Methods of Drug Interaction Studies

This document is an informal translation of the official text that was promulgated in Japanese on 4 June 2001 and intended for use as a reference in conducting drug interaction studies.

1. Introduction

1.1 Background and objectives

Medicines are often used concomitantly with other drugs, and some degree of drug-drug interaction occurs with concomitant use. Although only a small proportion of this interaction is clinically significant, it sometimes causes serious adverse reactions. For example, drug interactions, particularly with drugs having a narrow therapeutic range, may have serious adverse consequences. Therefore, in the evaluation and clinical application of drugs, appropriate efforts should be made to predict the nature and degree of drug interactions so that patients will not be adversely affected. Humans are genetically diverse, and disease states are likewise diverse. It should, therefore, be kept in mind that drug interactions might readily cause clinically significant changes in blood drug levels (concentration in whole blood, plasma, or serum) in patients having pharmacokinetic parameters markedly deviating from those of the standard population.

The purposes of this document are (1) to outline the basic principles of non-clinical tests necessary to predict the potential for drug interactions and to decide the need for clinical tests, (2) to outline the clinical test methods and decision criteria to determine the occurrence and magnitude of a drug interaction in humans, and (3) to indicate the factors to be considered when interpreting results. Appropriate systematic and step-by-step approaches to characterize the basic properties of drugs are needed to evaluate drug interactions in the drug development process. Moreover, case-by-case decision making, according to an evaluation of the characteristics of each drug, is also important. This document provides a tool for determining which studies are necessary. It is expected to contribute to the prevention of the development of drugs with a high possibility of causing adverse drug interactions and to promote the efficient development of drugs. As it provides appropriate information to the medical practitioner, reduction in adverse drug interactions and promotion of the proper use of drugs are also expected.

This document defines drug interactions as the phenomena that occur between concomitant drugs, or drugs and food, or lifestyle (e.g., smoking, alcohol drinking) that may affect a drug's pharmacokinetic profile and/or a drug's efficacy/side effects.

Pharmacokinetic data are useful to predict drug interactions, to clarify the mechanism of the interaction, and to avoid drug interactions. Therefore, most of this document will focus on pharmacokinetics-related drug interactions.

1.2 Scope of the document

This document indicates the approach to drug interaction studies. For the purpose of achieving efficient drug development, it is important to conduct appropriate drug interaction studies in the early stages of drug development. This document will also be applicable to post-marketing studies.

The purpose of this document is to outline the principles for predicting clinically significant drug interactions and for evaluating their occurrence in humans. Accordingly, *in vitro* studies using human tissue-derived samples and expression systems, including drug-metabolizing enzymes and transporters, and drug interaction studies in humans, that are conducted when necessary, are important. *In vitro* and *in vivo* drug interaction studies using experimental animals, however, may also be useful

for predicting interactions in humans during the process of drug development and for clarifying the mechanism of interactions.

Drug interactions may occur after administration by any route. All of these are included in the scope of this document. However, this document refers mainly to drug interactions that may occur after oral administration. In cases where drug interaction studies with drugs given by different routes of administration are needed, they should be conducted appropriately, by referring to the descriptions in this document.

Drug interactions are classified, based on mechanism, into pharmacokinetic and pharmacodynamic interactions. The former interaction is the phenomenon that is induced by changes in blood levels and tissue distribution of a drug or its active metabolites by the interaction of the drugs in the processes of absorption, distribution, metabolism, and excretion. For the prediction and evaluation of the clinical significance of a drug interaction, it is necessary to evaluate how much the process where the interaction was observed determines the pharmacokinetics of the drug. The latter interaction is the phenomenon that occurs when the effects of a drug are additive/synergistic or antagonistic to the effects of a concomitant drug, or when a drug changes the tissue sensitivity/reactivity to the investigational drug. In clinical practice, this phenomenon is occasionally used to the advantage of patients as a concomitant therapy. For the purpose of predicting pharmacodynamics-related drug interactions, a thorough understanding of the main pharmacologic effect that leads to a given therapeutic effect, any potential secondary pharmacologic effect that may lead to side effects, and toxicologic effects is necessary.

If there is a possibility of concomitant use in patients in which a clinically inappropriate pharmacodynamic drug interaction may occur, interaction studies in animals should first be considered. There may be a case where both pharmacokinetic and pharmacodynamic drug interactions occur at the same time. A wide variety of pharmacological drug interactions are possible, and therefore it is difficult to provide standard methods in this document. It is necessary to address the pharmacodynamic interaction potential on a case-by-case basis, depending on the pharmacological properties of the given drug and its intended clinical usage.

1.3 Principles of drug interaction studies

Drug interactions should be considered from the perspective both of new drugs under development (investigational drug) and drugs that have already been approved and are expected to be used concomitantly with other drugs. Interactions should also be considered from the perspective both of drugs that cause interactions (interacting drug) and drugs that are affected by the interaction (interacted drug).

For a drug used as a component of combination therapy or for a drug with a high possibility of use with other drugs, the possibility of the occurrence of interaction and the effect on clinical efficacy and safety of the drug should be assessed thoroughly, based on the results of non-clinical pharmacokinetic, toxicity, and pharmacologic studies. If necessary, non-clinical or clinical drug interaction studies should be conducted for the purpose of examining the possibility of interactions and to assess their influence on drug therapy. Drugs for which particular attention is required are those that may cause a

severe adverse reaction.

When unchanged drug concentrations in plasma are low and most of the drug equivalents present are metabolites, the possibility of major metabolites being present in the plasma in amounts sufficient to cause drug interactions should also be examined.

