Drug interaction guideline for drug development and labeling recommendations (draft for public comment)

_____ 2014

Table of Contents

1. Introduction

1.1. Background and objectives

It is common in clinical practice for several drugs to be administered concurrently to achieve a therapeutic goal, and under such circumstances, particular attention must be paid to possible interactions among the drugs. Although drug interactions of clinical concern are rare, the adverse effects may be severe in some cases and pose a serious problem in drug therapy. Therefore, in the evaluation and clinical application of drugs, it is necessary to appropriately predict the characteristics and severities of possible drug interactions, and to deal with such interactions so as to prevent any patient risk.

Evaluation of drug interactions during the drug development process requires stepwise accumulation of basic study data and accurate judgment depending on the situation. Thus, planned and systematic investigations are important. The purpose of this guideline is to provide general methods, criteria for judgment, and a general guide for interpretation of the study results and provision of information concerning nonclinical studies aimed at predicting drug interaction potentials and judging the need for implementation of clinical studies, and also information concerning clinical studies aimed at confirming the presence or absence and degree of drug interactions in human. If the possibility of drug interactions that may become major clinical problems is judged early development phase based on these guidelines, more efficient development of drugs is expected to become possible. In addition, adequate provision of information obtained during the drug development process to clinical practice may avoid the occurrence of harmful drug interactions and/or decrease in the efficacy of drug therapy. These actions may be expected to lead to an optimized risk-benefit balance of drugs, eventually promoting the proper use of drugs.

This guideline presents general procedures that are considered to be scientifically valid at the present moment. However, because the physical and chemical properties, pharmacological actions, pharmacokinetics, and clinical usage vary among individual drugs, the methods of evaluation of drug interactions also vary among investigational drugs. Therefore, while implementing drug interaction studies, it is necessary to select the appropriate methods of study according to the properties of the drug in question, based on the principles described in these guidelines. In case of need, new methods of study and means of providing information derived from advances in academic studies and scientific technologies may also be evaluated and adopted proactively.

1.2. Scope

This guideline presents the principles and methods for appropriate provision of information on drug interaction studies and their results during the development of new drugs. This guideline applies

to *in vitro* studies that are carried out in the early phase of drug development using human tissue-derived specimen and expression systems to predict drug interactions in humans and to judge the need to implement clinical studies. This guideline also applies to clinical drug interaction studies that are conducted as needed during drug development, drug interaction studies carried out as necessary after marketing, and provision of information about the results of such studies in the package inserts.

Drug interactions can occur in any route of administration. Although these guidelines mainly provide an outline of drug interactions following oral administration, other routes of administration are also dealt with as appropriate. Drug interactions associated with routes of administration other than the oral route should be studied with reference to this guideline, bearing in mind the fact that the degree of drug interactions would vary among different routes of administration.

This guideline defines drug interactions as interactions among the drugs administered concomitantly that may affect the beneficial effects, adverse effects, or pharmacokinetics of the drugs (including, biotechnological and biological products) and between the drug under study and foods, beverages, or nonessential grocery items (e.g., tobacco, alcohol, nutritional supplements).

Drug interactions are broadly classified into pharmacokinetic drug interactions and pharmacodynamic drug interactions, according to the mechanism of occurrence. The pharmacokinetic drug interactions are caused by changes in the blood concentrations or tissue distribution of the drugs or their active metabolites resulting from interactions occurring in the process of their absorption, distribution, metabolism, or excretion. The pharmacodynamic drug interactions result from overlapping or cancellation of pharmacological actions, or changes in the drug sensitivity due to concomitantly administered drugs. It is difficult for the present guideline to provide general procedures relevant to pharmacodynamic drug interactions. It is necessary to appropriately determine whether studies of pharmacodynamic drug interactions must be implemented according to the pharmacological actions of drugs and anticipated clinical indications. In this guideline, descriptions are focused on pharmacokinetic drug interactions mediated by general drug metabolizing enzymes or transporters. However, it should also be borne in mind that some drugs strongly inhibit enzymes other than the general drug metabolizing enzymes shown in this guideline, as in the case of harmful effects caused by concomitant use of sorivudine and 5-fluorouracil, exerting inhibitory influences on the metabolic disposition of concomitantly used drugs that are metabolized by the enzymes other than general drug metabolizing enzymes, resulting in pharmacokinetic drug interactions. Since it is difficult to conclude necessity of studies of drug interactions during the drug development process because of the currently limited knowledge, this guideline only introduces the possibilities for pharmaceutical interactions and drug effects on the clinical biochemical test.

1.3. Principles of drug interaction studies

Drug interactions between a drug under development (investigational drug) and approved drugs that may be used with the investigational drug should be studied from the two aspects, i.e., the case where the investigational drug is affected by concomitant drugs and the case where the investigational drug affects concomitant drugs. In regard to drugs that are commonly used or likely to be used concomitantly with the investigational drug in the clinical setting, it is necessary to investigate the basic factors contributing to drug interactions by nonclinical studies, prior to the implementation of clinical drug interaction studies. In general, to predict and evaluate the clinical influences of drug interactions, it is necessary to quantitatively determine to what degree the pathway associated with the drug interactions is contributed in the major clearance pathway of the investigational drug. Towards this objective, *in vitro* studies using human tissue−derived specimen and expression systems should be conducted to investigate the possibility of drug interactions occurring in the clinical setting, and clinical studies should be chosen as necessary based on the results of such *in vitro* studies. Clinical drug interaction studies should be performed to confirm drug interactions that should be avoided or that require special attention during drug therapy. It is important that the effects on the therapeutic effects of the drug are considered sufficiently in this manner. The information thus obtained should be appropriately provided to healthcare professionals.

