neurogenic potential. These findings reveal distinct VDR-dependent and VDR-independent pathways in neural differentiation, highlighting a previously unrecognized role of vitamin D metabolism in neural fate determination.

Keywords: vitamin D, astrocyte differentiation, neural differentiation

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Yoshioka H*1,2, Yokota S, Torimoto S*1, Horita H*1, Tsukiboshi Y*1, Maeda T*2, Miura N*3: Cry2 Alleviates Cisplatin-Induced Cytotoxicity In Mouse Renal Cortex Tubular Cell Lines.

Biological Pharm Bull. 2025;48(4):390-398. doi: 10.1248/bpb.b24-00811

Cisplatin is a platinum-based drug that is widely used to treat various types of cancer. However, cisplatin is known to cause severe adverse effects, such as nephrotoxicity and ototoxicity. Clock genes, such as Bmal1 and Clock, regulate cisplatin-related homeostasis genes, such as Oct2 and Mate1. Although these clock genes may be involved in cisplatin-induced nephrotoxicity, their associations with other clock genes remain unclear. The aim of the present study was to investigate whether seven clock genes (Ciart, cryptochrome 1 (Cry1), Cry2, Npas2, Per1, Per2, and Per3) regulate cisplatin-induced renal toxicity in a renal cortex tubule cell line (MuRTE61). Cisplatin treatment decreases MuRTE61 cell viability in a dose-dependent manner. Cry2 expression levels increased after treatment with cisplatin for 24 h. Notably, Cry2 overexpression alleviated cisplatin-induced suppression of cell proliferation, apoptosis, and platinum content in MuRTE61 cells. Moreover, Cry2 overexpression upregulated the efflux-related transporters (Atp7a and Mrp2). These results suggest that Cry2 protects against cisplatin toxicity by reducing Pt accumulation and increasing the expression of Atp7a and Mrp2.

Keywords: circadian rhythm, cisplatin, nephrotoxicity

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Ito T*1, Suzuki T*2,3, Sakai Y*4, Nishioka K*5, Itoh Y*2,3, Sakamoto K*6, Ikemura N*7, Matoba S*7, Kanda Y, Takagi J*6,8, Okamoto T*2,3, Tahara K*1,9, Hoshino A*7: Engineered ACE2 decoy in dry powder form for inhalation: A novel therapy for SARS-CoV-2 variants.

Mol Ther Methods Clin Dev. 2025;33:101459. DOI: 10.1016/j.omtm.2025.101459

The persistent threat of SARS-CoV-2 and the emergence of new variants has prompted the development of a novel, easily administered modality that can overcome viral mutations. The engineered ACE2 decoy shows neutralizing activity comparable to monoclonal antibodies and is broadly effective against SARS-CoV-2 variants and ACE2-utilizing sarbecoviruses. In addition to intravenous administration, this decoy has shown antiviral efficacy through nebulized aerosol inhalation in murine and primate models, offering a dose-sparing advantage. Clinically, dry powder formulation is ideal for convenience and storage but poses challenges for protein biologics. This study developed a freeze-dried spray formulation of the ACE2 decoy for inhalation. The trehalose and leucine-based excipient maintained neutralizing activity and prevented aggregate formation. The dry powder showed aerodynamic distribution from bronchi to alveoli, aiding protection against SARS-CoV-2 infections. Neutralizing activity, structural stability, and powder dispersibility were preserved after 6 months of storage. In a mouse model of SARS-CoV-2 infection, significant reductions in viral replication and lung pathology were observed with intratracheal administration 24 h post-infection. The ACE2 decoy retained activity against recent JN.1 and current KP.3 strains, confirming its robust efficacy against viral mutations. This ACE2 decoy powder inhalant is a self-administered, next-generation treatment addressing the ongoing immune-evading evolution of SARS-CoV-2.

Keywords: ACE2 decoy, SARS-CoV-2, dry powder formulation

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ALTEX. 2025;42:323-349. DOI: 10.14573/altex.2503191

The 5th International Conference on Developmental Neurotoxicity (DNT) Testing (DNT5) took place in April 2024 in Konstanz, Germany, organized by CAAT-Europe, the University of Konstanz, and scientists from the US EPA, SCAHT, and CAAT at Johns Hopkins University Bloomberg School of Public Health. The conference convened experts from regulatory agencies, industry, and academia to explore the latest advancements in DNT testing and the integration of animal-free new approach methodologies (NAMs) into next-generation risk assessment (NGRA). The key topic was the appli-cation and further development of the recently established DNT *in vitro* test battery (DNT-IVB). To support this, OECD held a satellite meeting to discuss necessary next steps for further implementation of the DNT-IVB in regulatory contexts. Validation of new DNT test methods and use of their data for in-vitro-to-in-vivo extrapolations in physiologically based kinetic models were also important themes of the main meeting. In this context, the question was raised when a comprehensive biological and chemical coverage by the DNT-IVB would be reached. A need for additional testing data was recognized. Context-specific validation approaches for the entire DNT-IVB and the potential for intelligent combinations of assays to enhance the predictive power of the test battery were also addressed. Many presentations demonstrated the field's embrace of novel developments, including the use of multi-endpoint embryonic zebrafish tests, the development of artificial intelligence-driven computational approaches, and the establishment of complex, electrically active brain organoids and other self-organizing structures. Through its highly interactive format, DNT5 promoted extensive collaborative efforts in advancing the field toward more human-relevant, scientifically reliable, and ethical toxicological assessments.

Keywords: DNT *in vitro* test battery (DNT-IVB), developmental neurotoxicity (DNT), key neurodevelopmental processes (KNDP)

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Kawagishi H*1, Nakajima T*1, Ramadhiani R*2, Nakada T*3, Tomita T*1, Kanda Y, Emoto N*2, Yamada M*1: Development of a Novel Therapeutic for Pediatric Heart Failure Targeting Angiotensin II Receptor.

Yakugaku Zasshi. 2025;145:275-280. DOI: 10.1248/yakushi.24-00171-1

Pediatric drug development is a global challenge. Children undergo organ growth and exhibit different drug reactions than adults, resulting in different pharmacological responses. Therefore, it is necessary to consider children's physiological characteristics when evaluating drug efficacy and safety in pediatric patients. We conducted drug discovery research for the treatment of pediatric heart failure, based on neonatal-specific physiological functions of angiotensin II. Pediatric heart failure is one of the most important causes of death in children, particularly neonates and infants. However, only a few therapeutic agents are available to treat pediatric heart failure. Angiotensin II binds to its receptors（angiotensin II receptor type 1: AT_1R）and regulates cellular functions through G protein and β-arrestin pathways. We previously found that the β-arrestin-biased AT_1R agonist, TRV027, induced a positive inotropic effect in the hearts of neonatal mice. Acute administration of TRV027 did not affect heart rate, oxygen consumption, or production of reactive oxygen species. The inotropic effect of TRV027 was also observed in human iPS cell-derived cardiomyocytes and a neonatal mouse model of dilated cardiomyopathy. Furthermore, chronic administration of TRV027 improved the survival rate of mice with dilated cardiomyopathy during the preweaning period. These results demonstrate that TRV027 has the potential to be a safe and effective drug for treating pediatric heart failure.

Keywords: TRV027, angiotensin receptor, biased agonist

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Kawagishi H, Kanda Y: Challenges in Development

of Appropriate Drugs for Children.
Yakugaku Zasshi. 2025;145:273-274. DOI: 10.1248/yakushi.24-00171-F
［No abstract available］

Izumi-Nakaseko H[*1], Sekino Y[*2], Kambayashi R[*1], Goto A[*1], Takei Y[*3], Himeno Y[*4], Okado-Matsumoto A[*5], Nagasawa Y[*6], Naito AT[*7], Kanda Y, Sugiyama A[*8]: Nilotinib impairs relaxation and temporal electro-mechanical integrity in human iPS-derived cardiomyocyte sheets.
Toxicol Appl Pharmacol. 2025;496:117258. DOI: 10.1016/j.taap.2025.117258

Introduction: Nilotinib, an anti-tumor tyrosine kinase inhibitor against BCR-ABL1, has been clinically reported to cause QT prolongation, but currently lacks evidence for a risk of torsade de pointes. Indeed, it is poorly understood why nilotinib rarely induces torsade de pointes.

Methods and results: We adopted two-dimensional human induced pluripotent stem cell-derived cardiomyocyte (hiPSC-CM) sheets to examine effects of nilotinib on their electrophysiological and mechanical properties besides intracellular calcium (Ca^{2+}) dynamics. Nilotinib prolonged repolarization in concentration- and reverse-frequency-dependent manners but shortened the contraction-relaxation duration (CRD), which made the electro-mechanical window negative in hiPSC-CM sheets. These effects would correspond to "trigger" of drug-induced torsade de pointes. The drug also suppressed mitochondrial maximum respiration and decreased the peak amplitude and the decay rate of Ca^{2+} transients, which shortened the CRD and impaired relaxation function of the cell sheets, partly explaining the onset mechanism of nilotinib-induced heart failure in patients. Additionally, nilotinib-induced early afterdepolarization (EAD) fluctuated the conduction speed and repolarization, and shifted the electro-mechanical window in a negative direction. These phenomena increased beat-to-beat variability of repolarization and electrical vulnerability of the heart. Meanwhile, nilotinib caused the conduction delay by Na channel blockade, thereby blocking "substrate" formation for the arrhythmia persistence.

