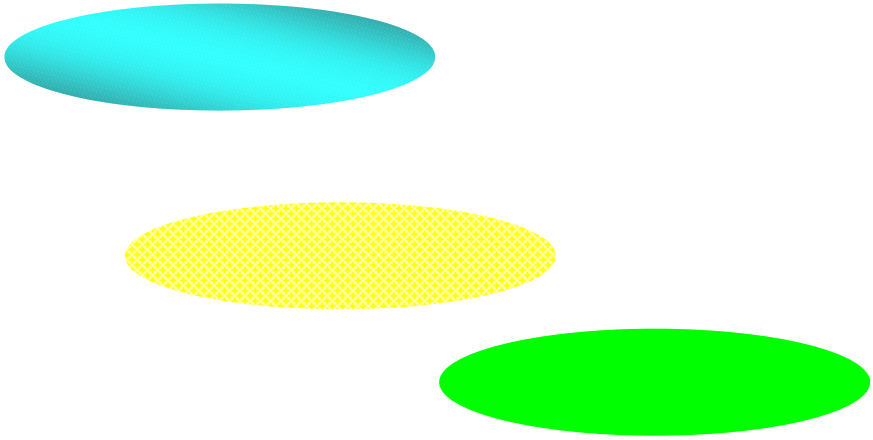
Guideline for Bioequivalence Studies of Generic Products for Topical Use

July 7, 2003

局所皮膚適用製剤の後発医薬品のための生物学的同等性試験ガイドライン



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Chapter 1. Introduction

This guideline states the principle of bioequivalence test for generic products for topical uses, which is simply described in the Guideline for Bioequivalence Studies of Generic products published on December 22 in 1997.

Chapter 2. Terminology

Terms used in the guideline are defined as follows:

- Bioavailability: The rate and extent of absorption of parent drugs or active metabolites from a dosage form into the active site.
- Bioequivalent products: Drug products having the same bioavailabilities.
- Therapeutically equivalent products: Drug products having the same therapeutic efficacies.
- Innovator products: Products being approved as new drugs by clinical trials or relating drug products.
- Generic products: Products whose active ingredients, strengths, dosage forms and regimen are the same as those of innovator's products. Generic and innovator products should be the same in the application area of dosage forms in case of sheet-like products (e.g, tape or cataplasm) and they should be identical in the drug content per weight in case of liquid or semisolid products. Their physiochemical characteristics should be similar to those of innovator products. The similarity of physiochemical characteristics will be judged based on W/O or O/W, oily or aqueous bases, and the physiochemical condition of the drug in the base, that is, suspension, emulsion or solution.

  Ointment, cream, gel, cataplasm, tapes, lotion, spray, powder, liniment are considered as different dosage forms which are out of bioequivalence tests for generic drugs.
- Bioequivalence range: The acceptable range of bioequivalence is generally 0.8 - 1.25 for the test / reference ratio of average values, when the parameters are logarithmically transformed. The acceptable range is generally -0.2 - +0.2 for the relative difference in vivo parameters between reference and test products, when the raw data are used.

Chapter 3. Test

Ⅰ. Reference and test products

In vitro release tests should be performed using 6 units or more for three lots of the innovator product at 32 °C, by appropriate methods such as USP paddle-over disk method or diffusion cell which can estimate their drug release. Aqueous test fluids, aqueous alcohol solutions or other alternative solutions may be used as a test fluid. When release tests are carried out using membranes to separate test fluids and dosage forms, the permeation of the membrane should not be the rate-determining step for the drug release. Among the three lots, the one which shows

intermediate release should be selected as the reference product.
As the test product of generic drugs, a industrial scale-lot should be used. However, a lot of 1/10 or larger of industrial scale can also be used as the test product which should be the same as the production lots in manufacturing method, quality and bioavailability. The drug content or potency of the reference product should be close to the label claim, and the difference in drug content or potency between test and reference products should be less than 5%.