Drug interaction studies are planned and implemented on the basis of the mechanism of drug interactions expected from preliminarily obtained physiological, chemical, pharmacological and pharmacokinetic properties of the investigational drugs. The results of *in vitro* studies and clinical drug interaction studies using strong inhibitors, etc., of drug metabolizing enzymes or transporters are useful for predicting interactions with other drugs that may be used concomitantly. The possibility of drug interactions with the metabolites of the investigational drug should also be studied if necessary, when the blood concentration of the unchanged drug is low, in contrast to high concentrations of metabolites, or when metabolites that may be harmful are produced. When the investigational drug is developed for the purpose of using concomitantly with other drugs, as in the case of development related to combination products or combination therapy, clinical drug interaction studies should be conducted among the drugs in question.

Drug interaction studies in the drug development process should be carried out in a stepwise manner based on the phase of development. *In vitro* studies to evaluate the effects of concomitant drugs on the pharmacokinetics of the investigational drug (when the investigational drug is affected by concomitant drugs) and the effects of the investigational drug on the pharmacokinetics of concomitant drugs (when the investigational drug affects concomitant drugs) should be carried out before the implementation of studies in a large number of patients or on long-term administration (usually before

the initiation of phase III studies),. Usually, *in vitro* metabolism studies are carried out before the initiation of phase I studies, in order to investigate the major metabolites produced and the plasma protein binding ratio. In addition, it is desirable that the results of mass balance studies be obtained before the initiation of phase III studies. Information obtained stepwise from *in vitro* or clinical drug interaction studies according to the aforementioned development policies should be provided appropriately at the time of implementing later clinical studies by providing appropriate descriptions in investigational brochures.

At each stage of drug development, studies using models and simulations such as the physiologically-based pharmacokinetic (PBPK) model may be useful for predicting the possibility of drug interactions and obtaining the information required for the implementation of clinical studies and their designs. In modeling and simulation, a full understanding of the model used and the simulation applied and verification of the reliability of modeling and simulation results are necessary according to the study purpose. In the case of New Drug Application (NDA), it is necessary to provide the assumption concerning the setting of the model and information about the process of model building, and to show the validity of the models and the simulation results from the physiological, medical and pharmaceutical viewpoints, as well as from the statistical viewpoint.

When the mechanism of the major drug interactions observed between the investigational drug and concomitant drugs in the clinical setting is inconclusive, it is recommended to elucidate the mechanism that produces such drug interactions by implementing additional studies.

When implementing clinical drug interaction studies, Good Clinical Practice (Standards for the Conduct of Clinical Trials of Medical Products) (GCP) should be observed, and pharmacokinetic drug interactions should be studied in accordance with "Clinical Pharmacokinetic Studies on Pharmaceuticals (2001)."

2. Drug interactions in absorption

Drug interactions of concern involving the process of absorption from the gastrointestinal (GI) tract are mainly associated with investigational drugs that are administered orally. However, drug interactions of the same type should also be considered for drugs administered by inhalation, nasal, or oral transmucosal route, etc., that may be absorbed from the GI tract after drug administration.

Not only concomitant drugs, but also components of food and beverage may exert significant influences on the process of drug absorption. Many of these influences can be predicted qualitatively based on full understanding of physical and chemical properties and pharmacological actions of the drugs and formulations. The applicability of the following items 2.1-2.2 should be primarily considered. In case of pharmacokinetics changes not predictable from these items are observed, the causes of the

changes should be investigated, including the possibility of drug interactions with drug metabolizing enzymes or transporters mentioned below, if necessary.

The influences of food on the drug absorption process should be examined using the final formulation of the drug, because the influences vary among different formulations. For the definition of the final formulation, "Clinical Pharmacokinetic Studies of pharmaceuticals (2001)" should be consulted.

2.1. Effects on gastrointestinal pH, complex/chelate formation, and solubility

2.1.1. Effects of concomitant drugs on the investigational drug

As for drugs or formulations whose solubility is pH-dependent, the need for clinical drug interaction studies with concomitant drugs that cause changes in the gastric pH (proton pump inhibitors, H_2 receptor antagonists, antacids, etc.) on the GI absorption should be considered.

In addition, because formation of complexes, chelates, micelles may occur as a result of the influence of concomitant drugs and components of food and beverage (e.g., calcium) that decrease or increase the GI absorption of the investigational drug, the possibility of complex formation should be evaluated *in vitro* if necessary, based on the physical and chemical properties of the drug. If the possibility that of the formation of complexes becoming a clinical concern is suggested by the physical and chemical properties and *in vitro* data, the need for clinical drug interaction studies with foods or/and beverages should be considered. In the case of pediatric drugs, the characteristics of the foods, such as ingestion of milk in neonates, should also be taken into consideration.

It is recommended that influence of meals is examined under the conditions that are most susceptible to foods. Drugs that are highly lipid-soluble and low solubility in the GI tract may show increased gastrointestinal absorption due to enhanced solubility in the GI tract caused by increased bile secretion after high-fat meal.

2.1.2. Effects of the investigational drug on concomitant drugs

If the investigational drug changes in gastric pH, the need for clinical drug interaction studies should be considered after predicting the influences on the GI absorption of other drugs that have pH dependency. According to the chemical structure of the investigational drug, the possibility of other mechanisms, such as inhibition of absorption with complex formation, should also be considered.