Conclusion: Nilotinib could deteriorate relaxation ability and temporal electrical integrity of the heart through impairing Ca^{2+} dynamics as well as repolarization phase, which were exacerbated by nilotinib-induced EAD. However, the drug only formed "trigger", which would explain the lower occurrence of nilotinib-induced torsade de pointes.
Keywords: Ca(2+) dynamics, Diastolic dysfunction, Human induced pluripotent stem cell-derived cardiomyocyte sheets

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加藤百合[*1]，中村祐也[*1]，近藤萌[*2]，諫田泰成，西田基宏[*3]：ヒトiPS細胞由来心筋細胞のミトコンドリア品質に着目した抗がん薬による心毒性リスク評価.
日本薬理学雑誌. 2025;160:9-12. DOI: 10.1254/fpj.24056
現代は２人に１人ががんに罹患すると言われており，がんを治療するために様々な抗がん薬が使用されている．抗がん薬は長期間継続して使用するため副作用のリスクもあり，主な副作用の１つとして心機能障害があげられる．例えば，アントラサイクリン系の抗がん薬であるドキソルビシンは用量依存的な心毒性を発症する．心毒性とは具体的には駆出率の低下，不整脈，うっ血性心不全など多岐にわたり，これらはすべて高い死亡率と関連している．そのため，予め抗がん薬の心毒性リスクを評価することは重要な意味をもつ．我々は，心筋細胞の拍動に必要な膨大なエネルギーを産生するミトコンドリアの形態機能に対する抗がん薬の影響に着目し，ヒト

iPS細胞由来心筋細胞（hiPSC-CMs）を用いた心毒性リスクの評価を行った．心不全が報告されている複数の抗がん薬をhiPSC-CMsに曝露すると，ミトコンドリアの過剰分裂が亢進し，ミトコンドリアの機能は有意に低下した．ミトコンドリアの分裂を促進するGTP結合タンパク質であるダイナミン関連タンパク質１（Drp1）をノックダウンすると，抗がん薬によるミトコンドリアの分裂亢進が抑制された．これらのことは，心筋細胞のミトコンドリア形態・機能を評価することが，抗がん薬による心毒性のリスクを議論する上で有用であることを示している．同時に，ミトコンドリアの品質を正常状態で維持することが抗がん薬による心毒性リスクを軽減する新たな治療戦略になる可能性がある．本稿では，抗がん薬におけるミトコンドリア品質を標的とした心毒性評価について，非小細胞肺がん治療薬であるオシメルチニブを例に紹介する．

Keywords：ヒトiPS細胞由来心筋細胞，抗がん薬，ミトコンドリア

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川岸裕幸，諫田泰成：ヒトiPS細胞由来心筋細胞を用いた医薬品安全性，有効性の評価．
日本薬理学雑誌. 2025;160:4-8. DOI: 10.1254/fpj.24043

本邦におけるドラッグ・ラグやドラッグ・ロスが深刻になっており，医薬品の迅速かつ安定な供給のための創薬力強化が求められている．医薬品開発には，安全かつ効率的な臨床試験の実施が不可欠であり，そのためには非臨床試験法の開発や高度化が重要である．近年，動物実験代替法の流れが国際的に加速しており，医薬品開発に関しても，ヒトiPS細胞技術やモデル＆シミュレーションなどのNew Approach Methodologies（NAMs）の利用が提唱されている．ヒトiPS細胞由来心筋細胞（ヒトiPS心筋）は，不整脈や心収縮障害などの心毒性のリスク評価における有用性が示されており，すでに承認申請で利用されている．またヒトiPS細胞の技術向上により，最近では成熟型ヒトiPS心筋やEngineered Heart Tissueの医薬品安全性，有効性評価への応用も検討されており，非臨床評価ツールとしての利用が進むと予想される．将来的に，小児や希少疾病患者などの特定集団の特性を反映したヒトiPS細胞技術が可能になれば，個人差を考慮した非臨床試験法の開発や高度化が期待される．本稿では，ヒトiPS細胞技術を利活用した非臨床評価に関する最新の動向と今後の展望について概説する．

Keywords：NAMs，安全性，ヒトiPS心筋

Nishimura A[*1,2,3], Ogata S[*4], Tang X[*1,2,3], Hengphasatporn K[*5], Umezawa K[*6], Sanbo M[*1], Hirabayashi M[*1], Kato Y[*7], Ibuki Y[*8], Kumagai Y[*7], Kobayashi K[*1], Kanda Y, Urano Y[*9,10], Shigeta Y[*5], Akaike T[*4], Nishida M[*11,12,13,14]: Polysulfur-based bulking of dynamin-related protein 1 prevents ischemic sulfide catabolism and heart failure in mice.
Nat Commun. 2025;16:276. DOI: 10.1038/s41467-024-55661-5

The presence of redox-active molecules containing catenated sulfur atoms (supersulfides) in living organisms has led to a review of the concepts of redox biology and its translational strategy. Glutathione (GSH) is the body's primary detoxifier and antioxidant, and its oxidized form (GSSG) has been considered as a marker of oxidative status. However, we report that GSSG, but not reduced GSH, prevents ischemic supersulfide catabolism-associated heart failure in male mice by electrophilic modification of dynamin-related protein (Drp1). In healthy exercised hearts, the redox-sensitive Cys644 of Drp1 is highly S-glutathionylated. Nearly 40% of Cys644 is normally polysulfidated, which is a preferential target for GSSG-mediated S-glutathionylation. Cys644 S-glutathionylation is resistant to Drp1 depolysulfidation-dependent mitochondrial hyperfission and myocardial dysfunction caused by hypoxic stress. MD simulation of Drp1 structure and site-directed mutagenetic analysis reveal a functional interaction between Cys644 and a critical phosphorylation site Ser637, through Glu640. Bulky modification at Cys644 via polysulfidation or S-glutathionylation reduces Drp1 activity by disrupting Ser637-Glu640-Cys644 interaction. Disruption of Cys644 S-glutathionylation nullifies the cardioprotective effect of GSSG against heart failure after myocardial infarction. Our findings suggest a therapeutic potential of supersulfide-based Cys bulking on Drp1 for ischemic heart disease.

Keywords: Heart failure, Pharmacology

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Lee CLM*1, Brabander CJ*2, Nomura Y*2, Kanda Y, Yoshida S*3: Embryonic exposure to acetamiprid insecticide induces CD68-positive microglia and Purkinje cell arrangement abnormalities in the cerebellum of neonatal rats.

Toxicol Appl Pharmacol. 2025;495:117215. DOI: 10.1016/j.taap.2024.117215

Concerns have been raised regarding acetamiprid (ACE), a neonicotinoid insecticide, due to its potential neurodevelopmental toxicity. ACE, which is structurally similar to nicotine, acts as an agonist of nicotinic acetylcholine receptors (nAChRs) and resists degradation by acetylcholinesterase. Furthermore, ACE has been reported to disrupt neuronal transmission and induce developmental neurotoxicity and ataxia in animal models. However, the prenatal ACE exposure and its pathological changes, including impacts on motor control, remains unclear. In this study, we investigated the effects of ACE exposure, focusing on the development of cerebellar neurons and glia, which are linked to motor impairment. ACE at doses of 20, 40-, and 60 mg/kg body weight was administered to Pregnant Wistar rats via feed on gestational day (G) 15. The developing cerebellum of the pups was examined on postnatal days (P) 7, 14, and 18, corresponding to the critical periods of cerebellar maturation in rodents. Our data revealed that ACE exposure at 40 and 60 mg/kg induced abnormal neuronal alignment on P14, and neuronal cell loss on P18. Additionally, ACE altered microglial behavior, with an increase in the number of CD68-positive microglia, suggesting that the exposure results in an increase in phagocytic microglia in response to neuronal abnormalities, ultimately leading to neuronal cell loss. Pups exposed to 60 mg/kg ACE exhibited hindlimb clasping during the hindlimb suspension test, indicating motor impairment. These findings suggest that ACE exposure causes neuronal cell loss of developing Purkinje cells and promotes a phase shift to the activate mode of microglia. This study further highlights the crucial role of neuron-glia interactions in ACE-induced motor impairment, thus contributing to our understanding of the potential risks associated with prenatal ACE exposure.

Keywords: Acetamiprid, Developmental Neurotoxicity, microglia

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Atef Y*1, Ito T*1, Masuda A*2, Kato Y*1, Nishimura A*3,4,5, Kanda Y, Kunisawa J*6,7, Kusakabe T*8, Nishida M*1,3,5,6: Diabetic Mice Spleen Vulnerability Contributes to Decreased Persistence of Antibody Production after SARS-CoV-2 Vaccine.

Int J Mol Sci. 2024;25:10379. DOI: 10.3390/ijms251910379

During the COVID-19 pandemic, diabetic and obese patients experienced higher rates of hospital admissions, severe illness, and mortality. However, vaccinations failed to provide those vulnerable populations the same level of protection against

COVID-19 severity as those without diabetic and obese phenotypes. Our study aimed to investigate how diabetes mellitus (DM) impacts the immune response following vaccination including the artificially designed trimeric SARS-CoV-2 spike (S)-protein. By using two diabetic mouse models, ob/ob mice (obese, hyperglycemic, and insulin-resistant) and STZ-treated mice (insulin-deficient and hyperglycemic), we observed a significant reduction in S-protein-specific IgG antibody titer post-vaccination in both diabetic models compared to wild-type (WT) mice. Both diabetic mouse models exhibited significant abnormalities in spleen tissue, including marked reductions in splenic weight and the size of the white pulp regions. Furthermore, the splenic T-cell and B-cell zones were notably diminished, suggesting an underlying immune dysfunction that could contribute to impaired antibody production. Notably, vaccination with the S-protein, when paired with an optimal adjuvant, did not exacerbate diabetic cardiomyopathy, blood glucose levels, or liver function, providing reassurance about the vaccine's safety. These findings offer valuable insights into potential mechanisms responsible for the decreased persistence of antibody production in diabetic patients.

Keywords: COVID-19, S-protein, SARS-CoV-2

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Kondo M*1, Nakamura Y*2, Kato Y*2, Nishimura A*3, Fukata M*4, Moriyama S*4, Ito T*2, Umezawa K*5, Urano Y*6, Akaike T*7, Akashi K*8, Kanda Y, Nishida M*9: Inorganic sulfides prevent osimertinib-induced mitochondrial dysfunction in human iPS cell-derived cardiomyocytes.