Ⅱ. Bioequivalence test

This section describes representative tests to assess bioequivalence of topical drug products, 1. Dermatopharmacokinetic test, 2. Pharmacological test, 3. Test for measuring the unabsorbed amount, 4. Pharmacokinetic test, 5. Clinical trial, 6. In vitro efficacy test, 7. Animal test. From among them, the most suitable test should be selected by considering the characteristics of topical products. If other alternative appropriate tests are available, they may be employed.
The testing procedure, analytical method, stability of drugs during storage and analysis, etc. should be validated. General remarks in human tests are shown below.

a) Subjects having normal skins should be employed except for clinical tests. The normal skins mean:
   - no eczema, dermatitis and dyschromatosis
   - no injury and scar
   - no inflammatory sunburn
   - no atopic dermatitis and anamnesis
   - no drug hypersensitivity
   - no abnormality at the skin sites where drugs are applied.
b) Suitable site of the skin such as back, thorax, forearm should be chosen for each topical drug product.
c) Avoid the physical damage of skin from irritations of surfactants and other chemicals. Keep the skin under normal conditions. Usually, 2 hours will be required for the skin to return the ordinary state when it was cleaned with surfactants.
d) Occlusive guards should not be applied to topical drug products except for the case in which the use is allowed in the dosage regimen. Nonocclusive guards may be used if necessary.
e) The duration of product application should be determined based on the pilot tests, since it differs depending on products and the test method selected.
f) Sample sizes should be determined by considering the residual variability. If a large variation is anticipated, it is recommended to obtain the average data by applying the product at multiple skin sites. Followings should be investigated to estimate the variability of the test.
   - Reproducibility between and within operators of the skin stripping and visual assay of skin blanching caused by a vasoconstrictor effect
   - Variability of in vivo measures between subjects and between skin sites of each subject
   - Variability of the extraction of drugs and assay.

g) The combination of comparison (test product vs, reference product) should be randomly assigned at the sites of skin in order to exclude biased data.
h) The test should be performed under a fixed dosing condition and constant environment, because the drug availability will be influenced by the circadian rhythm and environmental condition.
i) Detailed standard operational procedure (SOP) should be prepared for each test, involving the application and removal of products, recovery of the sample, measurements of pharmacological response, skin stripping procedures and analytical method, because the testing procedures for topical products are generally complicated.

1. Dermatopharmacokinetic test

The test is to assess bioequivalence by comparing the amounts absorbed in the stratum corneum between test and reference products. Topical drugs are generally distributed into the stratum corneum and reach the epidermal cells. Thus, the bioavailability in the skin can be estimated by measuring the amount of the drug in the stratum corneum by means of the skin stripping using adhesive tapes. This method is applicable to topical drugs whose site of the action is the stratum corneum itself or deeper, although inappropriate for the topical drugs which disrupt or damage the stratum corneum by the 1st application. The amount or layers of the stratum corneum stripped off with one adhesive tape will change depending on the stripping technique of each operator and will vary between and within subjects. Accordingly, the recoveries of the stratum corneum layers for the total layers will be variable even if the number of the adhesive tapes used for the stripping is specified in SOP, which lower the power of the test. In order to increase the power, it may be advantageous to use the average drug concentration which is determined by dividing the total amount of drug recovered by the total weight of the stratum corneum or to employ the drug concentration normalized for the thickness (L) of the stratum corneum as shown in the appendix 1.

(1) Pilot test

In the pilot test, followings should be investigated.

- The dose applied, the time and skin area for the product application and the skin stripping area
- To establish and validate the extraction of drugs from the adhesive tape and analytical method.
- The time to reach the steady state (the duration of drug products should be equal to or longer than the time to reach steady state).
- The number of sites per subjects treated with products which should be determined based on the variability of a pilot tests, and the selection of either synchronized or staggered application when the product is applied to multiple sites of each subject.
- When the drug uptake into the stratum corneum is estimated using TEWL according to the equation in the appendix 1, TEWL should be measured under a constant environmental

condition (temperature and humidity), since the measurements are influenced by the condition.

(2) Pivotal Test

The number of subjects and the number of treatment sites of the skin per each subject with test and reference products should be determined based on the variability of pilot test. The testing procedure is as follows.

A) Allocate randomly the skin sites treated with test and reference products (one or more site for each product) which should be marked using a template without disturbing or injuring the stratum corneum/skin.

B) Apply test and reference products to the allocated site.

C) Remove the products from the skin at the designated time and wipe off the ointment or cream remaining on the skin. Then, peel off the surface layers of stratum corneum with two adhesive tapes which are discarded since the drug contained in the layers are generally not considered to be absorbed.

D) Strip the stratum corneum sequentially with adhesive tapes which differs depending on whether the drug concentration in the stratum corneum is estimated or not according to the equation shown in appendix 1.

   a) When the equation in appendix 1 is not employed

      Strip the stratum corneum with a fixed number of adhesive tapes from 10 to twenty or until TEWL is below 50 g/m^2/h with the tapes which are collected in a suitable container.

   b) When the equation in appendix 1 is employed

      Strip the stratum corneum with an adhesive tape of known weight which is put in a suitable container after being weighed. The skin stripping should be repeated with twenty adhesive tapes or until 80 % of the stratum corneum layers are stripped off. The thickness of the stratum corneal layer of each subject is determined by measuring TEWL for every one or two tape-strips from the control skin site without drug treatment.