2.2. Effects on gastrointestinal motility

2.2.1. Effects of concomitant drugs on the investigational drug

Concomitant drugs that influence the gastric emptying rate (propantheline, metoclopramide, etc.) change the rate of absorption of the investigational drug from the GI tract by affecting the dissolution rate of drug formulation and passive into the small intestine. In addition, ingestion of food and beverage often delays absorption due to a delay in the gastric emptying rate. Among these, if pharmacokinetic changes associated with AUC change will be observed, attention should be paid to the possible influences of concomitant drugs on the metabolism and absorption process of the investigational drug.

2.2.2. Effects of the investigational drug on concomitant drugs

The investigational drug that influence on gastric emptying or intestinal motility could also affect the pharmacokinetics of other concomitant drugs. In this case, the possibility of occurrence of drug interactions of clinical concern should be considered, and if necessary, clinical drug interaction studies should be conducted using the appropriate marker drugs (e.g., acetaminophen as an index of the effect on gastric emptying). In many cases it should be borne in mind that such influences of investigational drug on gastric emptying or intestinal motility can result in the systemic effects of investigational drugs administered by the parenteral route. Absorption of drugs may also be influenced by activity of transporters in GI tract as well. See sections 2.3 and 5.6.2. for evaluation of effects of the investigational drug (or affect the investigational drug) on the active transport of other drugs.

2.3. Involvement of transporters in the absorption process

Some drugs that are absorbed by transporters expressed on the cell membrane on the luminal side of the GI tract epithelial cells may interact with other drugs or diet components that are subject to absorption by the same transporters, resulting in decreased absorption. Efflux transporters are expressed on the cell membrane on the luminal side of the GI tract epithelial cells, and some drugs taken up by epithelial cells from the luminal side are sometimes pushed back to the luminal side of the GI tract by efflux transporters before being reached to the basal side (portal side). It has been reported that certain drug interactions cause increased absorption through inhibition of these efflux process in the GI tract[1, 2] (see Table 6-1). In addition, some drugs are reported to induce the expression of efflux transporters (P-glycoprotein (P-gp)) in the GI tract, and decrease the absorption of other drugs[3, 4] (see Table 6-2).

It has been demonstrated that both breast cancer resistance protein (BCRP) and P-glycoprotein (P-gp) expressed on the luminal side of the GI tract epithelial cells function as efflux transporters that decrease the GI absorption of substrate drugs (see Table 6-1). Therefore, the contribution of P-gp or BCRP to the GI absorption of the investigational drug should be evaluated in *in vitro* studies.

Recommended *in vitro* studies are bidirectional trans-cellular transport studies using Caco-2 cells or transporter-expressing cell lines. If the results of the experiments are positive, the need of clinical drug interaction studies is recommended to be considered (see section 6.2 and Figure 6-2 for consideration procedures). If the results suggest that transporters other than P-gp or BCRP exert large effects on the absorption or efflux processes in the GI tract, it may be considered that the contributing transporters are identified, and the degree of their contribution is determined using Caco-2 cells or transporter-expressing cell lines. Implementation of clinical drug interaction studies may also be considered according to the need.

If the investigational drug inhibits P-gp and BCRP, the inhibitory effects of the investigational drug on P-gp and BCRP should also evaluated in *in vitro* studies, because absorption of the substrate drugs may be enhanced in the presence of concomitant substrate drugs for these transporters. Based on the results of such studies, the need to implement clinical drug interaction studies may be considered (see section 6.2 and Fig. 6-3 for consideration procedures). In addition, if there is a suggestion that the inhibitory effect of the investigational drug on transporters other than P-gp or BCRP might affect the absorption of concomitant drugs, it may be considered that the degree of this effect is determined by *in vitro* studies, and the implementation of clinical drug interaction studies may be considered according to the need.

As for interactions with diet components and nutritional supplements, induction of P-gp by St. John's Wort and inhibition of uptake transporters by grapefruit juice, orange juice, and apple juice were reported[5,6].

2.4. Drug interactions related to drug metabolizing enzymes in gastrointestinal tract

CYP3A is frequently expressed in the GI tract, particularly in the mucosa of the small intestine. In case of the investigational drug shows markedly decreased bioavailability due to first-pass metabolism by CYP3A in the small intestine, it may be caused unexpected adverse event with concomitant drugs that inhibit CYP3A by increasing the bioavailability of the investigational drug. On the other hand, in case of CYP3A in the small intestine as well as in the liver is induced by concomitant drugs that induce CYP3A, the blood concentration of the investigational drug may decrease. As a result, the blood concentration of the investigational drug may not reach the therapeutic range and the desired efficacy may not be achieved. Therefore, it is preferable to evaluate drug interactions in the small intestine if necessary, taking into account the degree of decrease in the bioavailability due to first-pass metabolism of the investigational drug. On the other hand, in case of the investigational drug inhibits CYP3A, *in vitro* studies should be carried out from the perspective of

metabolic inhibition in the small intestine, and the need to conduct clinical drug interaction studies should be considered (see sections 4.1-2 and Figs. 4-1-3).

Influences of food and beverage components that inhibit CYP3A should also be taken into consideration. For example, grapefruit juice contains a substance that strongly inhibits CYP3A. It has been reported that the bioavailability of oral drugs that are metabolized mainly by CYP3A were increased when they were taken with grapefruit juice[7)].