J Pharmacol Sci. 2024;156:69-76. DOI: 10.1016/j.jphs.2024.07.007

Despite the widespread recognition of the global concern regarding the onset of cardiovascular diseases in a significant number of patients following cancer treatment, definitive strategies for prevention and treatment remain elusive. In this study, we established systems to evaluate the influence of anti-cancer drugs on the quality control of mitochondria, pivotal for energy metabolism, using human induced pluripotent stem cell-derived cardiomyocytes (hiPSC-CMs). Osimertinib, an epidermal growth factor receptor tyrosine kinase inhibitor used for treatment in lung cancer, reportedly increases the risk of cardiovascular disease. However, its underlying mechanism is largely unknown. Here, we found that the treatment of hiPSC-CMs with osimertinib and doxorubicin, but not trastuzumab and cisplatin, revealed a concentration-dependent impairment of respiratory function accompanied by mitochondrial fission. We previously reported the significant role of sulfur metabolism in maintaining mitochondrial quality in the heart. Co-treatment with various inorganic sulfur donors (Na_2S, Na_2S_2, Na_2S_3) alongside anti-cancer drugs demonstrated that Na_2S attenuated the cardiotoxicity of osimertinib but not doxorubicin. Osimertinib decreased intracellular reduced sulfur levels, while Na_2S treatment suppressed the sulfur leakage, suggesting its potential in mitigating osimertinib-induced cardiotoxicity. These results imply the prospect of inorganic sulfides, such as Na_2S, as a seed for precision pharmacotherapy to alleviate osimertinib's cardiotoxic effects.

Keywords: Heart failure, Mitochondria, Onco-cardiology

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Satsuka A, Ribeiro AJS*1, Kawagishi H, Yanagida S, Hirata N, Yoshinaga T*2, Kurokawa J*3, Sugiyama A*4, Strauss DG*5, Kanda Y: Contractility assessment using aligned human iPSC-derived cardiomyocytes.
J Pharmacol Toxicol Methods. 2024;128:107530. DOI: 10.1016/j.vascn.2024.107530

Introduction: Cardiac safety assessment, such as lethal arrhythmias and contractility dysfunction, is critical during drug development. Human induced pluripotent stem cell-derived cardiomyocytes (hiPSC-CMs) have been shown to be useful in predicting drug-induced proarrhythmic risk through international validation studies. Although cardiac contractility is another key function, fit-for-purpose hiPSC-CMs in evaluating drug-induced contractile dysfunction remain poorly understood. In this study, we investigated whether alignment of hiPSC-CMs on nanopatterned culture plates can assess drug-induced contractile changes more efficiently than non-aligned monolayer culture.

Methods: Aligned hiPSC-CMs were obtained by culturing on 96-well culture plates with a ridge-groove-ridge nanopattern on the bottom surface, while non-aligned hiPSC-CMs were cultured on regular 96-well plates. Next-generation sequencing and qPCR experiments were performed for gene expression analysis. Contractility of the hiPSC-CMs was assessed using an imaging-based motion analysis system.

Results: When cultured on nanopatterned plates, hiPSC-CMs exhibited an aligned morphology and enhanced expression of genes encoding proteins that regulate contractility, including myosin heavy chain, calcium channel, and ryanodine receptor. Compared to cultures on regular plates, the aligned hiPSC-CMs also showed both enhanced contraction and relaxation velocity. In addition, the aligned hiPSC-CMs showed a more physiological response to positive and negative inotropic agents, such as isoproterenol and verapamil.

Discussion: Taken together, the aligned hiPSC-CMs exhibited enhanced structural and functional properties, leading to an improved capacity for contractility assessment compared to the non-aligned cells. These findings suggest that the aligned hiPSC-CMs can be used to evaluate drug-induced cardiac contractile changes.

Keywords: Alignment, Cardiomyocyte, Contractility

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Puglisi R[*8], Shi H[*9], Yang X[*10], Pugsley MK[*11]: Development of a pharmaceutical database as an aid to the nonclinical detection of drug-induced cardiac toxicity.

J Pharmacol Toxicol Methods. 2024;127:107507. DOI: 10.1016/j.vascn.2024.107507

The Health and Environmental Sciences Institute (HESI) Cardiac Safety Committee designed and created a publicly accessible database with an initial set of 128 pharmacologically defined pharmaceutical agents, many with known cardiotoxic properties. The database includes specific information about each compound that could be useful in evaluating hypotheses around mechanisms of drug-induced cardiac toxicity or for development of novel cardiovascular safety assays. Data on each of the compounds was obtained from published literature and online sources (e.g., DrugBank.ca and International Union of Basic and Clinical Pharmacology (IUPHAR) / British Pharmacological Society (BPS) Guide to PHARMACOLOGY) and was curated by 10 subject matter experts. The database includes information such as compound name, pharmacological mode of action, characterized cardiac mode of action, type of cardiac toxicity, known clinical cardiac toxicity profile, animal models used to evaluate the cardiotoxicity profile, routes of administration, and toxicokinetic parameters (i.e., Cmax). Data from both nonclinical and clinical studies are included for each compound. The user-friendly web interface allows for multiple approaches to search the database and is also intended to provide a means for the submission of new data/compounds from relevant users. This will ensure that the database is constantly updated and remains current. Such a data repository will not only aid the HESI working groups in defining drugs for use in any future studies, but safety scientists can also use the database as a vehicle of support for broader cardiovascular safety studies or exploring mechanisms of toxicity associated with certain pharmacological modes of action.

Keywords: Adverse event, Cardiac, Cardiac methods

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Yamada S, Hashita T[*1], Yanagida S, Sato H[*1], Yasuhiko Y, Okabe K[*2], Noda T[*2,3], Nishida M[*4,5], Matsunaga T[*1], Kanda Y: SARS-CoV-2 causes dysfunction in human iPSC-derived brain microvascular endothelial cells potentially by modulating the Wnt signaling pathway.

Fluids Barriers CNS. 2024;21:32. DOI: 10.1186/s12987-024-00533-9

Background: Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) causes coronavirus disease 2019 (COVID-19), which is associated with various neurological symptoms, including nausea, dizziness, headache, encephalitis, and epileptic seizures. SARS-CoV-2 is considered to affect the central nervous system (CNS) by interacting with the blood-brain barrier (BBB), which is defined by tight junctions that seal paracellular gaps between brain microvascular endothelial cells (BMECs). Although SARS-CoV-2 infection of BMECs has been reported, the detailed mechanism has not been fully elucidated.

Methods: Using the original strain of SARS-CoV-2, the infection in BMECs was confirmed by a detection of intracellular RNA copy number and localization of viral particles. BMEC functions were evaluated by measuring transendothelial electrical resistance (TEER), which evaluates the integrity of tight junction dynamics, and expression levels of proinflammatory genes. BMEC signaling pathway was examined by comprehensive RNA-seq analysis.

Results: We observed that iPSC derived brain microvascular endothelial like cells (iPSC-BMELCs) were infected with SARS-CoV-2. SARS-CoV-2 infection resulted in decreased TEER. In addition, SARS-CoV-2 infection decreased expression levels of tight junction markers CLDN3 and CLDN11. SARS-CoV-2 infection also increased expression levels of proinflammatory genes, which are known to be elevated in patients with COVID-19. Furthermore, RNA-seq analysis revealed that SARS-CoV-2 dysregulated the canonical

Wnt signaling pathway in iPSC-BMELCs. Modulation of the Wnt signaling by CHIR99021 partially inhibited the infection and the subsequent inflammatory responses.

Conclusion: These findings suggest that SARS-CoV-2 infection causes BBB dysfunction via Wnt signaling. Thus, iPSC-BMELCs are a useful *in vitro* model for elucidating COVID-19 neuropathology and drug development.

Keywords: BBB, Brain microvascular endothelial cells, CNS barrier

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Nakayama-Kitamura K, Shigemoto-Mogami Y, Piantino M*1, Naka Y*1, Yamada A*2, Kitano S*2, Furihata T*3, Matsusaki M*1, Sato K: Collagen I Microfiber Promotes Brain Capillary Network Formation in Three-Dimensional Blood-Brain Barrier Microphysiological Systems.

Biomedicines. 2024;12(11):doi;10.3390/biomedicines12112500.

Background: The blood-brain barrier (BBB) strictly regulates the penetration of substances into the brain, which, although important for maintaining brain homeostasis, may delay drug development because of the difficulties in predicting pharmacokinetics/pharmacodynamics (PKPD), toxicokinetics/toxicodynamics (TKTD), toxicity, safety, and efficacy in the central nervous system (CNS). Moreover, BBB functional proteins show species differences; therefore, humanized *in vitro* BBB models are urgently needed to improve the predictability of preclinical studies. Recently, international trends in the 3Rs in animal experiments and the approval of the FDA Modernization Act 2.0 have accelerated the application of microphysiological systems (MPSs) in preclinical studies, and *in vitro* BBB models have become synonymous with BBB-MPSs. Recently, we developed an industrialized humanized BBB-MPS, BBB-NET. In our previous report, we reproduced transferrin receptor (TfR)-mediated transcytosis with high efficiency and robustness, using hydrogels including fibrin and collagen I microfibers (CMFs). Methods: We investigated how adding CMFs to the fibrin gel benefits BBB-NETs. Results: We showed that CMFs accelerate capillary network formation and maturation by promoting astrocyte (AC) survival, and clarified that integrin β1 is involved in the mechanism of CMFs. Conclusions: Our data suggest that the quality control (QC) of CMFs is important for ensuring the stable production of BBB-NETs.

Keywords: blood-brain barrier (BBB), microphysiological system (MPS), collagen I microfiber (CMF)

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Shigemoto-Mogami Y, Nakayama-Kitamura K, Sato K*: The arrangements of the microvasculature and surrounding glial cells are linked to blood-brain barrier formation in the cerebral cortex.

Front Neuroanat. 2024 Aug 7;18:1438190: doi: 10.3389/fnana.2024.1438190.

The blood-brain barrier (BBB) blocks harmful substances from entering the brain and dictates the central nervous system (CNS)-specific pharmacokinetics. Recent studies have shown that perivascular astrocytes and microglia also control BBB functions, however, information about the formation of BBB glial architecture remains scarce. We investigated the time course of the formation of BBB glial architecture in the rat brain cerebral cortex using Evans blue (EB) and tissue fixable biotin (Sulfo-NHS Biotin). The extent of the leakage into the brain parenchyma showed that the BBB was not formed at postnatal Day 4 (P4). The BBB gradually strengthened and reached a plateau at P15. We then investigated the changes in the configurations of blood vessels, astrocytes, and microglia with age by 3D image reconstruction of the immunohistochemical data.