E) Measure the amount of the drug recovered in each container. When the drug concentration in the stratum corneum is estimated according to the equation in appendix 1, calculate the concentration. When the equation is not used, determine the drug amount recovered from the stratum corneum or the average drug concentration which is determined by dividing the total amount of drug recovered by the total weight of the stratum corneum.

F) When the same product is applied to multiple sites per each subject, the average drug uptake should be determined and used for the assessment of bioequivalence..

(3) Statistics

In vivo parameters at a steady state should be logarithmically transformed, and the 90 % confidence interval of the test / reference ratio of the average values is calculated by a nonparametric method.

2. Pharmacological test

This test is to assess bioequivalence using the pharmacological response of topical products, which correlates with the clinical efficacy or bioavailability. Topically applied corticosteroids produce a vasoconstrictor effect depending on the drug uptake into the skin that results in skin blanching. This pharmacological response correlates with the clinical efficacy and can be used as a measure for the bioequivalence assessment, except for those with mild pharmacological responses, not producing a clear skin blanching. The procedure for the use of vasoconstrictor effect is shown below.

(1) Skin blanching assay

There are two types in skin blanching assay, visual assay by human observers and chromameter assay.

In the visual assay, trained, blinded observers estimate the degree of blanching on a multiple scale(0 – 3 or 4 or higher) based on a visual comparison of the treated sites to the untreated control. Usually, more than one observer independently estimates the degree of skin blanching at each site and the average score is employed for the assessment of bioequivalence. All of the control and treated sites with test and reference drugs should be randomly blinded for observers to avoid the biased estimation and also for subjects if possible.

A chromameter has L-, a- and b-scales showing the degree of darkness, white – black, and the degrees of color, red – green and blue – yellow, respectively. The change in the tone of color is expressed as ΔE ($=\sqrt{(\Delta a^*)^2+(\Delta b)^2+(\Delta L)^2}$). The skin blanching has sometime been estimated only on the a-scale readings. The skin blanching due to the drug treatment is generally adjusted for the baseline measurement determined at that site 1 hr prior to the drug treatment. The baseline-adjusted values are employed for bioequivalence assessment or further adjusted with the control values at the untreated skin sites.

(2) Duration of product application and observation time

When the bioequivalence is assessed using skin blanching, the duration (T_{50}) of product application should be the time at which the effect is half-maximal (ED_{50}) in the Emax-model (Emax shows the maximal effect). The details are explained in the appendix 2. The observation of skin blanching should be continued until the time, generally 24 hr, when the blanching effect disappears. The observation period and time points should be pre-determined based on the pilot test data.

(3) Selection of responder for corticosteroids

Coricosteroid responders should be used for the skin blanching tests which should be selected as follows. The durations of topical application, T_1 (T_{50}/ n) and T_2 (T_{50} ×n) are determined where T_{50} is the time of E_{50} of Emax model and n is 2 or 3. The area under the effect concentration time curves from zero to t_1 ($AUEC_1$) and to t_2 ($AUEC_2$) were calculated for each subject, and the subject who shows more than 1.25 for the ratio ($AUEC_2$/ $AUEC_1$) is judged to be a responder. It is desirable to

employ responder subjects only for the skin blanching test but it is acceptable to select the pharmacological date of responders after the blanching test where more subjects should be used so as to satisfy the statistical assessment after removal of non-responders.

(4) Pivotal skin blanching test

The number of subjects and the number of treatment sites of the skin per each subject with test and reference products should be determined based on the variability of pilot test. The testing procedure is as follows.

A) Allocate randomly the skin sites treated with test and reference products (one or multiple sites for each product), two control sites untreated and responder-checking sites which is needed when the pharmacological date of responders are selected after the blanching test. Treatment and control sites are marked using a template without disturbing or injuring the stratum corneum/skin.
B) When chromameter assay is used, the baseline color should be measured prior to the treatment of topical drugs.
C) Apply the products. When the pharmacological data of responders are selected by the visual assay, the times of application of two reference products with different durations, T1 and T2, should be adjusted so that the observers are blinded for the two products.
D) Remove the products from the skin at the designated time and wipe off the ointment or cream remaining on the skin.
E) Measure the skin blanching at each site at designated time intervals. The skin blanching at treatment site should be adjusted for the baseline measurements when the chromameter is employed, and, if necessary, with control measurements.
F) Calculate AUEC of treatment sites for each subject. If an identical product is applied to multiple sites of each subject, the average AUEC should be calculated. When the tests are performed without selecting responders, the pharmacological data of non-responders should be disregarded.