Since physical and chemical properties, pharmacologic action, pharmacokinetics, and clinical usage differ for each drug, the procedures of drug interaction studies should be appropriately selected depending on the property of the investigational drug and in accordance with the principles outlined in this document. This document is based on currently available information, and it is therefore desirable to adopt new methods continually, in keeping with medical and technical advances.

Prior to performing clinical studies in humans, basic items that may be factors in drug interactions need to be adequately examined, particularly from the pharmacokinetic perspective. Information on the nature of possible adverse effects induced by concomitant use with the other drugs should be examined. Decisions on the necessity of conducting interaction studies and on the timing and design of the studies should also be based on information on the stage of clinical development of the drug candidate, its intended indication and therapeutic and safety ranges, and the estimated frequency of concomitant use. It is also important to consider the clinical dose and route of administration.

Clinical drug interaction studies should be conducted in accordance with Good Clinical Practice (GCP). Pharmacokinetic drug interaction studies should be conducted in accordance with the "Clinical Pharmacokinetic Studies of Pharmaceuticals (2001)".

1.4 Related guidelines and guidances

Descriptions of drug interactions are included in the guidelines and guidances listed below. They are also found in the guidelines for the method of clinical evaluation of drugs for specific diseases such as anti-hyperlipidemics, anxiolytics, hypnotics, antibiotics, blood products, antiarrhythmics, and anti-inflammatory analgesics. This document integrates these guidelines/guidances and incorporates new findings. We recommend referring to these guidelines/guidances for studying drug interactions within a specific category of drugs. The "Guideline for Bioequivalency Studies for Generic Drugs (1997)" can also serve as a reference for determining the absence of a drug interaction.

1)	Guideline for Clinical Studies of Drugs for Use in the Elderly (Yakushinyaku no. 104, 12/2/93)
2)	Guideline for Design and Evaluation of Slow-release Products (oral) (Yakushin 1-5, 3/11/88)
3)	Guideline for Format and Contents of Clinical Study Reports (Iyakushin no. 335, 5/1/96)
4)	Guideline for Non-clinical Pharmacokinetic Studies (Iyakushin no. 496, 6/26/98)
5)	Clinical Pharmacokinetic Studies of pharmaceuticals (2001)
6)	Guideline for Bioequivalency Studies (of generics) (Iyakushin no. 487, 12/22/97)
7)	General Guideline for Clinical Evaluation (Iyakushin no. 380, 4/21/98)
8)	Guidance on the Ethnic Factors to Accept Clinical Data Obtained in a Foreign Country (Iyakushin no 672, 11/8/98)
9)	Notice for the Utilization of Clinical Data Obtained in a Foreign Country (Iyakuhatu no.739, 11/8/98)

2. Drug Interactions in Absorption

This section will focus on drug interactions in gastrointestinal absorption.

Not only concomitant drugs but also food constituents may exert significant effects on the process of drug absorption. Many of these effects can be predicted qualitatively from adequate knowledge of the physical and chemical properties and pharmacologic actions of the drug or pharmaceutical. The focus should be on the factors listed below. If unexpected results are observed, such as changes in bioavailability, the cause should be examined, which may necessitate an investigation of drug's metabolism.

The effect of food on the absorption process should be examined using the final formulation of the drug. A definition of final formulation can be found in the "Clinical Pharmacokinetic Studies of pharmaceuticals (2001)".

2.1 Effect on complex/chelate formation and solubility in the gastrointestinal tract

When the solubility of a given drug is dependent on pH, changes in gastric pH due to a concomitant drug may influence its intestinal absorption. Complex/chelate and micelle formation may occur as a result of the influence of concomitant drugs and food components that reduce or enhance the gastrointestinal absorption of a drug. Among highly lipid-soluble drugs, some show increased absorption due to enhanced solubility caused by high-fat diet, which increases bile secretion.

2.2 Effect on gastrointestinal motility

Drugs that affect the gastric emptying rate may change the rate of absorption of a drug from the gastrointestinal tract by affecting the dissolution rate of tablets and passage into the small intestine. Ingestion of food often delays absorption due to a delay in the gastric emptying rate.

2.3 Drugs absorbed and excreted by transporters

Transporters expressed on the plasma membrane of intestinal epithelial cells participate in the absorption process of some drugs, and therefore competitive inhibition of absorption may occur when the same transporter participates in the absorption of drugs or food components. Also, some transporters, such as P-glycoprotein, expressed in the intestinal wall, contribute to the efflux of a drug that is taken up by epithelial cells from the luminal side before going to the basal side (or into the portal vein). Inhibition of this process may lead to increased absorption.

When active transport is suspected to contribute to the absorption and efflux process in the gastrointestinal tract, it may be useful to examine the extent of this contribution using in vitro test systems, such as cells in which transporters are expressed.

3. Drug Interactions in Tissue Distribution

Many drugs bind to plasma proteins and to proteins and/or other components in tissues. Since only unbound drug is available for transport between plasma and tissues, changes in the unbound fraction due to displacement may lead to drug interactions. Some transporters have also been reported to play a role in tissue distribution.

3.1 Plasma protein binding of drugs

Albumin is a major plasma protein to which drugs bind. Some drugs also bind to α_1-acid glycoprotein, lipoprotein, and other proteins. When *in vitro* plasma protein binding is high, it is necessary to clarify the identity of the binding protein and the extent of binding.