The endfeet of astrocytes covered the blood vessels, and the coverage rate rapidly increased after birth and reached a plateau at P15. Interestingly, microglia were also in contact with the capillaries, and the coverage rate was highest at P15 and stabilized at P30. It was also clarified that the microglial morphology changed from the amoeboid type to the ramified type, while the areas of the respective contact sites became smaller during P4 and P15. These results suggest that the perivascular glial architecture formation of the rat BBB occurs from P4 to P15 because the paracellular transport and the arrangements of perivascular glial cells at P15 are totally the same as those of P30. In addition, the contact style of perivascular microglia dramatically changed during P4-P15.

Keywords: BBB, astrocytes, microglia

Horiuchi S, Koda N, Ikeda Y*, Tanaka Y, Masuo Y*, Kato Y*, Yamazaki D: Examination of common culture medium for human hepatocytes and engineered heart tissue: Towards an evaluation of cardiotoxicity associated with hepatic drug metabolism *in vitro*.

PLoS One. 2024 Dec 23;19(12):e0315997. doi: 10.1371/journal.pone.0315997.

Cardiotoxicity associated with hepatic metabolism and drug-drug interactions is a serious concern. Predicting drug toxicity using animals remains challenging due to species and ethical concerns, necessitating the need to develop alternative approaches. Drug cardiotoxicity associated with hepatic metabolism cannot be detected using a cardiomyocyte-only evaluation system. Therefore, we aimed to establish a system for evaluating cardiotoxicity via hepatic metabolism by co-culturing cryopreserved human hepatocytes (cryoheps) and human iPS cell-derived engineered heart tissues (hiPSC-EHTs) using a stirrer-based microphysiological system. We investigated candidate media to identify a medium that can be used commonly for hepatocytes and cardiomyocytes. We found that the contraction length was significantly greater in the HM Dex (-) medium, the medium used for cryohep culture without dexamethasone, than that in the EHT medium used for hiPSC-EHT culture. Additionally, the beating rate, contraction length, contraction speed, and relaxation speed of hiPSC-EHT cultured in the HM Dex (-) medium were stable throughout the culture period. Among the major CYPs, the expression of CYP3A4 alone was low in cryoheps cultured in the HM Dex (-) medium. However, improved oxygenation using the InnoCell plate increased CYP3A4 expression to levels comparable to those found in the human liver. In addition, CYP3A4 activity was also increased by the improved oxygenation. Furthermore, expression levels of hepatic function-related gene and nuclear receptors in cryoheps cultured in HM Dex (-) medium were comparable to those in the human liver. These results suggest that the HM Dex (-) medium can be applied to co-culture and may allow the evaluation of cardiotoxicity via hepatic metabolism. Moreover, CYP induction by typical inducers was confirmed in cryoheps cultured in the HM Dex (-) medium, suggesting that drug-drug interactions could also be evaluated using this medium. Our findings may facilitate the evaluation of cardiotoxicity via hepatic metabolism, potentially reducing animal testing, lowering costs, and expediting drug development.

Keywords: Microphysiological systems, Coculture, Engineered heart tissues

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Akane H, Toyoda T, Matsushita K, Morikawa T, Kosaka T*, Tajima H*, Aoyama H*, Ogawa K: Comparison of the sensitivity of histopathological and immunohistochemical analyses and blood hormone levels for early detection of antithyroid effects in rats treated with thyroid peroxidase inhibitors.

J Appl Toxicol. 2024;44:1084-1103. doi: 10.1002/jat.4604.

Although measurements of blood triiodothyronine (T3), thyroxine (T4), and thyroid-stimulating hormone (TSH) levels in rodent toxicity studies are useful for detection of antithyroid substances, assays for these measurements are expensive and can show high variability depending on blood sampling conditions. To develop more efficient methods for detecting thyroid disruptors, we compared histopathological and immunohistochemical findings in the thyroid and pituitary glands with blood hormone levels. Six-week-old male and female Sprague-Dawley rats (5 rats/group) were treated with multiple doses of the thyroid

peroxidase inhibitors propylthiouracil (PTU) and methimazole (MMI) by gavage for 28 days. Significant decreases in serum T3 and T4 and increases in TSH were observed in the ≥ 1 mg/kg PTU and ≥ 3 mg/kg MMI groups. An increase in TSH was also detected in male rats in the 0.3 mg/kg PTU group. Histopathological and immunohistochemical analyses revealed that follicular cell hypertrophy and decreased T4 and T3 expression in the thyroid gland were induced at doses lower than doses at which significant changes in serum hormone levels were observed, suggesting that these findings may be more sensitive than blood hormone levels. Significant increases in thyroid weights, Ki67-positive thyroid follicular cell counts, and TSH-positive areas in the pituitary gland were detected at doses comparable to those at which changes in serum T4 and TSH levels were observed, indicating that these parameters may also be useful for evaluation of antithyroid effects. Combining these parameters may be effective for detecting antithyroid substances without relying on hormone measurements.
Keywords: endocrine disruptor, histopathology, thyroid grand

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Matsushita K, Toyoda T, Akane H, Morikawa T, Ogawa K: CD44 expression in renal tubular epithelial cells in the kidneys of rats with cyclosporine-induced chronic kidney disease.
J Toxicol Pathol. 2024;37:55-67. doi: 10.1293/tox.2023-0111

Renal tubular epithelial cell (TEC) injury is the most common cause of drug-induced kidney injury (DIKI). Although TEC regeneration facilitates renal function and structural recovery following DIKI, maladaptive repair of TECs leads to irreversible fibrosis, resulting in chronic kidney disease (CKD). CD44 is specifically expressed in TECs during maladaptive repair in several types of rat CKD models. In this study, we investigated CD44 expression and its role in renal fibrogenesis in a cyclosporine (CyA) rat model of CKD. Seven-week-old male Sprague-Dawley rats fed a low-salt diet were subcutaneously administered CyA (0, 15, or 30 mg/kg) for 28 days. CD44 was expressed in atrophic, dilated, and hypertrophic TECs in the fibrotic lesions of the CyA groups. These TECs were collected by laser microdissection and evaluated by microarray analysis. Gene ontology analysis suggested that these TECs have a mesenchymal phenotype, and pathway analysis identified CD44 as an upstream regulator of fibrosis-related genes, including fibronectin 1 (*Fn1*). Immunohistochemistry revealed that epithelial and mesenchymal markers of TECs of fibrotic lesions were downregulated and upregulated, respectively, and that these TECs were surrounded by a thickened basement membrane. *In situ* hybridization revealed an increase in *Fn1* mRNA in the cytoplasm of TECs of fibrotic lesions, whereas fibronectin protein was localized in the stroma surrounding these tubules. Enzyme-linked immunosorbent assay revealed increased serum CD44 levels in CyA-treated rats. Collectively, these findings suggest that CD44 contributes to renal fibrosis by inducing fibronectin secretion in TECs exhibiting partial epithelial-mesenchymal transition and highlight the potential of CD44 as a biomarker of renal fibrosis.
Keywords: cyclosporine, CD44, kidney

Uneyama M, Toyoda T, Doi Y*, Matsushita K, Akane H, Morikawa T, Ogawa K: A 13-week subchronic toxicity study of linalool oxide in Crl:CD (SD) rats.
J Toxicol Pathol. 2024;37:151-61. doi: 10.1293/tox.2024-0012

Linalool oxide is frequently used as a flavoring agent, however, data on its toxicity is limited. In this study, we performed a 13-week subchronic toxicity study of linalool oxide (furanoid) in male and female Crl:CD(SD) rats. Doses of 0, 80, 250, and 800 mg/kg body weight (bw) per day were orally administered by gavage, using corn oil as the vehicle. Abnormal gait in both sexes and decreased locomotor activity in males were observed in the 800 mg/kg group. Reduced body weight gain was noted in both sexes at 800 mg/kg and at 250 mg/kg in males. In the 800 mg/kg group, serum biochemistry showed increased γ-glutamyl transpeptidase and decreased glucose in both sexes, increased total protein in males, and increased total cholesterol and phospholipids in females, suggesting that linalool oxide may have adverse effects on the liver. Increased relative and/or absolute liver weights, centrilobular hepatocellular hypertrophy in both sexes, and periportal

microvesicular fatty changes in females were observed in the 800 mg/kg group. Increased relative liver weights and decreased serum glucose levels were observed in the 250 mg/kg male and female groups, respectively. Increased serum magnesium levels and relative kidney weights were observed in both sexes in the 800 mg/kg group, suggesting possible adverse effects of linalool oxide. Although histopathology showed accumulation of hyaline droplets in the male kidneys, immunohistochemistry revealed α_{2u}-globulin nephropathy, which was not considered toxicologically significant. These results indicate that the no-observed-adverse-effect level of linalool oxide was 80 mg/kg bw/day for both sexes.

Keywords: flavoring agent, linalool oxide, subchronic toxicity

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Nakamura K, Ishii Y, Takasu S, Namiki M, Soma M, Takimoto N, Matsushita K, Shibutani M*, Ogawa K: Chromosome aberrations cause tumorigenesis through chromosomal rearrangements in a hepatocarcinogenesis rat model.

Cancer Sci. 2024;115:3612-3621. doi: 10.1111/cas.16324

Chromosome aberrations (CAs), a genotoxic potential of carcinogens, are believed to contribute to tumorigenesis by chromosomal rearrangements through micronucleus formation. However, there is no direct evidence that proves the involvement of CAs in tumorigenesis *in vivo*. In the current study, we sought to clarify the involvement of CAs in chemical carcinogenesis using a rat model with a pure CA-inducer hepatocarcinogen, acetamide. Whole-genome analysis indicated that hepatic tumors induced by acetamide treatment for 26-30 weeks showed a broad range of copy number alterations in various chromosomes. In contrast, hepatic tumors induced by a typical mutagen (diethylnitrosamine) followed by a nonmutagen (phenobarbital) did not show such mutational patterns. Additionally, structural alterations such as translocations were observed more frequently in the acetamide-induced tumors. Moreover, most of the acetamide-induced tumors expressed c-Myc and/or MDM2 protein due to the copy number gain of each oncogene. These results suggest the occurrence of chromosomal rearrangements and subsequent oncogene amplification in the acetamide-induced tumors. Taken together, the results indicate that CAs are directly involved in tumorigenesis through chromosomal rearrangements in an acetamide-induced hepatocarcinogenesis rat model.