(5) Statistics

When chromameter assay is employed, the AUEC values should not log-transformed since they are usually negative values. The 90 % confidence interval of the difference in mean AUEC between test and reference products is calculated using raw data by a nonparametric method.

When visual assay is employed, the 90 % confidence interval of the difference in mean AUEC between test and reference products is calculated by a nonparametric method or parametric method after the logarithmic transformation of the AUEC values.

3. Test for measuring the unabsorbed drug

This test is to estimate the amount of drug absorbed into the skin from the amount remaining in the product. However, the use of this test is generally limited, because the drug uptakes of usual topical

products is very low, making it difficult to estimate the uptake precisely, although this test may be useful if the precise estimation is possible.

(1) Pilot study

Followings should be investigated prior to the pivotal test.

- To determine the relation between drug content and the weight of product, if necessary.
- To establish and validate the analytical method and extraction of the drug from products, cotton swab and cleaning solution used for removing the drug product remaining on the skin.

(2) Pivotal test

The duration of drug product application should follow the dosage regimen or is equal to or longer the time to reach the steady state.

The number of subjects and number of treatment sites of the skin per each subject with test and reference products should be determined based on the variability of pilot test. The testing procedure is as follows.

(A) Allocate randomly the skin sites treated with test and reference products (one or multiple sites for each product) and control sites. Treatment and control sites are marked using a template without disturbing or injuring the stratum corneum/skin.

(B) Weigh the products applied to the skin.

(C) Apply test and reference products to the allocated treatment and control sites.

(D) Remove the products on control sites immediately after their application, and wipe off the drug or products remaining on the skin with cotton swab. All of products recovered, cotton swabs and guard, if it is contaminated with the drug products, are put in the container designated.

(E) Remove the products on the sites at the designated time, t, after their application, and remove the drug or products remaining on the skin with cotton swab. All of products recovered, cotton swabs and guard, if it is contaminated with the drug products are put in the container designated.

(F) All amounts of drugs in the container indicate the amount recovered from the skin site.

(G) When there are two or more treatment and control sites per each subject, the average amount of drug should be used for the assessment of bioequivalence..

(H) The difference between the amount of drug recovered from control site and that from treatment site at the time, t, is considered as the amount absorbed into the skin.

(3) Statistics

In principle, the data should be logarithmically transformed, and the 90 % confidence interval of the test / reference ratio of the average value is calculated by a parametric method.

4. Pharmacokinetic test

This test is to assess bioequivalence using pharmacokinetic parameters of blood concentration – time curves after the product application. The test is useful when the active site of the drug is the stratum corneum and/or below and if the clinical efficacy or drug concentration in the active site correlates with the pharmacokinetic parameters

(1) The duration of the product application

The duration of drug product application should follow the dosage regimen or is equal to or longer the time to reach the steady state.

(2) Pivotal test

The tests should be performed according to the Guideline for Bioequivalence Studies of Generic products (1997). AUC or Css (steady state drug concentration) is used as the parameter to assess bioequivalence.

(3) Statistics

In principle, the data should be logarithmically transformed, and the 90 % confidence interval of the test / reference ratio of the average value is calculated by a parametric method.

5. Clinical test

The test is to assess bioequivalence using a suitable clinical response which should be selected by considering the clinical property of the drug. This test should be performed using statistically sufficient number of patients, when clinical assessment is preferable or when other tests are impossible or inappropriate.

The acceptance criteria of equivalence in this study should be established for each drug by considering the clinical characteristics.

6. In vitro efficacy test

The test is to assess bioequivalence using an in vitro activity as the index. The test may be applicable for topical drugs such as bactericides, disinfectants and antiseptics whose active site is the surface of the skin and which is not needed to be absorbed in the stratum corneum. The in vitro efficacy tests do not include drug release tests for topical drugs.

The acceptance criteria of equivalence in this study should be established for each drug by considering the characteristics of the efficacy.

7. Animal test

The test is to assess bioequivalence using a pharmacological activity on the skin as the index. The test may be applicable for topical drugs such as bactericides, disinfectants, antiseptics, hemostatics and wound repair agents whose active site is the surface of the skin and which is not needed to be

absorbed in the stratum corneum.
The acceptance criteria of equivalence in this study should be established for each drug by considering the pharmacological characteristics.