Displacement of a drug bound to plasma proteins is the most usual cause of changes in the distribution of a drug due to drug interactions. Injection of a drug that binds tightly to plasma proteins may cause the release of an already bound investigational drug and thereby increase the plasma concentration of its unbound form. In most cases, however, displacement does not result in a clinically significant change. Cases where displacement from plasma proteins causes increases in the plasma concentration of the unbound form of an investigational drug, and in turn a clinically significant drug interaction, occur only when the extent of protein binding is more than 90%, the therapeutic range of the drug is narrow, and one of the following conditions is met:

(1) Drug with a small volume of distribution (Note 1): In this case, clearance of the drug and route of administration of the investigational drug are not critical.

(2) Elimination of the investigational drug is mainly via the liver, its clearance is high, and the drug is administered intravenously.

(3) Elimination of the investigational drug is mainly via the kidneys, and the clearance is high. In this case, the route of administration is not critical.

In the case of investigational drugs for which the volume of distribution in humans is large (Note 1) and hepatic clearance is low, displacement of plasma protein binding reduces the total drug concentration in plasma, but no clinically important result occurs, since the unbound drug concentration does not change appreciably.

Interactions with plasma protein binding should be examined mostly by *in vitro* methods when the conditions described above are present. If marked displacement of protein binding is observed, studies should be conducted in humans from the perspective of plasma protein binding and/or pharmacological effect.

If metabolites are present in high concentrations and the above conditions are satisfied, the metabolites should be studied for their potential to cause drug interaction by binding to plasma protein.

3.2 Special tissue distribution

Methods for the prediction of drug interactions in humans due to the following factors are not established. Information on the binding of drugs to special tissue components and drug interactions with transporters, however, will help in understanding any unanticipated clinical events.

3.2.1 Binding with special tissue components

There are drugs that bind selectively with receptors, proteins, or lipids in tissue. The concentration of an unbound drug in tissue may change as a result of competition for binding, which may in turn cause a drug interaction.

3.2.2 Involvement of transporters in the uptake and excretion processes of tissues

The involvement of transporters in distribution to the liver, kidneys, and brain has been reported for certain drugs. In particular, interactions involving the active transport process may change the unbound drug concentration in tissue and thereby alter the effects and side effects of the drug. When an interaction occurs in a major organ of distribution and elimination, such as the liver and kidneys, the volume of distribution of the drug and its systemic clearance may also be affected.

4. Drug Interactions in Drug Metabolism

Although there are cases of interactions involving other enzymes such as dihydropyrimidine dehydrogenase in the case of sorivudine and fluorouracil anticancer drug, most known drug interactions are related to oxidative metabolism, particularly by cytochrome P450 (P450). Therefore, the focus of this section will be on P450. Representative human P450 isoforms and their substrates, inhibitors, inducers, and marker drugs are listed in Table 1. For the estimation of the clinical significance of drug interactions caused by the inhibition and induction of drug metabolism, information on to what extent in systemic clearance the metabolic pathway participates is necessary.

4.1 Inhibition of cytochrome P450

As shown in Table 1, P450 has many known isoforms, such as CYP1A2, CYP2C9, CYP2C19, CYP2D6, and CYP3A4. Because the substrate specificity of these P450s is broad, competitive inhibition occurs between drugs metabolized by the same isoforms. However, isoforms involved in the metabolism of a drug do not necessarily correspond to the isoforms that are inhibited by the drug. For example, quinidine is metabolized mainly by CYP3A4, but strongly inhibits CYP2D6. P450 enzymes, CYP3A4 as a representative, are also found in the human intestine as well as in the liver. Therefore, when the enzyme participates in the first pass effect of an investigational drug, orally administered inhibitors or those in food or beverages frequently cause drug interactions.

In vitro inhibition studies using representative marker drugs and/or substrates are conducted in order to predict drug interactions caused by the inhibition of P450. Since there are marked species differences in drug metabolism by P450, the use of available human tissue-derived samples and P450 expression systems is important from an early stage of drug development. If the metabolism of an investigational drug is not inhibited by representative marker drugs *in vitro* and vice versa, no further study is necessary. If a significant inhibition is observed, kinetic parameters such as K_i (inhibition constant) should be determined, and the potential for drug interactions should be assessed. Irreversible enzyme inhibition may lead to a loss of enzyme activity and may cause abrupt increases in the blood concentration of the drug. Therefore, it will also be necessary to determine whether the inhibition is competitive and whether it is reversible or irreversible (Note 2). For the purpose of predicting drug interactions, it is necessary to calculate the ratio of the estimated maximum concentration of unbound drug in major metabolic organs (Note 3 and Note 4) following the administration of the expected clinical dose to K_i. If the ratio thus obtained is large, if participation of the metabolic enzymes in the systemic clearance of the interacted drug is high, and if a marked increase in clinical blood concentration is anticipated, it will be important to administer the expected clinical dose to healthy volunteers to determine the actual degree of drug interaction (Note 4). In the case of irreversible inhibition, however, prediction of *in vivo* outcomes from only K_i values obtained *in vitro* is difficult.

In some cases, drug interaction studies involving experimental animals may be conducted to obtain information on the *in vitro*/*in vivo* relationship according to the degree of drug interaction. The results may be useful for predicting *in vivo* drug interactions in humans based on *in vitro* information. This procedure will be applied when it is known that the inhibition of metabolism by the investigational drug in animals and humans is similar in terms of the P450 isoforms involved, type, and strength of inhibition.

Since there is a large amount of the CYP3A4 enzyme present in human ileal epithelium, it is necessary to consider the influence of the P450 inhibition by compounds in food for drugs given orally. For example, grapefruit juice contains a potent inhibitor of CYP3A4, and caution must be taken to avoid interactions when prescribing orally administered drugs that are metabolized mainly by CYP3A4.