Keywords: carcinogenesis, chromosomal rearrangement, chromosome aberration

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Hibi D, Soma M, Suzuki Y, Takasu S, Ishii Y, Umemura T: Appearance of sex-determining region Y-box 9 (SOX9)- and glutathione S-transferase placental form (GST-P)-positive hepatocytes as possible carcinogenic events in the early stage of furan-induced hepatocarcinogenesis.

J Appl Toxicol. 2024;44: 1976-85. doi: 10.1002/jat.4691

Furan, the basic skeleton of various flavoring agents, induces cholangiocellular tumors with higher incidences in the caudate lobe and hepatocellular tumors without the lobe specificity in rats, but the mechanism is unclear. We investigated the lobe distribution of possible carcinogenic events. Furan caused proliferation/infiltration of oval and inflammatory cells prominently in the caudate lobe as early as 4 weeks and cholangiofibrosis in this lobe at 8 weeks. *In vivo* mutagenicity assays using DNA extracted from the caudate or left lateral lobe of male *gpt* delta rats, the reporter gene-transgenic rats, treated with 8 mg/kg furan for 4 or 8 weeks showed negative outcomes. The distribution of glutathione S-transferase placental form (GST-P)-positive or sex-determining region Y-box 9 (SOX9)-positive hepatocytes was examined. Significant increases in the number of GST-P-positive hepatocytes were observed in all lobes of furan-treated rats at 8 weeks. By contrast, SOX9-positive hepatocytes, liver injury-inducible progenitor cells, were also found in all lobes of treated rats, the incidences of which were by far the highest in the caudate lobe. In addition, some of these hepatocytes also co-expressed delta like 1 homolog (DLK1), a hepatoblast marker, particularly in areas with a predominant presence of inflammatory cells. Overall, furan induced liver injury, leading to the appearance of SOX9-positive hepatocytes, some of which were subjected to dedifferentiation in the inflammatory microenvironment of a

cholangiocarcinoma-prone lobe. Thus, the appearance of SOX9-positive hepatocytes together with GST-P-positive hepatocytes could be initial events in furan-induced hepatocarcinogenesis via non-genotoxic mechanisms.

Keywords: DLK1, furan, SOX9

Takimoto N, Ishii Y, Mitsumoto T, Takasu S, Namiki M, Toyoda T, Shibutani M*, Ogawa K: Involvement of nuclear atrophy of binucleated hepatocytes in the large micronucleus formation induced by rat hepatocarcinogen acetamide.

Toxicol Appl Pharmacol. 2025;496:117243. doi: 10.1016/j.taap.2025.117243

Acetamide is a hepatocarcinogen in rats. We previously revealed that acetamide induces characteristic large micronuclei in rat liver, suggesting the possible involvement of chromosome aberrations in acetamide-induced hepatocarcinogenesis. To elucidate the mechanism of large micronuclei formation, in this study we examined time-dependent changes in rat hepatocytes after administration of acetamide. Male 6-week-old F344 rats were gavaged with a single-dose administration of acetamide. A liver micronucleus test showed large micronuclei formation 48 and 72 h after acetamide administration. Histopathological analysis showed binucleated hepatocytes with a unilateral atrophic nucleus beginning 6 h after acetamide administration, and the number reached a maximum at 24 h. At 48 h, the number of binucleated hepatocytes with an atrophic nucleus decreased, and apoptotic hepatocytes and large micronucleated hepatocytes appeared. The changes in the frequency of these abnormal binucleated hepatocytes demonstrated a transition from atrophic nuclei to large micronuclei. Immunohistopathological examinations of binucleated hepatocytes showed loss of nuclear lamina, accumulation of barrier-to-autointegration factor (BAF) and chromatin condensation with heterochromatinization at the atrophic site of nuclei. Results of a BrdU-labeling assay were negative. The abnormal expression of BAF in morphologically normal nuclei suggested that nuclear envelope aberration in hepatocytes was an initial event of the nuclear atrophy. In addition, lack of involvement of cell division in the nuclear atrophy and large micronucleus formation was also demonstrated by BrdU-labeling assay. Overall, our data suggest that large micronuclei induced by acetamide are formed in binucleated hepatocytes through nuclear atrophy.

Keywords: acetamide, binucleated hepatocyte, micronucleus

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Arakawa H*1, Higuchi D*1, Takahashi E*2, Matsushita K, Nedachi S*1, Peng H*1, Kadoguchi M*1, Morimura K*2, Araki A*2, Kondo M*2, Ishiguro N*3, Jimbo Y*2, Tamai I*1: Three-dimensional culture of human proximal tubular epithelial cells for an *in vitro* evaluation of drug-induced kidney injury.

J Pharm Sci. 2024;113:3255-64. doi: 10.1016/j.xphs.2024.08.009.

Drug-induced kidney injury (DIKI) is the major cause of acute kidney injury (AKI). Renal proximal tubular epithelial cells (RPTECs) are the primary target sites of DIKI and express transporters involved in renal drug disposition. In the present study, we focused on three-dimensionally cultured human RPTECs (3D-RPTECs) with elevated expression of drug transporters to investigate their utility in DIKI evaluation. Intracellular ATP levels in 3D-RPTECs are reduced by tenofovir and cisplatin that are substrates of an organic anion transporter 1 and an organic cation transporter 2, respectively. In addition, 3D-RPTECs were exposed to 17 and 15 drugs that are positive and negative to RPTEC toxicity, respectively, for up to 28 d. The 20% decreasing concentration of drugs for ATP amount (EC_{20}) was obtained, and the ratio of EC_{20} values and clinical maximum concentration (C_{max}) ≤ 100 were used as cut-off value to evaluate potential of DIKI. The sensitivities of 3D-RPTECs were 82.4% and 88.2% after 7 d and 28 d of drug exposure, respectively, and the specificities were 100% and 93.3%, respectively. The predictive performance of 3D-RPTECs was higher than that of two-dimensional cultured RPTECs and the kidney cell line HK-2. In conclusion, 3D-RPTECs are useful for *in vitro* evaluation of RPTEC injury by measuring intracellular ATP levels.

Keywords: drug-induced kidney injury, *in vitro* assay, spheroid

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Iwasaki K*1, Tojo A*1, Kobayashi H*1, Shimizu K*1, Kamimura Y*2, Horikoshi Y*2, Fukuto A*2, Sun J*2, Yasui M, Honma M, Okabe A*1, Fujiki R*1,3, Nakajima NI*4, Kaneda A*1, Tashiro S*2, Sassa A*1, Ura K*1: Dose-dependent effects of histone methyltransferase NSD2 on site-specific double-strand break repair.

Genes Cells. 2024;29:951-965. doi: 10.1111/gtc.13156.

Histone modifications are catalyzed and recognized by specific proteins to regulate dynamic DNA metabolism processes. NSD2 is a histone H3 lysine 36 (H3K36)-specific methyltransferase that is associated with both various transcription regulators and DNA repair factors. Specifically, it has been implicated in the repair of DNA double-strand breaks (DSBs); however, the role of NSD2 during DSB repair remains enigmatic. Here, we show that NSD2 does not accumulate at DSB sites and that it is not further mobilized by DSB formation. Using three different DSB repair reporter systems, which contained the endonuclease site in the active thymidine kinase gene (*TK*) locus, we demonstrated separate dose-dependent effects of NSD2 on homologous recombination (HR), canonical-non-homologous end joining (c-NHEJ), and non-canonical-NHEJ (non-c-NHEJ). Endogenous NSD2 has a role in repressing non-c-NHEJ, without affecting DSB repair efficiency by HR or total NHEJ. Furthermore, overexpression of NSD2 promotes c-NHEJ repair and suppresses HR repair. Therefore, we propose that NSD2 has functions in chromatin integrity at the active regions during DSB repair.

Keywords: DNA double-strand break repair, homologous recombination, nonhomologous end joining

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Yamada H*, Odagiri M*, Yamakita K*, Chiba A*, Ukai A, Yasui M, Honma M, Sugiyama K, Ura K*, Sassa A*: Dual-directional epi-genotoxicity assay for assessing chemically induced epigenetic effects utilizing the housekeeping *TK* gene.

Sci Rep. 2025;15:7780. doi: 10.1038/s41598-025-92121-6.

Numerous chemicals are associated with carcinogenesis through epigenetic alterations in cells. To detect global epigenetic changes induced by carcinogens, the housekeeping gene can serve as a reporter locus, offering a baseline for identifying shifts in epigenetic marks. To investigate this potential, we developed a simple, cost-effective, and quantitative reporter system to assess chemically induced epigenetic effects, utilizing the thymidine kinase (*TK*) gene mutation assay as a foundation. Using a standard genotoxicity test cell line, human lymphoblast TK6, we edited the CpG promoter loci of the endogenous *TK* gene using the CRISPR/dCas9-SunTag-DNMT3A system. This epi-genotoxicity assay, employing modified mTK6 cells, provides a simple method for quantifying chemically induced epigenetic effects. The assay successfully detects both increased TK reversion rates induced by DNMT inhibitors, such as 5-Aza-2′-deoxycytidine and GSK-3484862, and, for the first time, a significant reduction in *TK* revertant frequency caused by the non-genotoxic carcinogen 12-O-tetradecanoylphorbol-13-acetate (TPA). Chromatin immunoprecipitation and western blotting analyses revealed that TPA treatment led to a global decrease in H3K27Ac levels, likely driven by TPA-mediated inflammation. These results demonstrate the utility of the epi-genotoxicity assay as a valuable tool for evaluating dual-directional epigenetic changes triggered by chemical exposure.