Because the substrates of CYP3A are often also the substrates of P-gp, it is currently difficult to evaluate the separate contribution of CYP3A and P-gp to drug interactions. Therefore, drug interactions should be evaluated bearing in mind the risk of interactions due to inhibition or induction of both.

3. Drug interactions related to tissue distribution

Many drugs bind to plasma proteins, and bind to proteins and/or other components in tissues. Since unbound form drugs is available for transport between the plasma and tissue, changes in the unbind fraction due to displacement may lead to drug interactions. For the case of some drugs, transporters are involved in their distribution in tissues.

3.1. Plasma protein binding

Although the major protein to which drugs bind in the plasma is albumin, some drugs also bind to α_1-acid glycoprotein, lipoprotein and other proteins. If the investigational drug shows high binding ratios to plasma proteins *in vitro*, it is necessary to determine the type of binding proteins and the degree of binding ratio.

The most common cause of changed distribution of the investigational drug due to drug interactions is displacement of the drug bound to plasma protein. In the presence of concomitant drugs that strongly bind to plasma protein, the investigational drug becomes detached from the binding protein, resulting in an increased plasma concentration of the unbound form of the investigational drug. Although, displacement of the investigational drug does not cause clinically significant changes, in the case of the investigational drug which have a plasma protein binding ratio of about 90% or higher, a narrow therapeutic range and fulfill any one of the following conditions, it should be considered that significant drug interactions may occur between the investigational drug and concomitant drugs that strongly bind to plasma proteins.

1) The investigational drug shows a small distribution volume. In this case, the clearance rate of the drug and route of administration of the investigational drug are irrelevant.

2) The investigational drug eliminates mainly via the liver. In particular, the investigational drug is a high liver clearance and is administered intravenously.
3) The investigational drug eliminate mainly via the kidney, and its renal clearance is high. In this case, the route of administration is irrelevant.

On the other hand, it is limited that the drug that changes pharmacokinetics of concomitant drugs through displacement of protein binding is at least same level of plasma concentration compared to binding protein concentration. And because the occurrence of adverse events and changes in drug efficacy that are clinically significant depend on the concentration of the unbound form of the drug, it should be considered that the concentration of the unbound form of the drug is measured in clinical drug interaction studies. In the case of the investigational drug for which the volume of distribution in humans is large and hepatic clearance is low, displacement of plasma protein binding reduces the total plasma concentration of the drug in plasma, but no clinically significant consequences occurs, since the unbound concentration does not change appreciably. As an example of this case, it has been reported that phenytoin in the steady state which is administered with valproic acid shows no changes the unbound plasma concentration whereas the total plasma concentration decreases[8)].

3.2. Tissue distribution

In addition to drug interactions due to variations in binding to specific tissue components, we should bear in mind the possibility that the tissue distribution of the investigational drug might vary in response to variations in the functions of uptake or efflux transporters expressed in each tissue.

3.2.1. Binding to specific tissue components

Some drugs specifically bind to receptors, proteins, lipid, etc., in tissues, and may be associated with drug interactions due to changes in the tissue concentration of the unbound form of the drug as a result of competition in binding.

3.2.1. Binding to specific tissue components

Some drugs specifically bind to receptors, proteins, lipid, etc., in tissues. The concentration unbound drug in tissue may change as a result of competition for binding, which may result in a drug interaction.

3.2.2. Involvement of transporters in the process of tissue uptake and excretion

Transporters are expressed in the liver, kidney and barrier tissue in the brain, placenta, retina, etc. and are involved in the distribution (uptake/efflux) of the drug in each tissue. When drug

interactions involving the active transport process via transporters take place, the unbound concentration of the drug in the tissue in question may be affected (the concentration is decreased by inhibition of uptake, and increased by inhibition of efflux), resulting in some changes in the pharmacological/adverse effects in the tissues. *note (1)

Drug interactions involving tissue distribution are not necessarily reflected in changes in the plasma concentrations of the drugs. In particular, when such drug interactions are found only in the tissues with a small distribution volume relative to the distribution volume of the whole body, variations in the drug concentration in the tissue in question are not reflected in changes in the plasma concentrations of the drugs, necessitating careful attention to be paid. On the other hand, when drug interactions occur in major distribution and elimination organs such as the liver and kidney, they may affect the distribution volume and systemic clearance of the drug, causing variations in the plasma concentrations of the drugs (See 5.1 and 5.2).

4. Drug interactions in drug metabolism

In drug interaction studies involving drug metabolism, it is important to identify the metabolic pathway associated with the interactions, and to quantitatively determine the importance of the metabolic pathway in the overall elimination pathway when the investigational drug is the "affected drug" (or victim drug, the investigational drug is affected by other drugs) or evaluate the effects on the activity of the metabolic pathway according to the mechanism of the interactions such as inhibition and induction when the investigational drug is the "interacting drug" (or perpetrator drug, the investigational drug affects other drugs). In drug metabolism, a single enzyme is frequently involved in the elimination of many drugs. In particular, the most important drug metabolizing enzyme, cytochrome P450 3A (CYP3A), has a wide substrate specificity and is associated with drug interactions of a number of drugs, and therefore, it is difficult to carry out numerous clinical studies. Use of modeling and simulation based on the results of a relatively limited number of clinical drug interaction studies, with due consideration of the reliability, may be helpful.