4.2 Induction of cytochrome P450

As shown in Table 1, many drugs and environmental substances induce P450 enzymes. Smoking cigarettes strongly induces CYP1A2. Drug interactions caused by P450 induction generally result in a reduced therapeutic effect by accelerating metabolism. For example, the administration of rifampicin causes the acceleration of metabolism of female steroid hormones, and may, in turn, decrease the efficacy of contraceptive drugs. On the other hand, potentiation of a therapeutic effect by active metabolites can occur for drugs that require metabolic activation. In addition, disappearance of induction caused by abrupt cessation of concomitant administration can result in adverse events related to increased plasma drug levels.

There may be a case where levels of cytochrome P450 isoforms and metabolic activities of representative substrates in experimental animals like rats are examined following the repeated administration of investigational drugs to obtain information on induction of P450 isoforms. This may be useful at present for the screening of candidates in the early stage of drug development. However, predictions of human outcomes with animal data must be made with care (Note 5). On the other hand, cultured human hepatocytes can be useful tools for the qualitative or semi-quantitative assessment of induction of enzymes by using appropriate positive controls. The induced enzyme(s) should be identified whenever total P450 in the liver and/or metabolism of representative substrates increases significantly.

On the other hand, information on the presence, absence, and degree of enzyme induction with long-term administration may be obtained from toxicokinetic data. Caution is required, however, since the administered drug does not necessarily induce the P450 isoforms involved in its own metabolism.

As the clinical dose is generally lower if calculated based on body weight rather than on the dose used for experimental animals in an induction study, the observed enzyme induction in humans is often not as strong as that in animals. However, medicines such as rifampicin markedly induce enzyme activity in humans, but not in rats. Therefore, when the possibility of drug interactions is studied in human hepatocyte culture, drug concentration and exposure time that cause enzyme induction and the relationship between dose and blood concentrations of the drug in humans should be considered.

The presence or absence of enzyme induction in humans can sometimes be evaluated by measuring metabolites in urine after caffeine loading (CYP1A2) and the ratio of 6β-hydroxycortisol/cortisol in urine (CYP3A4). Information can also sometimes be obtained from reductions in plasma concentration of the drug with repeated administration

in a phase I clinical study. In the case of drugs with a high possibility for drug interactions, it is desirable to examine the effects of repeated administration of the clinical dose before concomitant use in clinical studies, if necessary, while considering the expected frequency of concomitant use and influences on the effects and adverse effects of concomitant drug.

4.3 Inhibition and induction of enzyme systems other than cytochrome P450

It is also expected that inhibition will occur in enzyme systems other than P450, but reports of adverse interactions are rare, except for the appearance of fluorouracil toxicity due to inhibition of dihydropyrimidine dehydrogenase by sorivudine and the inhibition of aldehyde dehydrogenase by disulfiram and related compounds. At times, however, there is the possibility of serious interactions when enzymes related to the metabolism of nucleic acids and hormones are affected. On the other hand, mild induction of glutathione transferase and glucuronosyl transferase and an increase in cofactors have been reported. However, such reactions rarely cause clinically significant drug interactions.

4.4 Points to consider in the study of metabolic drug interactions

4.4.1 Drug concentration and dose

The degree of inhibition of drug metabolism and induction of enzymes is strongly dependent on the inhibitor concentration, substrate concentration, dose, and dosing interval. In animal studies, the administered dose is generally high, and clear inhibition or induction readily occurs. On the other hand, drugs with much higher clearance in animals than in humans may not cause clear drug interactions. Therefore, it is important to predict the possibility and degree of drug interactions in humans while considering the changes in unbound drug concentrations in the liver when the predicted clinical dose is administered, and not to conduct unnecessary animal and clinical studies.

4.4.2 Inhibition and induction caused by metabolites

Metabolites may cause drug interactions depending on the amount produced and their enzyme inhibition strength. A representative example is the occurrence of Antabuse syndrome due to inhibition of aldehyde dehydrogenase by metabolites of cephalosporins. It is also known that P450 induction is caused by some lipophilic metabolites.

4.4.3 Special cases of inhibition

Some drugs are metabolized to reactive intermediates that irreversibly inactivate the enzyme involved in their metabolism. Such drugs are called suicide substrates. It is important to note that repeated administration of those drugs may cause strong inhibition of the metabolism of the drug and other drugs.

Information on this type of drug can often be obtained from *in vitro* metabolic studies and from the abnormal increase in plasma concentration of a drug following its repeated administration in toxicity studies incorporating toxicokinetics and phase I clinical studies (Note 2). Commonly known examples include loss of CYP3A4 activity caused by macrolide antibiotics, such as troleandomycin, erythromycin, and clarithromycin that are metabolized by CYP3A4. Sorivudine potentiation of fluorouracil toxicity is caused by the inhibition by the sorivudine metabolites of dihydropyrimidine dehydrogenase, the enzyme that metabolizes fluorouracil anticancer drugs.

4.4.4 Interaction of drugs demonstrating blood flow-dependent clearance in metabolism

Changes in hepatic blood flow cause changes in plasma concentrations of drugs only when drugs demonstrating blood flow-dependent hepatic clearance are administered intravenously. It is therefore necessary to examine interactions with drugs that have a marked effect on hepatic blood flow.