Keywords: epi-genotoxicity, reporter assay, *TK* gene

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Nakano T*1, Akamatsu K*1, Kohzaki M*2, Tsuda M, Hirayama R*3, Sassa A*4, Yasui M, Shoulkamy MI*6, Hiromoto T*4,7, Tamada T*4,7, Ide H*8, Shikazono N*1: Deciphering repair pathways of clustered DNA damage in human TK6 cells: insights from atomic force microscopy direct visualization.

Nucleic Acids Res. 2025;53:gkae1077. doi: 10.1093/nar/gkae1077.

Ionizing radiation induces various types of DNA damage, and the reparability and lethal effects of DNA damage differ depending on its spatial density. Elucidating the structure of radiation-induced clustered DNA damage and its repair processes will enhance

our understanding of the lethal impact of ionizing radiation and advance progress toward precise therapeutics. Previously, we developed a method to directly visualize DNA damage using atomic force microscopy (AFM) and classified clustered DNA damage into simple base damage clusters (BDCs), complex BDCs and complex double-strand breaks (DSBs). This study investigated the repair of each type of damage in DNA-repair-deficient human TK6 cells and elucidated the association between each type of clustered DNA damage and the pathway responsible for its repair postirradiation with low linear energy transfer (LET) radiation (X-rays) and high-LET radiation (Fe-ion beams) in cells. We found that base excision repair and, surprisingly, nucleotide excision repair restored simple and complex BDCs. In addition, the number of complex DSBs in wild-type cells increases 1 h postirradiation, which was most likely caused by BDC cleavage initiated with DNA glycosylases. Furthermore, complex DSBs, which are likely associated with lethality, are repaired by homologous recombination with little contribution from nonhomologous-end joining.

Keywords: atomic force microscopy, clustered DNA damage, TK6 cell

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Muto S*1, Furuhama A, Yamamoto M*2, Otagiri Y*3, Koyama N*4, Hitaoka S*4, Nagato Y*5, Ouchi H*6, Ogawa M*7, Shikano K*7, Yamada K*8, Ono S*8, Hoki M*9, Ishizuka F*10, Hagio S*11, Takeshita C*12, Omori H*13, Hashimoto K*14, Chikura S*15, Honma M, Sugiyama K, Mishima M: Local QSAR based on quantum chemistry calculations for the stability of nitrenium ions to reduce false positive outcomes from standard QSAR systems for the mutagenicity of primary aromatic amines.

Genes Environ. 2024;46:24. doi: 10.1186/s41021-024-00318-4.

Background: Primary aromatic amines (PAAs) present significant challenges in the prediction of mutagenicity using current standard quantitative structure activity relationship (QSAR) systems, which are knowledge-based and statistics-based, because of their low positive prediction values (PPVs). Previous studies have suggested that PAAs are metabolized into genotoxic nitrenium ions. Moreover, ddE, a relative-energy based index derived from quantum chemistry calculations that measures the stability nitrenium ions, has been correlated with mutagenicity. This study aims to further examine the ability of the ddE-based approach in improving QSAR mutagenicity predictions for PAAs and to develop a refined method to decrease false positive predictions.

Results: Information on 1,177 PAAs was collected, of which 420 were from public databases and 757 were from in-house databases across 16 laboratories. The total dataset included 465 Ames test-positive and 712 test-negative chemicals. For internal PAAs, detailed Ames test data were scrutinized and final decisions were made using common evaluation criteria. In this study, ddE calculations were performed using a convenient and consistent protocol. An optimal ddE cutoff value of -5 kcal/mol, combined with a molecular weight ≤ 500 and ortho substitution groups yielded well-balanced prediction scores: sensitivity of 72.0%, specificity of 75.9%, PPV of 65.6%, negative predictive value of 80.9% and a balanced accuracy of 74.0%. The PPV of the ddE-based approach was greatly reduced by the presence of two ortho substituent groups of ethyl or larger, as because almost all of them were negative in the Ames test regardless of their ddE values, probably due to steric hindrance affecting interactions between the PAA and metabolic enzymes. The great majority of the PAAs whose molecular weights were greater than 500 were also negative in Ames test, despite ddE predictions indicating positive mutagenicity.

Conclusions: This study proposes a refined approach to enhance the accuracy of QSAR mutagenicity predictions for PAAs by minimizing false positives. This integrative approach incorporating molecular weight, ortho substitution patterns, and ddE values, substantially can provide a more reliable basis for

evaluating the genotoxic potential of PAAs.

Keywords: nitrenium ion, primary aromatic amine, QSAR

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Furuhama A, Sugiyama K, Honma M: Ames mutagenicity of 15 aryl, benzyl, and aliphatic ring *N*-nitrosamines.

Regul Toxicol Pharmacol. 2025;156:105763. doi: 10.1016/j.yrtph.2024.105763.

The Ames mutagenicity test is an effective means of screening compounds for their carcinogenic potential. Here, we conducted Ames tests on 15 aryl, benzyl, and aliphatic ring *N*-nitrosamines. Then, by using two indicators of mutagenicity strength calculated from the Ames test results, namely, maximum specific activity (MSA; number of revertant colonies) and maximum fold increase (MFI; relative ratio of increased colonies), we examined the relationship between Ames mutagenicity strength and Carcinogenic Potency Categorization Approach (CPCA) potency category, which is a structure-activity-relationship-based prediction of the carcinogenic potency of nitrosamines. Eleven of the test compounds were Ames positive and four were negative. Of the 11 positive compounds, three were categorized as strong positive (MSA ≥ 1000), five as medium positive (100 ≤ MSA <1000), and three as weak positive (MSA <100). The compounds with an aliphatic ring showed a negative relationship between mutagenicity strength (i.e., MSA or MFI) and carcinogenic potential (i.e., CPCA category), whereas, the alpha-methyl aryl *N*-nitrosamines did not. Overall, MSA and MFI were found to be detailed indicators of the carcinogenic potency of the *N*-nitrosamines and can potentially be used to support CPCA categorization.

Keywords: Ames mutagenicity, *N*-nitrosamine, maximum specific activity

Furihata C*, Suzuki T: Four functional genotoxic marker genes (*Bax*, *Btg2*, *Ccng1*, and *Cdkn1a*) discriminate genotoxic hepatocarcinogens from non-genotoxic hepatocarcinogens and non-genotoxic non-hepatocarcinogens in rat public toxicogenomics data, Open TG-GATEs.

Genes Environ. 2024;46:28. doi: 10.1186/s41021-024-00322-8.

Background: Previously, Japanese Environmental Mutagen and Genome Society/Mammalian Mutagenicity Study Group/Toxicogenomics Study Group (JEMS/MMS toxicogenomic study group) proposed 12 genotoxic marker genes (*Aen*, *Bax*, *Btg2*, *Ccnf*, *Ccng1*, *Cdkn1a*, *Gdf15*, *Lrp1*, *Mbd1*, *Phlda3*, *Plk2*, and *Tubb4b*) to discriminate genotoxic hepatocarcinogens (GTHCs) from non-genotoxic hepatocarcinogens (NGTHCs) and non-genotoxic non-hepatocarcinogens (NGTNHCs) in mouse and rat liver using qPCR and RNA-Seq and confirmed in public rat toxicogenomics data, Open TG-GATEs, by principal component analysis (PCA). On the other hand, the U.S. Environmental Protection Agency (US EPA) suggested seven genotoxic marker genes (*Bax*, *Btg2*, *Ccng1*, *Cgrrf1*, *Cdkn1a*, *Mgmt*, and *Tmem47*) with Open TG-GATEs data. Four genes (*Bax*, *Btg2*, *Ccng1*, and *Cdkn1a*) were common in these two studies. In the present study, we examined the performance of these four genes in Open TG-GATEs data using PCA.

Results: The study's findings are of paramount significance, as these four genes proved to be highly effective in distinguishing five typical GTHCs (2-acetylaminofluorene, aflatoxin B1, 2-nitrofluorene, *N*-nitrosodiethylamine and *N*-nitrosomorpholine) from seven typical NGTHCs (clofibrate, ethanol, fenofibrate, gemfibrozil, hexachlorobenzene, phenobarbital, and WY-14643) and 11 NGTNHCs (allyl alcohol, aspirin, caffeine, chlorpheniramine, chlorpropamide, dexamethasone, diazepam, indomethacin, phenylbutazone, theophylline, and tolbutamide) by PCA at 24 h after a single administration with 100%

accuracy. These four genes also effectively distinguished two typical GTHCs (2-acetylaminofluorene and *N*-nitrosodiethylamine) from seven NGTHCs and ten NGTNHCs by PCA on 29 days after 28 days-repeated administrations, with a similar or even better performance compared to the previous 12 genes. Furthermore, the study's analysis revealed that the three intermediate GTHC/NGTHCs (methapyrilene, monocrotaline, and thioacetamide, which were negative in the *Salmonella* test but positive in the *in vivo* rat liver test) were located in the intermediate region between typical GTHCs and typical NGTHCs by PCA.

Conclusions: The present results unequivocally demonstrate the availability of four genotoxic marker genes ((*Bax*, *Btg2*, *Ccng1*, and *Cdkn1a*) and PCA in discriminating GTHCs from NGTHCs and NGTNHCs in Open TG-GATEs. These findings strongly support our recommendation that future rat liver *in vivo* toxicogenomics tests prioritize these four genotoxic marker genes, as they have proven to be highly effective in discriminating between different types of hepatocarcinogens.

Keywords: genotoxic marker gene, open TG-GATE, toxicogenomics

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Hosoi S*, Hirose T*, Matsumura S*, Otsubo Y*, Saito K*, Miyazawa M*, Suzuki T, Masumura K, Sugiyama K: Effect of sequencing platforms on the sensitivity of chemical mutation detection using Hawk-Seq™.

Genes Environ. 2024;46:20. doi: 10.1186/s41021-024-00313-9.