Many of the drug interactions involving drug metabolism are related to oxidative metabolism, particularly that related to cytochrome P450 (P450). Non-P450 enzymes such as UDP glucuronosyl transferase (UGT) are also known to be involved in drug interactions[9]. This section mainly addresses studies of the possibility of drug interactions mainly involving P450. Practical methods are described in the following sections: section 4.1 deals with identification of the major elimination pathway and evaluation of the degree of contribution of its pathway to drug interactions, and section 4.2 deals with investigation of the possibility of drug interactions with P450 and other drug metabolizing enzymes separately. In addition, representative examples of *in vitro* P450 marker enzyme reactions, inhibitors

and inducers, and those of *in vivo* P450 inhibitors, inducers and substrates are shown (see Table 4-1 to 4-3 and 7-1 to 7-3).

4.1. Evaluation of the major elimination pathway of the investigational drug and the *in vivo* contribution ratio

To investigate the possibility of the effects of an orally administered investigational drug being affected by other drugs, and to quantitatively evaluate the degree of contribution of drug interactions, an important factor is the *in vivo* contribution ratio (CR) of the pathway involved in the drug interactions to the clearance (CL/F) of the investigational drug after oral administration[10]. If the major elimination pathway of the investigational drug is metabolism, drug metabolizing enzymes contributing highly should be identified according to the investigation procedures shown in sections 4.1.1. and 4.1.2., and the degree of the contribution should be clarified as much as possible (see Fig. 4-1). In general, when estimating the CR from *in vitro* metabolism studies, the fraction metabolized (f_m) by the drug metabolizing enzyme in question in human liver microsomes, etc., should be used in substitution.*[note (2)] When the CR of the elimination pathway controlled by a certain drug metabolizing enzyme to the overall elimination of the investigational drug is estimated to be 25% or more from the results of *in vitro* metabolism studies and clinical pharmacokinetic studies, implementation of clinical drug interaction studies using drugs that affect the enzyme in question (inhibitors, inducers: see Tables 7-1 and 7-2) should be considered. Even in the case of orally administered drug in the clinical indication, intravenous administration study of the investigational drug would be useful since the contribution of renal excretion to the total clearance of the drug becomes clear.

When the investigational drug is a pro-drug, and the main action is exerted by the active metabolite, when pharmacologically active metabolite is produced and the *in vivo* pharmacologic effect of the metabolite estimated from the *in vitro* activity and the AUC of the unbound form of the investigational drug accounts for at least 50% of the entire pharmacologic effect, or when it is suspected that adverse effects may be induced by the metabolite, the drug metabolizing enzyme involved in the major production pathway and elimination pathway of the metabolite in question should be identified, and studied in the same manner.

4.1.1. Identification of enzymes involved in the major elimination pathway by *in vitro* metabolism studies

For implementation of *in vitro* studies, the experimental method, study system, appropriate substrate/interacting drugs and their concentrations should be chosen to reflect the *in vivo* metabolic profile of the investigational drug. Usually, human liver and small intestine microsomes, S9 fraction,

human hepatocytes, microsomal fraction of the expression system (recombinant cells) of human enzymes, etc., are chosen according to the type of the enzyme. P450 and UGT are present in all of the aforementioned systems (recombinant cells usually expresses only one species of enzyme in high level). Enzymes present in the soluble fraction, such as sulfotransferase, glutathione transferase, aldehyde dehydrogenase and alcohol dehydrogenase are contained in the S9 fraction and hepatocytes. Transporters are also expressed in the hepatocytes. Interpretation of the study results requires adequate consideration of the characteristic features of the *in vitro* study system used.

In vitro metabolism studies, in general, should be carried out using therapeutically relevant concentrations of the investigational drug, if possible, under linear conditions. In a multi-enzyme system, it is possible to evaluate the contribution of each enzyme to the metabolism of the investigational drug by adding enzyme-specific inhibitors (see Table 4-2). If the specificity of the inhibitor is not high enough, use of *in vitro* study systems expressing only a specific drug metabolizing enzyme is recommended. If an antibody for which specificity has been sufficiently corroborated is available, it can be used as a substitute for inhibitors. In addition, use of positive control (marker substrate) for enzymatic activity in the study is recommended. To identify the major enzymes involved in metabolism under *in vitro* conditions, evaluation of the investigational drug in multiple *in vitro* study systems and verification of the results by comparison are recommended.*[note (3)]

Metabolic rate is evaluated as the elimination rate of the investigational drug or the formation rate of its metabolites. When evaluating the activity of the enzyme that catalyzes a specific metabolic pathway, investigation of the formation of metabolites rather than decreases of the investigational drug or marker substrate drug is recommended. On the other hand, for the purpose of determining the contribution of the metabolic pathway in question to the overall elimination pathway of the investigational drug, it is very important to evaluate decreases of the investigational drug in question.

4.1.2. Identification and quantitative evaluation of the major elimination pathway by mass balance studies

Human mass balance studies are studies that determine the mass balance of drugs in the human body; they provide information on the pharmacokinetics of the unchanged drug and metabolites and useful information for presuming the major elimination pathway. Combining the data obtained from mass balance studies with the results of *in vitro* studies, we can determine the *in vivo* major elimination pathway of the investigational drug and estimate the CR of the enzymes involved in the elimination pathway. However, mass balance studies are particularly useful for identification of the major elimination pathway when the metabolism is relatively slow and when the metabolic pathway is simple. On the other hand, when multistep metabolism occurs in a vigorous fashion, interpretation of the

results requires caution. When the recovery of the unchanged form of the investigational drug and its known metabolites is high and when there are few unknown metabolites of the investigational drug, use of a radiolabeled material is not necessary in mass balance studies.