4.4.5 Genetic polymorphisms and drug interactions

Due to variant alleles of the genes encoding drug-metabolizing enzymes, there are some individuals who cannot metabolize certain drugs as efficiently as others (genetic polymorphism). If they are given ordinary doses of a drug, plasma drug concentrations tend to increase to a greater extent than in ordinary patients. Therefore, side effects are likely to occur in those patients whose P450 activities for the investigational drug are low (individuals who have one or two mutant alleles encoding a low activity or no enzymes) if interactions due to inhibition of metabolism occur. In other words, when further reduction in metabolic activity occurs with inhibition of the remaining activity or with inhibition of the other enzymes that participates also to the metabolism of a drug, the risk of drug interaction appears to be higher. In addition, when a genetic polymorphism affects the metabolism of a concomitant drug with enzyme-inducing activity, the plasma concentration of the drug tends to be higher in those deficient in the metabolizing enzyme than that in others, therefore the risk of inducing a drug interaction appears to increase. There are few instances of elevated metabolic activity due to a genetic polymorphism.

The relationship between genetic polymorphism and drug interactions is complicated and cannot be discussed in general terms. If polymorphically expressed enzyme is largely responsible for the metabolism of an investigational drug, it is necessary to discuss the possibility of drug interactions considering the phenotype and/or genotype of each patient.

The presence of ethnic differences in the type of genetic polymorphism and its frequency should also be considered.

4.4.6 Differences between drugs metabolized by a single enzyme and multiple enzymes

In the case of drugs metabolized by a single enzyme, drug concentrations are markedly elevated when that enzyme is inhibited. Therefore, the possibility of drug interaction of these drugs is high. On the other hand, when multiple enzymes are involved in metabolism of a drug, inhibition of the major metabolizing enzyme causes a shift in metabolism to the other enzymes, and thus does not cause a dramatic increase in drug concentration. Drug concentrations in the body tend to increase more with the inhibition of major metabolizing enzymes when the alternative metabolizing enzyme is deficient (e.g., due to genetic polymorphism).

In the case of enzyme inducers, drug concentrations are markedly reduced only if the induced enzyme metabolizes the drug, but the effect is relatively slight if other enzymes are also involved.

5. Drug Interactions in Excretion

5.1 Drug interaction in the process of renal excretion

Most drugs are filtered through the renal glomeruli, and many are passively reabsorbed by the renal tubules. However, highly polarized drugs are usually excreted in the urine without reabsorption. In the case of drugs with a high reabsorption rate (weakly acidic or basic drugs), concomitant administration of drugs that affect urinary pH may change their urinary excretion.

On the other hand, there are many highly polarized drugs that are excreted into the renal tubular fluid or actively reabsorbed by the renal tubules via transporters. Inhibitory interactions may occur in these processes among acidic drugs or among basic drugs. Caution is therefore required. There are more reports of drug interactions with acidic drugs than with basic drugs. Some drugs interact with concomitant drugs through their metabolites.

It is necessary to consider the possibility of drug interactions in the process of urinary excretion for a drug that is extensively secreted into the renal tubular fluid and whose renal clearance is high.

Patients with renal disease or of advanced age often show low drug excretion rates, and thus blood concentrations of renal clearance-dependent drugs tend to be high. Therefore, appropriate caution is required in these patients for side effects due to drug interactions particularly in the urinary excretion process.

5.2 Drug interactions in biliary excretion

Drugs are often conjugated and excreted in bile. Some drugs are excreted in bile without biotransformation. For example, in humans, most water-soluble drugs and metabolites of relatively high molecular weight (more than about 450) are excreted largely in the bile. This excretion is mainly via transporters, and the possibility exists for drug interactions with concomitant administration. Conjugates such as glucuronides are often excreted in bile and then deconjugated in the intestinal tract and reabsorbed (enterohepatic circulation). Drug interactions in the process of biliary excretion may affect the residence time and area under the curve (AUC) of the unchanged drug in plasma.

To predict interactions in the processes of urinary and biliary excretion, *in vitro* inhibition studies using human tissue-derived samples, cells expressing transporters, and membrane vesicles may be useful.

6. Situations in Which Clinical Interaction Studies are Required

Clinical studies must be both ethical and scientifically rational. It is desirable to avoid conducting unnecessary studies in humans. Therefore, it is important to select drug candidates that do not require drug interaction studies in humans. *In vitro* studies using human tissue-derived samples, human enzyme expression systems, and animal studies conducted when necessary should contribute to this selection. Results of studies with related drugs and/or other drugs are useful for the extrapolation of *in vitro* data to the *in vivo* situation and of animal data to humans.

If serious adverse drug interactions are anticipated, for example, with the inhibition of drug metabolism, further development of the drug candidates should be reconsidered. However, there may be cases where benefits of the drug outweigh the risk of the drug interaction, and clinical developments are considered necessary. In such

situations, the following should be considered: potency of inhibition in human tissue-derived samples and/or human enzyme expression systems, intended clinical dosage and plasma/tissue concentrations (unbound drug), and clinical implications of the drug interaction. It is important to estimate the contribution of the interacting pathway or enzyme to the total clearance while considering whether the drug is of a hepatic or renal clearance-type and whether it is metabolized by a single or multiple enzymes. Furthermore, care should be taken not to make false predictions, especially false-negative predictions, for *in vivo* outcomes using *in vitro* results, since the correlation between results of *in vitro* and *in vivo* interaction studies is sometimes not good.

In planning clinical drug interaction studies for examining the occurrence and the severity of drug interactions that are predicted by non-clinical studies, it is necessary to be very careful in considering the side effects that might be caused by the interaction.

The decision as to the necessity of conducting drug interaction studies in humans should be based on the above points.

7. Design of Clinical Studies

For the purpose of evaluating drug interactions quantitatively, clinical drug interaction studies considering the clinical dose and method of administration are necessary. Drug metabolism-related interaction studies are usually conducted under steady-state conditions after repeated dosing. Except for the case of examining induction of drug-metabolizing enzymes, single-dose studies are also acceptable when the half-life of an investigational drug and/or concomitant drug is shorter than the dosing interval, and marked accumulation does not occur. In addition, multiple-dose inhibition studies are necessary in cases where the investigational drug causes irreversible inhibition (loss of enzyme activity). On the other hand, in the case of investigational drugs with enzyme induction potential, several days of pre-treatment are usually necessary for adequate enzyme induction to occur.