Background: Error-corrected next-generation sequencing (ecNGS) technologies have enabled the direct evaluation of genome-wide mutations after exposure to mutagens. Previously, we reported an ecNGS methodology, Hawk-Seq™, and demonstrated its utility in evaluating mutagenicity. The evaluation of technical transferability is essential to further evaluate the reliability of ecNGS-based assays. However, cutting-edge sequencing platforms are continually evolving, which can affect the sensitivity of ecNGS. Therefore, the effect of differences in sequencing instruments on mutation data quality should be evaluated.

Results: We assessed the performance of four sequencing platforms (HiSeq2500, NovaSeq6000, NextSeq2000, and DNBSEQ-G400) with the Hawk-Seq™ protocol for mutagenicity evaluation using DNA samples from mouse bone marrow exposed to benzo[*a*]pyrene (BP). The overall mutation (OM) frequencies per 10^6 bp in vehicle-treated samples were 0.22, 0.36, 0.46, and 0.26 for HiSeq2500, NovaSeq6000, NextSeq2000, and DNBSEQ-G400, respectively. The OM frequency of NextSeq2000 was significantly higher than that of HiSeq2500, suggesting the difference to be based on the platform. The relatively higher value in NextSeq2000 was a consequence of the G:C to C:G mutations in NextSeq2000 data (0.67 per 10^6 G:C bp), which was higher than the mean of the four platforms by a ca. of 0.25 per 10^6 G:C bp. A clear dose-dependent increase in G:C to T:A mutation frequencies was observed in all four sequencing platforms after BP exposure. The cosine similarity values of the 96-dimensional trinucleotide mutation patterns between HiSeq and the three other platforms were 0.93, 0.95, and 0.92 for NovaSeq, NextSeq, and DNBSeq, respectively. These results suggest that all platforms can provide equivalent data that reflect the characteristics of the mutagens.

Conclusions: All platforms sensitively detected mutagen-induced mutations using the Hawk-Seq™ analysis. The substitution types and frequencies of the background errors differed depending on the platform. The effects of sequencing platforms on mutagenicity evaluation should be assessed before experimentation.

Keywords: error-corrected sequencing, mutational signature, next generation sequencing

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Shimizu N*1, Izawa K, Washif M*2, Morozumi R, Hirota K*2, Tsuda M: Role of TDP2 in the repair of DNA damage induced by the radiomimetic drug Bleomycin.

Genes Environ. 2025;47:1. doi: 10.1186/s41021-025-00329-9.

Background: Bleomycin (Bleo) is a glycopeptide with potent antitumor activity that induces DNA double-strand breaks (DSBs) through free radical generation, similar to ionizing radiation (IR).

Therefore, Bleo is considered a radiomimetic drug. However, differences in DNA repair mechanisms between IR- and Bleo-induced DNA damage have not been fully elucidated. Therefore, in the present study, we examined a panel of repair-deficient human TK6 cell lines to elucidate the relative contributions of individual repair factors.

Results: Our comprehensive profiling indicated that both non-homologous end joining (NHEJ) and homologous recombination (HR) contributed to DSB repair induced by X-rays and Bleo. Furthermore, tyrosyl-DNA phosphodiesterase (TDP)-related repair was a significant factor for cellular sensitivity to Bleo treatment. *TDP1*$^{-/-}$/*TDP2*$^{-/-}$ cells exhibited greater sensitivity to Bleo than *TDP1*$^{-/-}$ or *TDP2*$^{-/-}$ cells, but not to X-rays. In addition, we determined whether TDP2 is involved in the repair of Bleo-induced DSBs using a neutral comet assay. In TDP1-deficient cells, knockout of *TDP2* resulted in a significant delay in the repair kinetics of DSBs induced by Bleo, but not by X-rays.

Conclusions: The contribution of the TDP-related pathway to DSB repair significantly differed between IR and radiomimetic drugs. The discovery of this novel TDP2-dependent repair of DSBs resulting from radiomimetic drug exposure indicates that TDP1 and TDP2 inhibition in combination with radiomimetic drugs represents a strategy for cancer treatment.

Keywords: DNA double-strand break, radiomimetic drug, tyrosyl-DNA phosphodiesterase

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Nishida A*, Sawada Y*, Arai R*, Ishibashi N*, Suzuo M*, Ohno A, Ashikaga T, Iijima K*: Evaluation of the immunotoxicity potential of nanomaterials using THP-1 cells.

Front Toxicol. 2024;6:1293147. doi: 10.3389/ftox.2024.1293147.

With the expansion of nanomaterials (NMs) usage, concerns about their toxicity are increasing, and the wide variety of NMs makes it difficult to assess their toxicity. Therefore, the development of a high-throughput, accurate, and certified method to evaluate the immunotoxicity of NMs is required. In this study, we assessed the immunotoxicity potential of various NMs, such as nanoparticles of silver, silica, and titanium dioxide, using the human Cell Line Activation Test (h-CLAT) at the cellular level. After exposure to silver nanoparticle dispersions, the expression levels of CD86 and CD54 increased, suggesting the activation of antigen-presenting cells (APCs) by silver nanoparticles. Quantification of silver ions eluted from silver nanoparticles and the activation of APCs by silver ions suggested that it was due to the release of silver ions. Silica nanoparticles also increased the expression of CD86 and/or CD54, and their activation ability correlated with the synthesis methods and hydrodynamic diameters. The ability of titanium dioxide to activate APCs differed depending on the crystal type and hydrodynamic diameter. These results suggest a potential method to evaluate the immunotoxicity potential of various NMs based on their ability to activate APCs using human monocytic THP-1 cells. This method will be valuable in assessing the immunotoxicity potential and elucidating the immunotoxic mechanisms of NMs.

Keywords: nanomaterial, immunotoxicity, THP-1 cell

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Ashikaga T, Hatano K*1, Iwasa H*1, Kinoshita K*2, Nakamura N*1, Ambe K*2, Tohkin M*2: Next Generation Risk Assessment Case Study: A Skin Sensitization Quantitative Risk Assessment for Bandrowski's Base Existing in Hair Color Formulations.

J Jp Cosmetic Sci Soc. 2024;48:73-77. doi: 10.11469/koshohin.48.73.

Bandrowski's base (BB) is produced from p-phenylenediamine (pPD) in hair dye products during application and is known to have extreme skin-sensitizing potency. We aimed to conduct a quantitative skin sensitization risk assessment using both the predicted EC3 value and the percutaneous absorption rate of BB generated from a machine learning model. We purchased 22 domestically available hair dye products containing pPD and measured the amount of BB produced under simulated product usage conditions. The consumer exposure level (CEL) to BB was estimated using the following parameters: amount of hair dye product applied (100 mL), measured concentration of BB, retention

factor (10%), estimated dermal percutaneous rate of BB, and scalp surface area (551 cm^2). The acceptable exposure level (AEL) of BB was determined by converting the local lymph node assay (LLNA) EC3 value of BB to the no expected sensitization induction level (NESIL) value and using a sensitization assessment factor (SAF) of 30, which is commonly set for hair color products. We then used the predicted value of our independently developed machine learning prediction model. Finally, the AEL value was divided by the CEL value for each product to calculate the margin of safety (MOS). The amount of BB generated differed for each product and ranged widely, from below the limit of detection to 38.1 ppm. We calculated the MOS for each product, which was 1 or higher for all products and conditions, similar to the actual measured values of the LLNA EC3. These findings suggest that the use of hair dye products that could generate BB under the evaluated exposure scenarios would unlikely induce skin sensitization. For the practical use of next generation risk assessment for skin sensitization, in addition to comparisons with conventional methods using animals, further verification is necessary, including examination of the validity of tentative set values, test methods, and case studies with other skin sensitizers.

Keywords: skin sensitization, Bandrowski's base, next generation risk assessment

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Kasahara T[*1], Yamamoto Y[*1], Nakashima N[*1], Imamura M[*1], Mizumachi H[*2], Suzuki S[*2], Aiba S[*3], Kimura Y[*3], Ashikaga T, Kojima H[*4], Ono A[*5], Matsumoto K[*6]: Borderline Range Determined Using Data From Validation Study of Alternative Methods for Skin Sensitization: ADRA, IL-8 Luc Assay, and EpiSensA.

J. Applied Toxicol. 2025;45:432-439. doi: 10.1002/jat.4712

Most predictive models that use alternatives to animal experiments divide judgements into two classes with a cutoff value for each model. However, if the results of alternative methods are close to the cutoff values, the true result may be ambiguous because of variability in the data. Therefore, the OECD GL497 uses a judgement method that establishes a borderline range (BR) around a cutoff value using a statistical method. However, because there is no detailed description of how the BR is calculated, we clarified two specific points. The scale-constant correction method was used to calculate the median absolute deviation (MAD) around the median. In addition, the bottom-raised transformation method was used when the data were "0" because calculation of the BR requires that all data are logarithmic. Indeed, the BRs for the amino acid derivative reactivity assay (ADRA), interleukin-8 reporter gene assay (IL-8 Luc), and epidermal sensitization assay (EpiSensA) were calculated using data from each validation study. The results showed that the BR for ADRA and IL-8 Luc ranged from 4.1 to 5.9 and 1.25 to 1.57, respectively. Furthermore, the BRs of four genes (ATF3, GCLM, DNAJB4, and IL-8) evaluated using EpiSensA ranged from 10.71 to 21.02, 1.64 to 2.45, 1.61 to 2.52, and 3.11 to 5.16, respectively. The difference (deviation) between the lower and upper BR limits and cutoff value for each alternative method were comparable to those of the alternative methods listed in the guidelines (DPRA, KerarinoSens, and h-CLAT) and thus were considered as adequate.

Keywords: ADRA (amino acid derivative reactivity assay), BR (borderline range), EpiSensA (epidermal sensitization assay)

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Tanabe S, Quader S[*1], Cabral H[*2], Perkins EJ[*3], Yokozaki H[*4], Sasaki H[*5]: Master regulators of causal networks in intestinal- and diffuse-type gastric cancer and the relation to the RNA virus infection pathway.