In mass balance studies, the investigational drug should be radiolabeled at a metabolically stable position usually. After administration, the AUC of the total radioactivity, AUC of the unchanged drug and metabolites, and the urinary and fecal excretions are determined. It is desirable to identify as many drug-related substances as possible. In general, it is recommended to identify the structure of metabolites whose AUC exceed 10% of the total of AUCs of the drug-related substances.

Estimation of the *in vivo* major elimination pathway of the investigational drug and the CR of the enzymes involved in the elimination pathway, based on the information obtained from mass balance studies and the results of *in vitro* studies, should be carried out by the following procedures. Based on the metabolic reactions expected from the chemical structure of the investigational drug and the metabolites determined in mass balance studies, the metabolic pathways are presumed. Then, based on the amount of drug-related substances excreted as primary and secondary metabolites in a specific pathway, the quantitative CR of each metabolic pathway to elimination is estimated. The estimated CR of the major pathway to the total elimination (including first pass) of the investigational drug is calculated by dividing the total amount of all metabolites derived from a major pathway by the dose of the investigational drug or the total amount of drug-related substances found in the excreta. When a considerable amount of unchanged drug is found in the feces and when this is not confirmed to be derived from biliary excretion (or GI tract wall secretion), the denominator of the calculation formula should be the value obtained by subtracting the amount of the unchanged drug found in the feces from the amount of drug-related substances found in the excreta. By these procedures, the *in vivo* CR (maximum estimate) of each (major) elimination pathway is calculated.

4.2. Evaluation of the need of clinical drug interaction studies based on *in vitro* studies

In vitro enzyme inhibition studies use human liver microsomes, human hepatocytes, microsomal fraction of the expression system (recombinant cells) of the target enzymes, etc. If the investigational drug is metabolized extensively in the reaction mixture, a marker substrate drug with a sufficiently high metabolic rate should be used to minimize reduction in the concentration of the investigational drug, and the K_i (inhibition constant: dissociation constant of the inhibitor from the enzyme-inhibitor complex) should be evaluated. Positive control studies should be carried out using a known selective inhibitor (see Table 4-2), and the results should be compared with K_i values previously evaluated in the same manner and reported in the literature.

It is desirable to use hepatocytes in primary culture (fresh or cryopreserved) in *in vitro* enzyme induction/down-regulation studies. At present, data obtained from other *in vitro* study systems, such as human hepatic tumor-derived cell lines (e.g., HepaRG), nuclear receptor-binding assay, and reporter gene assay, are positioned as auxiliary data to support the data obtained from the primary culture hepatocyte system. In general, the results obtained by the use of hepatocytes in primary culture show large variations among different individuals and lots. Therefore, evaluation should be carried out using hepatocytes derived from 3 or more donors and include appropriate vehicle control and positive control to guarantee the validity of the study system[*note (4)] (see Table 4-3). Use of changes in the mRNA expression level of the target gene as an evaluation item is recommended in order to avoid overlooking the enzyme induction effect because of enzyme inhibition by the investigational drug. However, if it is apparent that the investigational drug is devoid of enzyme inhibition (especially time-dependent inhibition, see section 4.2.1.3), it would be possible to use the enzyme activity as an evaluation item. If concentration-dependent changes in the enzyme activity is observed, the need for clinical drug interaction studies should be judged by the similar criteria as for changes in the mRNA expression level (see section 4.2.1.6).

4.2.1. Methods of drug interaction studies related to cytochrome P450 (P450)

Although it is known that cytochrome P450 (P450) has a large number of isozymes, the major isozymes are CYP1A2, 2B6, 2C8, 2C9, 2C19, 2D6, and 3A (including CYP3A4 and CYP3A5). If the investigational drug undergoes metabolism by these P450 isozymes, their contribution to clearance should be estimated by *in vitro* metabolism studies and clinical pharmacokinetic studies. When *in vitro* studies suggest the possibility of inhibition or induction of the metabolism of the investigational drug, clinical drug interaction studies should be implemented (see Figs. 4-1-3). If major P450 isozymes are not involved in the metabolism of the investigational drug, the possibility for the investigational drug to serve as the substrate of other P450 (e.g., CYP2A6, 2E1, 2J2, 4F2) or phase I enzymes other than P450 should be investigated.

4.2.1.1. *In vitro* studies to examine the possibility for effects of concomitant drugs on the investigational drug

For estimation of the CR of the P450 isozyme, study systems using human liver microsomes are generally adopted. The validity of the study system is usually confirmed by evaluating the reaction time dependency and microsomal protein content dependency in terms of the rate of formation of metabolites as an index. Because the fm of P450 isozymes may vary according to the study conditions,

such as the concentration of the investigational drug used, it is necessary to use a study system that reflects the *in vivo* physiological conditions[11-13].