In the case of an investigational drug for which serious adverse drug interactions are predicted and further development is considered justifiable, clinical studies should be conducted prohibiting the concomitant use of the interacting drug to prevent the occurrence of the adverse drug interaction. To confirm the drug interaction, careful performance of the clinical drug interaction study considering the occurrence and severity of the adverse effects is necessary.

In terms of the study design, random crossover designs (single administration of investigational drug and co-administration with concomitant drug), add-on designs (single-dose studies followed by co-administration studies), and parallel study designs (single-dose study and co-administration study conducted separately with the other volunteer group) can be considered. Timing of the administration of the investigational drug and the indicator drug should be planned appropriately to allow detection of the drug interaction. When a drug interaction due to enzyme inhibition is expected, the concomitant drug should be selected while considering the following: (1) The drug should be very safe, and non-clinical studies should indicate that the drug is metabolized by the enzyme affected by the investigational drug, and that its clearance is markedly changed by the inhibition of metabolism. (2) The drug will frequently be used concomitantly, and there is a possibility that serious adverse events will result from an interaction. When a drug interaction is due to inhibition or induction of the metabolism of an investigational drug, the concomitant drug should be selected from those that affect the main metabolic pathways and that are safe and do not have any pharmacological effects or have only the least pharmacological effects.

In this case, due caution, such as use of safe concomitant drugs or beginning with lower doses, is needed to ensure the safety of volunteers.

The method of population pharmacokinetics may also be useful when data from many patients are available, depending on the disease indication.

7.1 Timing of clinical drug interaction studies

When developing drug with a high potential for causing drug interactions in humans, it is desirable to conduct drug interaction studies in healthy volunteers using the investigational drug (at a clinical dose determined in a phase Ⅱ study) together with a marker drug and a possible concomitant drug. The results would provide information necessary for the decision as to whether to continue further development of the drug, for the selection of an alternative drug, and for setting contraindicated drugs in a phase Ⅲ study. On the other hand, there may be situations where predicted serious adverse drug interactions make it necessary to conduct the drug interaction study before phase Ⅱ, in order to select candidates for further development and to establish contraindicated drugs in clinical studies. If the potential to cause a serious adverse drug interaction is demonstrated, the drug should not be used as a concomitant drug until its safety has been demonstrated. When a drug interaction is observed in these studies, additional clinical drug interaction studies in patients should be conducted using representative drugs before submitting a new drug application, taking into consideration (1) the properties of any drug with a high possibility of concomitant use in patients, (2) the frequency of drug interaction, and (3) the clinical significance of predicted adverse effects. There may be a need for further clinical drug interaction studies when new drug interactions are demonstrated after approval.

7.2 Indices of drug interaction to be examined

Taking into consideration the mechanism of interaction, appropriate pharmacokinetic parameters should be selected as indices to allow quantitative evaluation of the interaction (refer to the "Clinical Pharmacokinetic Studies of pharmaceuticals (2001)" and "Guideline for Bioequivalency Studies [of generics [1997)"). Evaluation of drug efficacy and side effects may also yield valuable parameters for drug interaction, depending on the type of co-administered drug.

7.3 Criteria for the absence of a drug interaction based on the results of a clinical study

The presence or absence of a drug interaction in clinical studies is determined by statistical analysis. If the 90% confidence interval for the ratio of pharmacokinetic parameters (C_{max} and AUC) falls between 0.80-1.25, a pharmacokinetic drug interaction is generally considered negative. It is necessary, however, to remember that drug interactions within this range may cause clinically significant adverse effects.

7.4 Consideration of Special Populations

Special concern over drug interactions is necessary if the investigational drug is to be used primarily in a special population or if the drug is to be used in special diseases.

Drug clearance is often low in neonates/infants, the elderly, and patients with severe hepatic or renal dysfunction. Side effects may therefore develop in such patients even

with mild inhibition of metabolism or excretion. On the other hand, the clearance of certain types of drugs that are metabolized by P450 is somewhat greater in pediatric subjects than in adults. It is desirable to consider the necessity of conducting drug interaction studies while taking into account the characteristics of each patient population and the properties of the investigational drug. When the patient numbers are large, population pharmacokinetics is a useful method to reduce the burden on each patient.

8. Terminology

Concomitant drug : In a broad sense, this term refers to a drug used in combination with another drug. In a narrow sense, it refers to a drug used in addition to the basic drug treatment.

Interacted drug : Refers to a drug whose kinetics are influenced by combination therapy. Examples are those for which metabolism is reduced or increased by the inhibition or induction of responsible drug-metabolizing enzymes.

Interacting drug : Refers to a drug that affects the kinetics of another drug when used in combination. Examples are inhibitors and inducers of drug metabolism.

Investigational drug : Refers to the drug or drug candidate for which the possibility of causing drug interactions or of being affected by drug interactions is being investigated.

Marker drug : Refers to a drug that is specific to certain drug-metabolizing enzymes, transporters, and plasma proteins for binding, and one that can be used effectively to indicate changes in these processes. It is necessary for the drug to be relatively easy to analyze quantitatively and safe for clinical test.

Multiple-metabolizing enzyme drug : Refers to a drug that is metabolized by two or more enzymes. The influence of metabolic drug interactions is less than that of a single-metabolizing enzyme drug.