Int J Mol Sci. 2024;25(16):8821. doi: 10.3390/ijms25168821

Causal networks are important for understanding disease signaling alterations. To reveal the network pathways affected in the epithelial-mesenchymal transition (EMT) and cancer stem cells (CSCs), which

are related to the poor prognosis of cancer, the molecular networks and gene expression in diffuse- and intestinal-type gastric cancer (GC) were analyzed. The network pathways in GC were analyzed using Ingenuity Pathway Analysis (IPA). The analysis of the probe sets in which the gene expression had significant differences between diffuse- and intestinal-type GC in RNA sequencing of the publicly available data identified 1099 causal networks in diffuse- and intestinal-type GC. Master regulators of the causal networks included lenvatinib, pyrotinib, histone deacetylase 1 (HDAC1), mir-196, and erb-b2 receptor tyrosine kinase 2 (ERBB2). The analysis of the HDAC1-interacting network identified the involvement of EMT regulation via the growth factors pathway, the coronavirus pathogenesis pathway, and vorinostat. The network had RNA-RNA interactions with microRNAs such as mir-10, mir-15, mir-17, mir-19, mir-21, mir-223, mir-25, mir-27, mir-29, and mir-34. The molecular networks revealed in the study may lead to identifying drug targets for GC.

Keywords: causal network, epithelial-mesenchymal transition (EMT), gastric cancer (GC)

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Nymark P*1, Clerbaux L-A*2,3, Amorim M-J*4,5, Andronis C*6, de Bernardi F*7, Bezemer GFG*8,9, Coecke S*3, Gavins FNE*10, Jacobson D*11, Lekka E*6, Margiotta-Casaluci L*12, Martens M*13, Mayasich SA*14, Mortensen HM*15, Kim YJ*16, Sachana M*17, Tanabe S, Virvilis V*6, Edwards SW*18, Halappanavar S*19,20: Building an Adverse Outcome Pathway network for COVID-19.
Front Syst Biol. 2024;4:1384481. doi: 10.3389/fsysb.2024.1384481

The COVID-19 pandemic generated large amounts of data on the disease pathogenesis leading to a need for organizing the vast knowledge in a succinct manner. Between April 2020 and February 2023, the CIAO consortium exploited the Adverse Outcome Pathway (AOP) framework to comprehensively gather and systematically organize published scientific literature on COVID-19 pathology. The project considered 24 pathways relevant for COVID-19 by identifying essential key events (KEs) leading to 19 adverse outcomes observed in patients. While an individual AOP defines causally linked perturbed KEs towards an outcome, building an AOP network visually reflect the interrelatedness of the various pathways and outcomes. In this study, 17 of those COVID-19 AOPs were selected based on quality criteria to computationally derive an AOP network. This primary network highlighted the need to consider tissue specificity and helped to identify missing or redundant elements which were then manually implemented in the final network. Such a network enabled visualization of the complex interactions of the KEs leading to the various outcomes of the multifaceted COVID-19 and confirmed the central role of the inflammatory response in the disease. In addition, this study disclosed the importance of terminology harmonization and of tissue/organ specificity for network building. Furthermore the unequal completeness and quality of information contained in the AOPs highlighted the need for tighter implementation of the FAIR principles to improve AOP findability, accessibility, interoperability and re-usability. Finally, the study underlined that describing KEs specific to SARS-CoV-2 replication and discriminating physiological from pathological inflammation is necessary but requires adaptations to the framework. Hence, based on the challenges encountered, we proposed recommendations relevant for ongoing and future AOP-aligned consortia aiming to build computationally biologically meaningful AOP networks in the context of, but not limited to, viral diseases.

Keywords: COVID-19, adverse outcome pathway (AOP), key event (KE)

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Kawashima A, Inoue K: Re-evaluation of the reduced heart weights in male rats in a 28-day oral repeated-dose toxicity study of tetramethylammonium hydroxide.
Regul Toxicol Pharmacol. 2024;153:105712. doi: 10.1016/j.yrtph.2024.105712

We recently conducted a detailed hazard assessment of tetramethylammonium hydroxide (TMAH), a priority chemical substance under the Japan Chemical Substances Control Law. During this assessment, there was debate regarding the reduced heart weight observed in the treated male groups in the 28-day rat oral repeated-dose toxicity study. This finding was not observed in females in this study and in both sexes of oral toxicity studies for tetramethylammonium chloride (TMAC) or tetramethylammonium hydrogen phthalate (TMAHP). Unpublished individual data from the oral TMAH developmental and reproductive toxicity (DART) screening study were also obtained; no effect on heart weight was observed. In addition, background data on rat heart weight from six 28-day oral toxicity studies conducted in the same facility, year, strain, age, and breeder as the TMAH study were obtained from the Japan Existing Chemical Substances Database (JECDB). These investigations suggest that the statistically significant lower heart weight in the treated males in the 28-day toxicity study is likely caused by an incidental skewing of individuals with heavier heart weights toward control male groups and is not due to TMAH treatment. Thus, it is worthwhile to include as much relevant data as possible to confirm or refute unexpected findings in toxicity studies.
Keywords: tetramethylammonium hydroxide (TMAH, CAS No. 75-59-2), oral toxicity study, heart weight

Kawashima A, Inoue K, Ushida K, Kai K, Yoshida-Yamashita LS, Masumura K: Derivation of human health hazard assessment values for tetramethylammonium hydroxide (TMAH) under the Japan Chemical Substances Control Law.
Fundam Toxicol Sci. 2024;11:267-278. doi: 10.2131/fts.11.267

Tetramethylammonium hydroxide (TMAH) and substances that release tetramethylammonium (TMA) are classified as Priority Assessment Chemical Substances (PACSs) under registration number 17 of the Japan Chemical Substances Control Law (CSCL 1973). This classification requires a through human health hazard assessment and derivation of Hazard Assessment Values (HAVs) for the oral and inhalation exposure at the Assessment II stage. We analyzed their general, developmental, reproductive toxicity, genotoxicity, and carcinogenicity using hazard data from both domestic and international risk assessment agencies and subsequently proposed an HAV. For oral exposure, a no observed adverse effect level (NOAEL) of 1 mg/kg/day, based on transient or lasting salivation in parent rats from a TMAH developmental and

reproductive toxicology (DART) screening study, was chosen as the point of departure (POD). The POD was then divided by uncertainty factors (UFs) totaling 1,000 (interspecies variation: 10; intraspecific variation: 10; short duration: 10), resulting in an oral HAV of 0.001 mg/kg/day for TMAH. Due to a lack of hazard data for humans and animals via inhalation, an HAV for the inhalation route was not established.

Keywords: tetramethylammonium hydroxide (TMAH, CAS No. 75-59-2), Chemical Substances Control Law (CSCL), Assessment II for human health effects

Yamamoto S, Yoshida K, Matsumoto M, Yamada T: Construction and evaluation of an open-source database for inhalation-based physiologically based kinetic modeling of selected categories for industrial chemicals.

J Toxicol Sci. 2025;50:57-68. doi: 10.2131/jts.50.57

A physiologically based kinetic (PBK) model is used for predicting chemical concentrations of toxicological concern in target tissues. Such models are important for understanding toxicokinetics. However, it is challenging to obtain chemical-specific empirical parameter values used for PBK modeling. Thus, developing methods predicting these values is necessary. Herein, we researched PBK models of inhalation exposure to industrial chemicals and developed a database of parameters of approximately 200 chemicals in humans and rodents. Next, the chemicals in the database were classified into three categories (I, IIA, and IIB) based on the intermolecular interactions for humans and rats. Quantitative relationships between blood/air and tissue/blood partition coefficients and physicochemical parameters were derived for the chemicals in each category. Regression analyses of blood/air and fat/blood partition coefficients against Henry's law constant and log D at pH 7.4 for chemicals in category IIA for humans, in which van der Waals and dipole-dipole interactions were involved, yielded 0.88 and 0.54 coefficients of determination, respectively. Moreover, these methods worked for other categories and species. The metabolic parameters maximal velocity (*V*max) and Michaelis-Menten constant (*K*m) of the chemicals that are primarily metabolized by cytochrome P450 were calculated for humans and rats. Multiple regression analyses of logs *V*max and *K*m against the occurrence frequency of molecular fragments showed good correlations, respectively. The aforementioned models predicted values close to the reported values for test chemicals within the applicability domains. Our approach could also be applied to other chemicals within the domains that are not included in the database.

Keywords: database, physiologically based kinetic (PBK) modeling, *in silico* prediction

Ninomiya Y[*1], Watanabe H[*1,2], Yamagishi T[*1,2], Maruyama-Komoda T, Yamada T, Yamamoto H[*1,2]: Prediction of chronic toxicity of pharmaceuticals in *Daphnia magna* by combining ortholog prediction, pharmacological effects, and quantitative structure-activity relationship.

Ecotoxicol Environ Saf. 2024;282:116737. doi: 10.1016/j.ecoenv.2024.116737

To develop a method for predicting chronic toxicity of pharmaceuticals in *Daphnia*, we investigated the feasibility of combining the presence of drug-target orthologs in *Daphnia magna*, classification based on pharmacological effects, and ecotoxicity quantitative structure-activity relationship (QSAR) prediction. We established datasets on the chronic toxicity of pharmaceuticals in *Daphnia*, including information on therapeutic categories, target proteins, and the presence or absence of drug-target orthologs in *D. magna*, using literature and databases. Chronic toxicity was predicted using ecotoxicity prediction QSAR (Ecological Structure Activity Relationship and Kashinhou Tool for Ecotoxicity), and the differences between the predicted and measured values and the presence or absence of drug-target orthologs were examined. For pharmaceuticals without drug-target orthologs in *D. magna* or without expected specific actions, the ecotoxicity prediction QSAR analysis yielded acceptable predictions of the chronic toxicity of pharmaceuticals. In addition, a workflow model to assess the chronic toxicity of pharmaceuticals in *Daphnia* was proposed based on these evaluations and verified using an additional dataset. The addition of biological aspects such as drug-target orthologs and pharmacological effects would support the use of QSARs for predicting the chronic toxicity of pharmaceuticals in *Daphnia*.

Keywords: chronic toxicity in *Daphnia*, ecotoxicological

QSAR, drug-target orthologs

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