4.2.1.2. Need for clinical studies to examine the possibility for effects of concomitant drugs on the investigational drug

When the contribution of a certain P450 metabolic pathway accounts for 25% or more of the overall elimination pathway of the investigational drug as determined by the results of *in vitro* metabolism studies, mass balance studies, etc.,*[note (5)] it is necessary to implement clinical drug interaction studies using appropriate drug metabolizing enzyme inhibitors/inducers (see Tables 7-1, 7-2) because the investigational drug may be a drug affected by the interaction in which P450 is involved (see Fig. 4-1). In such studies, a strong inhibitor (see section 7.7 and Table 7-1) should be used first as much as possible to evaluate the degree of changes in the pharmacokinetics of the investigational drug. When the study results are judged to be negative (see section 7.3 and Fig. 4-1) or when interactions are minimal, the contribution of the enzyme in question to the overall elimination of the investigational drug is likely to be small, and thus there is no need to implement additional clinical drug interaction studies. On the other hand, when the results of interaction studies using strong inhibitors have suggested that the investigational drug is affected by drug interactions that may require dose adjustment, the effects of other inhibitors in the same metabolic pathway should be evaluated in clinical drug interaction studies according to need, taking into consideration the possibility that they are used concomitantly in clinical practice. Interactions with other inhibitors can be investigated on the basis of data on cases of concomitant use in usual clinical studies, or by simulations using an appropriate PBPK model. Clinical drug interaction studies with inducers are required when the risk of clinically significant drug interactions is inferred by simulations or other procedures based on the results of clinical drug interaction studies with inhibitors. Because St. John's Wort, a supplement, contains substances that strongly induce CYP3A, caution is necessary in use with investigational drugs that are mainly metabolized by CYP3A.

4.2.1.3. *In vitro* studies to examine the possibility for effects of the investigational drug on concomitant drugs (inhibition of P450)

In vitro studies should be carried out to determine whether the investigational drug exerts an inhibitory effect on P450 (see Fig. 4-2). Usually, inhibitory effects on major P450 isozymes such as CYP1A2, 2B6, 2C8, 2C9, 2C19, 2D6, and 3A are investigated. Table 4-1 shows the representative marker reactions of P450 isozymes used in the *in vitro* studies. Literature should be referred to for the concentrations of the marker substrates to be used in *in vitro* studies. Inhibition of CYP3A should be

evaluated using multiple marker substrates which differ in the substrate binding site on the enzyme, such as midazolam and testosterone[14].

The inhibitory effects of the investigational drug should be evaluated within a certain range of concentrations to determine the K_i value for the marker reaction of the P450 isozyme in question. The range of concentrations of the investigational drug should be set to include a sufficiently high concentration to allow appropriate evaluation of clinically possible inhibition. The set range of investigational drug concentrations may vary according to the expected site of enzyme inhibition (liver, small intestine), method of administration, dosage form, and pharmacokinetic parameters (C_{max} or AUC). However, the concentration range is usually set to include 10-fold or higher values of the C_{max} (bound + unbound forms), and the K_i value is calculated for cases of 50% or higher inhibition. For calculating the K_i value in the *in vitro* study system, the estimated or measured value of the concentration of the unbound drug in the reaction mixture is used if the concentration of the unbound form of the investigational drug in the reaction mixture is expected to be markedly lower than the total concentration of the investigational drug[15]. This applies to cases where the investigational drug is likely to be prominently adsorbed to the test tube wall.

It is desirable to investigate the enzyme inhibition effects of major metabolites in addition to those of the unchanged drug. The target metabolites should be chosen according to the following criterion: any phase I metabolite whose AUC accounts for at least 25% of the unchanged drug and at least 10% of the total AUC of drug-related substances. Inhibitory effects of other metabolites should also be investigated if there is some reason for suspecting strong enzyme inhibition. When it has been shown that the drug interactions observed in *in vivo* studies are attributable to a specific metabolite, implementation of *in vitro* enzyme inhibition studies using the metabolite would be helpful for designing clinical drug interaction studies and interpreting their results. Determination of the blood concentrations of metabolites that are possibly related to the drug interactions are also recommended in clinical drug interaction studies.

When investigating the inhibitory effect of a metabolite, the concentration should be set in a range containing 10-fold or higher of the C_{max} (bound + unbound forms) of the metabolite, similarly as the case for the unchanged drug. When calculation of the K_i value is necessary, the binding ratio in microsomes, etc., should be estimated or measured to achieve correction for the concentration of the unbound form.

When the inhibitory effect is enhanced by pre-incubation in *in vitro* studies, it should be judged that there is time-dependent inhibition (TDI). When there is TDI, the k_{inact} value (maximum inactivation kinetic constant) and the K_I value (the concentration of the inhibitor that yields a 50% rate of the maximum inactivation) should be estimated[16]. The TDI should be evaluated taking due account

of the possibility that the conditions of the *in vitro* studies (e.g., evaluation of nonspecific binding is necessary when the concentration of protein in the reaction mixture is high, so that the concentration of the unbound form of the investigational drug is expected to be markedly low) may affect the results.

4.2.1.4. Need for clinical studies to examine the possibility for effects of the investigational drug on other concomitant drugs (inhibition of P450)

Whether to implement clinical drug interaction studies to evaluate the possibility of the investigational drug serving as an inhibitor should be determined according to the following cutoff criteria based on *in vitro* data, etc. (see Fig. 4-2). In addition to evaluation by the cutoff criteria, investigation using study models are available, such as the mechanism-based static pharmacokinetic (MSPK) model and the dynamic physiologically-based pharmacokinetic (PBPK) model (see section 4.3.). For evaluation by the cutoff criteria, the ratio of the intrinsic clearance value of the substrate for a specific enzyme marker reaction (R value) in the presence and absence of the investigational drug should be calculated. The presence/absence of the need to implement clinical drug interaction studies should be judged based on the estimated R value. If a value in excess of this criterion is observed in the evaluation of the investigational drug, the risk should be investigated in clinical drug interaction studies using a substrate drug that is susceptible to pharmacokinetic drug interactions (see section 7.9 and Table 7-3).