Single-metabolizing enzyme drug : Refers to a drug that is metabolized mainly by a single enzyme. For drugs belonging to this category, changes in the responsible enzyme's metabolic activity will greatly influence the drug's metabolic clearance and tend to cause drug interactions.

Substrate : Drugs that are metabolized or transported by specific enzymes or transporters. They generally refer to the drug when used *in vitro* studies.

Transporter：Refers to a carrier, including P-glycoprotein, that transports a drug across a biological membrane.

9. Notes

Note 1: If the volume of distribution has been shown to be close to or less than the extracellular fluid volume, which is about 0.25 L/kg or less in humans, it is considered to be small. If it is about 0.8 L/kg or more in humans, it is considered to be large.

Note 2: When irreversible enzyme inhibition occurs, the inhibition tends to last for a relatively long period of time, and potent drug interactions may appear. Therefore, it is important to examine the possibility, for example, by preincubation of an enzyme preparation with the potential inhibitor. In the case of highly lipid-soluble inhibitors, it is also important to consider the absorption of the drug by constituents of the reaction mixture, such as microsomes, and to correct K_i values by measuring the unbound fraction of the inhibitors.

Note 3: The maximum concentration of the unbound inhibitor in the liver ($C_{uHi\ max}$), which is the major metabolic organ, can be assumed to be nearly equal to the unbound concentration in hepatic capillaries except for cases where the inhibitor is actively uptaken by transporters into the liver or where it is extensively metabolized or excreted by the liver. To avoid false-negative predictions by *in vitro* experiments, $C_{uHi\ max}$ should not be underestimated. Therefore, the maximum unbound concentration of the inhibitor in the blood close to the entrance of the liver is supposed to be the same as that of the unbound drug concentration in hepatic capillary. It is important when assessing an orally administered drug to estimate the concentration while considering both concentrations in circulating blood and intestinal absorption. A $C_{uHi\ max}$(appr), an approximation of $C_{uHi\ max}$, can be estimated by the following equation.

$$C_{uHi\ max}\ (appr) = fu \times (C_{p,max} + (k_a \times Dose \times F_a/Q_h))$$

f_u: fraction of the unbound drug in the blood.
$C_{p\ max}$: maximum concentration in the blood.
k_a: absorption rate constant.
F_a: fraction of intestinal absorption.
Q_h: hepatic blood flow.

k_a and F_a values are often not clear. In these cases, they are assumed to be their maximum value (0.1/ml/l) and 1, respectively, to avoid false-negative predictions.

If active transport of the inhibitors into the liver is anticipated, it may be necessary for obtaining an approximate of $C_{uHi\ max}$ to multiply $C_{uHi\ max}$ by the assumed concentration ratio.

Note 4: When competitive or non-competitive inhibition occurs, and the unbound concentration of the inhibited drug is much less than K_m (this is the case frequently observed with ordinary medication), inhibition is determined by the affinity of the inhibitor for the enzyme and the concentration of the inhibitor, and their relationship is indicated by the following equation:

$$Cp^*/Cp = 1 + C_{ui,max}/K_i$$

Cp^*/Cp indicates the rate of increase in plasma concentration of the inhibited drug (AUC or (average) plasma concentration at steady state), $C_{ui,max}$, maximum concentration of the unbound form of the inhibitor in a major metabolic organ (in the case of the liver, it is indicated as $L_{uHi\ max}$), and K_i, inhibition constant of the inhibitor. This equation suppose that the contribution of the enzyme to systemic clearance to be 100%. That is, it is valid only when the contribution is high. When it is low, the increase is lower than that predicted by this equation. The tissue concentration of the inhibitor decreases over time. However, this procedure utilizes the maximum concentration ($C_{ui,max}$). Therefore,

it should be kept in mind that this procedure tends to overestimate the extent of drug interaction. However, there are cases where no drug interactions are observed even when Cp*/Cp value is large. Therefore, this simple method is useful to indicate that the drug interaction is negative. When possibility of drug interaction is indicated by the calculation, it is useful to employ more accurate pharmacokinetic models like physiology based pharmacokinetic model that incorporate the changes in blood concentration of the inhibitors.
Whenever metabolism by intestinal enzymes like CYP3A4 cannot be ruled out, the unbound concentration of the orally administered drug in the intestinal epithelium tends to be higher than in the liver. In these cases, drug interactions may reach a level that cannot be explained by assuming only inhibition of hepatic metabolism.

Note 5: Species differences in the induction of metabolic enzymes should be considered. For example, induction of CYP3A by rifampicin is observed in humans and is not evident in the rat. On the contrary, induction of CYP4A by clofibrate is observed in the rat and not in humans.

In the case of induction studies in experimental animals, a non-toxic dose is administered repeatedly over a short period of time. Because hepatic drug-metabolizing activities in male rats change depending on androgen status, and tend to be influenced by many factors, female rats are more appropriate models for humans.

10. Questions and Answers

Q1: Is this document applicable to drugs produced by biotechnology or to protein drugs? Is this document also applicable to pharmaceuticals intended for inhalation or topical use, or to pharmaceuticals for which blood concentrations are quite low and are not suitable for an index of the effects?
A1: For drugs produced by biotechnology, identification/quantification of the active molecular species and/or their metabolites responsible for the pharmacological effect is usually more difficult than for chemical compounds, and the amount of interaction information about such drugs is limited. Therefore, it is difficult to apply this document uniformly to drugs produced by biotechnology. Case-by-case measures based on a thorough understanding of the purposes of this document are considered probable. In contrast, pharmaceuticals intended for inhalation or topical use are within the scope of this document. If these pharmaceuticals are said to have a low potential for drug interactions, one should also explain the reason for this, based on *in vitro* results and blood concentrations in clinical studies.