III. Exposure

The barrier function of the dermatoses skin is generally lower than the normal skin, which increases the absorption of the drug into the systemic circulation and may produce significant adverse effects. Accordingly, it is necessary to compare the exposures between test and reference products using the skin of a poor barrier function for the drugs which may brought about significant side effects such as immunosuppressants, steroids with potent pharmacological actions, retinoids, carcinostatics, chloramphenicol and related drugs. The exposure of test products should be equivalent with or less than that of the reference one or below the permissible level which should be proven by a suitable method including pharmacokinetic studies.

IV. Reporting of bioequivalence test result

The test results should be described as shown in the Guideline for Bioequivalence Studies of Generic products (1997).

Appendix 1.

Estimation of drug concentration in stratum corneum according to a diffusion equation

(1) Estimation of the stratum corneum thickness

The water under the skin is lost by the diffusion through the stratum corneum according to the Fix's law. The water lost from the surface of the skin is called as the transepidermal water loss (TEWL)

$$TEWL = \frac{K_W D_W \bullet \Delta C}{L} \qquad (1)$$

K_w : Partition coefficient of water between stratum corneum and epidermal cell

D_w : Diffusion constant of water through the stratum corneum

ΔC : The difference in concentration of water between the surface and deepest point of the stratum corneum

L : Thickness of the stratum corneum

TEWL after the removal of the stratum corneum of "x" in the thickness is shown as equation (2).

$$TEWL = \frac{K_W D_W \bullet \Delta C}{L - x} \qquad (2)$$

The reciprocal of equation (2) is :

$$\frac{1}{TEWL_x} = \frac{L - x}{K_W D_W \bullet \Delta C} = \frac{L}{\gamma D_W} - \frac{x}{\gamma D_W} \qquad (3)$$

where, $\gamma = K_W \cdot \Delta C$.

If the density of the stratum corneum is 1 g/cm^3 and the skin stripping area is constant, the weight of the stratum corneum layers stripped is converted to the thickness, "x", and 'L" can be determined from the intercept of the plot of 1/TEWL vs. x.

(2) Estimation of drug concentration in the stratum corneum

The drug concentration in stratum corneum is shown as equation (4) according to the Fick's second law of diffusion.

$$C_x = KC_{veh}\left[\left(1-\frac{x}{L}\right)-\frac{2}{\pi}\sum_{n=1}^{3}\frac{1}{n}\sin(\frac{n\pi x}{L})\exp(-\frac{Dn^2\pi^2 t}{L^2})\right] \quad (4)$$

C_x: Drug concentration at the depth of "x" in stratum corneum

C_{veh} : Drug concentration in vehicle

D : Diffusion constant of drug in stratum corneum,

K : Partition coefficient of the drug between stratum corneum and formulation

t : Duration of product application

D and K are determined by plotting C_x to x by the least square method.

The drug concentration in stratum corneum can be determined by inserting the values of L, D, K into equation (5).

$$A=\int_0^L C_x d(\frac{x}{L}) \quad = KC_{veh}\left[\frac{1}{2}-\frac{4}{\pi^2}\left(\exp(-\frac{D\pi^2 t}{L^2})+\frac{1}{9}\exp(-9\frac{Dn^2\pi^2 t}{L^2})\right)\right] \quad (5)$$

When the duration of the product application is enough long, equation(5) is reduced to equation (6).

$$A=\frac{KC_{veh}}{2} \quad (6)$$

The drug concentration in stratum corneum is proportional to the K, when the concentration in the vehicle is homogeneous, that is, C_{veh} is considered constant.

Appendix 2.

Determination of the duration of drug application according to the Emax model.

Maximal effect (Emax) and half maximal effect (E_{50}) are determined from the dose-response curves according to the Emax model, where the amount of the drug absorbed in the skin, in principle, should be used instead of the dose for topical products, because of the incomplete absorption. Usually, the duration of product application is used for the absorbed amount, since the absorbed amount is linearly proportional to the duration except for the initial phase showing the burst absorption and latter stage where the drug release significantly decreased.

AUEC values are calculated after removal of the product by changing the duration (T). Then, AUEC values are plotted against the T, and $AUEC_{50}$ is determined by a least square method according to the following equation. $AUEC_0$ and $AUEC_{max}$ show the baseline and maximal response of skin blanching. Thus, the duration,T_{50} corresponding to $AUEC_{50,}$ can be determined.

$$AUEC = AUEC_0 + \frac{AUEC_{\max} \times T}{AUEC_{50} + T}$$