Q2: This document stresses the usefulness of *in vitro* studies and *in vivo* studies in animals for the prediction of interactions in humans and the clarification of the mechanisms of interaction. Please present a clear example.
A2: Depending on the mechanism of drug interaction, there are cases where useful information can be obtained from animal experiments. For example, ① Inhibition of absorption by chelate formation during the absorption process. ② Inhibition of excretion by retardation of secretion in the renal tubules. ③ Concentration changes in plasma and tissue through inhibition of efflux by P-glycoprotein. ④ Inhibition of biliary excretion. It is necessary to note that biliary excretion varies widely among species.

The results of animal studies may be used when safety concerns prohibit human studies. Examples include the following: ① Increased risk of convulsion induced by concomitant

therapy with a quinolone drug and a non-steroidal antiinflammatory drug. ② Cases where irreversible inhibition can be predicted, such as with sorivudine and fluorouracil noted in the document.

Q3: It is noted in the document that when active transport is suspected to contribute greatly to the absorption and efflux processes in the gastrointestinal tract, it may be useful to examine the extent of this contribution using cells that express transporters (2.3). What case does the term "is suspected" suggest?
A3: It suggests cases where the plasma concentration-versus-time curve after oral administration differs greatly from the predicted curve, and the concentration is not proportional to the dose (cases of non-linearity).

Q4: There is a statement of "its clearance (hepatic clearance) is high ・・・" in section 3.1. Please give the clear criterion that determines high or low clearance.
A4: Comparison with hepatic blood flow rate is appropriate. Based on the ratio of clearance to the blood flow rate (extraction ratio), when the ratio is smaller than 0.3, clearance is considered to be low; when the ratio is larger than 0.7, clearance is considered to be high.

Q5: It is stated that when the inhibition of cytochrome P450 is irreversible, extrapolation from *in vitro* to *in vivo* is difficult. Does this mean that a clinical study should be always conducted in such cases?
A5: The extent of inhibition should be predicted based on analyses that involve contact time with the enzyme and/or the turnover rate of the enzyme protein. In general, when the *in vitro* inhibitory concentration is low, and a clinical adverse interaction is predicted, the suspension of drug development would be advised. However, when it is predicted that a drug in development has a benefit that exceeds its risks, a clinical study is conducted with careful consideration for the safety of the subjects, after examining the results of animal studies.

Q6: It is stated that based on the results of drug interactions after metabolic inhibition *in vitro*, animal studies are useful to predict *in vivo* responses in humans. Why are animal studies "useful"?
A6: It is necessary to consider the complex involvement of biological factors in the occurrence of drug interactions *in vivo*. For example, a drug may accumulate in the body after active uptake into tissues or the intracellular space. In a case like this, it is difficult to predict drug interactions in humans from the results of *in vitro* drug interaction studies using enzymes or microsomes and from information on blood concentrations. In a case like this, a clarification of the relationship between unbound concentrations in the blood and the cells by animal experiments is useful. However, existence of species similarities between animals and humans in metabolizing enzymes and in the mechanism and extent of inhibition is a prerequisite for the prediction.

Q7: To determine the hepatic concentration of unbound drug, is any method of estimation permitted if its validity is shown? Please give possible methods (other than that noted in Note 3).
A7: From the current scientific standard, estimation would be considered difficult by the method other than that described in Note 3. New methods for estimation or determination may be developed in the future, and, if so, a method with higher precision should be used.

Q8: Not only for drugs metabolized by a single enzyme but also drugs metabolized by multiple enzymes, is it necessary to estimate the relative contribution of each enzyme to

metabolism?
A8: Estimations of the metabolic route and extent of contribution of the enzymes involved in metabolism are always important for understanding the effect of changes in metabolic activity by drug interaction on the pharmacokinetics of a drug.

Q9: Please definitively explain the method used to judge whether a drug interaction is present.
A9: This judgment is made mainly by using parameters such as maximum concentration (C_{max}) and area under the blood concentration-versus-time curve (AUC), referring to the "Guideline for Bioequivalency Studies (of Generics)." For example, when these parameters have a logarithmic normal distribution, no pharmacokinetic interaction is judged to exist in cases where the 90% confidence intervals of the geometric mean ratios are within 80%-125%. The above criterion should be satisfied both for C_{max} and AUC in order for a conclusion of no interaction to be made. Since the time to reach maximum blood concentration, clearance, volume of distribution, elimination half-life, etc. are important for estimating the results, they should be calculated and discussed. For a drug whose effects and adverse effects are known to depend on a specific pharmacokinetic parameter, selection of the appropriate pharmacokinetic parameter is important after clarification of the reason.
The item noted above is to indicate the absence of a pharmacokinetic drug interaction. Overall, it should be remembered that the estimation of the clinical relevance of an interaction is important, considering the specific features of the drug.

Q10: In this document, the importance of using the unbound drug concentration is discussed. However, it can be interpreted that the criterion for judgment of whether or not a drug interaction exists is based on the total drug concentration. It is possible, however, that the unbound drug concentration may be changed, even though no change in the total drug concentration is observed. How should one describe the relationship between the unbound drug concentration and the criteria for drug interaction?
A10: When drug interactions are examined using the total drug concentration, it is necessary first to present the results yielded by the criteria for the total concentration. It is possible to discuss further with data on protein binding ratio that the unbound concentration of drug may be changed even though no change in the total drug concentration is observed, along with the scientific basis for it. When drug interactions are examined within the time course of the unbound drug, the criterion should be used based on the concentration of unbound drug.