In investigations using models, 90% confidential interval for AUC ratios (AUCR) of 0.8-1.25 used in the evaluation of bioequivalence can be used as the initial criteria. More specifically, if the AUCR estimated from models falls outside the range of 0.8-1.25, clinical drug interaction studies will be required. In addition, because there is only limited experience with the use of models in the quantitative evaluation of drug interactions caused by bidirectional actions of inhibition (reversible or TDI) and induction, conservative judgments for the need to implement clinical drug interaction studies should be made at present, based on separate evaluation of inhibition and induction[17)].

1-1) Reversible inhibition

The R value is determined from the *in vitro* inhibition constant (K_i) and the maximum concentration [I] of the inhibitor (investigational drug or metabolite) achieved *in vivo*, according to the following formula.

Formula 1

$$R = 1 + [I]/K_i$$

[I]: C_{max} (concentration of the bound form + concentration of the unbound form), or $[I]_g$: dose/250 mL

672 K_i: inhibition constant measured *in vitro*

673 In place of the K_i value, the 50% inhibitory concentration (IC_{50}) may be used. However, when
674 the IC_{50} is used, its scientific basis should be shown. For example, it is possible to consider $K_i=IC_{50}/2$
675 assuming competitive inhibition when the substrate concentration is close to its K_m value, or to
676 consider $K_i=IC_{50}$ under the linear condition where the substrate concentration is much lower than its
677 K_m value.

678 Usually, the maximum systemic blood concentration (C_{max}) of the inhibitor (concentration of
679 the bound form + unbound form of the drug) should be used as the conservative [I], and a cutoff
680 criterion of 1.1 should be used for the R value[17, 18]. In the case of orally administered drugs, the
681 possibility of inhibition of P450 (example: CYP3A) that is highly expressed in the GI tract should be
682 borne in mind. The use of dose (molar dose)/250 mL as the maximum concentration in the GI tract, $[I]_g$,
683 may reflect the maximum concentration of the inhibitor more appropriately than the systemic blood
684 concentration. If $[I]_g$ is used, a cutoff criterion of 11 should be used for the alternate R value ($R = 1 +$
685 $[I]_g / K_i$). If the R value is less than 1.1 or 11 (alternate R value), implementation of clinical drug
686 interaction studies is not required. If these criteria are exceeded, clinical drug interaction studies using
687 substrate drugs susceptible to pharmacokinetic drug interactions (section 7.9, Table 7-3) should be
688 carried out for the P450 with the greatest R value, taking into consideration the results obtained from
689 investigation using the models described in section 4.3. If the results of such clinical drug interaction
690 studies are judged to be negative, implementation of clinical drug interaction studies involving other
691 P450 isozymes are not required.

692

693 1-2) Time-dependent inhibition (TDI) *[note (6)]

694 Although most drug interactions that cause inhibition of P450 are reversible, TDI may be
695 observed when the inhibitory effect increases over time and is not completely reversible. TDI seems to
696 be attributable to irreversible covalent binding or semi-irreversible and strong non-covalent binding of
697 the produced intermediate to the enzyme that mainly catalyzes the formation of the highly chemically
698 reactive metabolic intermediate.

699 The standard *in vitro* method of TDI evaluation involves pre-incubation of the investigational
700 drug in the study system before adding the substrate. If the formation rate of the metabolite of the
701 substrate decreases in a time-dependent manner, TDI is suggested, warranting calculation of the
702 parameters (k_{inact} and K_I) of TDI in *in vitro* studies[16]. In general, evaluation is based on the assumption
703 that the level of the inhibited enzyme has reached another steady state in the presence of the inhibitor,
704 and that the inhibitor does not affect de novo synthesis of the enzyme. Unlike reversible inhibition, the

R value of TDI is dependent on the degradation rate constant (k_{deg}) of the inhibited enzyme, as well as on the exposure of the inhibitor and the parameters of TDI (k_{inact} and KI) (Formula 2).

Formula 2

$R = (k_{obs} + k_{deg}) / k_{deg}$, provided that $k_{obs} = k_{inact} \times [I]/(K_I + [I])$

[I]: C_{max} (concentration of the bound form + unbound form of the drug), or $[I]_g$: dose/250 mL

KI: concentration of the inhibitor that yields 50% of the maximum inactivation rate

k_{deg}: degradation rate constant of the enzyme

$k_{inac}t$: maximum inactivation rate constant

When the possibility of TDI is suggested by the results of *in vitro* studies (for instance, R > 1/1 in the liver, or R > 11 in the small intestine), clinical drug interaction studies using substrate drugs (Table 7-3) susceptible to pharmacokinetic drug interactions should be implemented, taking into consideration the results obtained from investigation using the models described in section 4.3, similarly as in the case of reversible inhibition.

4.2.1.5. *In vitro* studies to examine the possibility for effects of the investigational drug on concomitant drugs (induction and down-regulation of P450)

The possibility that the investigational drug causes induction or down-regulation*[note (7)] of the drug metabolizing enzyme mediated by influences on the nuclear receptors or other regulation pathways of P450 expression, resulting in drug interactions, should be investigated (see Fig. 4-3). Although, in general, the need for clinical drug interaction studies is examined based on the results of *in vitro* studies, induction may be evaluated directly by *in vivo* studies in some cases.

The enzyme induction effect is usually investigated in *in vitro* studies, focusing on CYP1A2, 2B6, and 3A. CYP3A and CYP2C are co-induced by activation of pregnane X receptor (PXR), which is a nuclear receptor. Therefore, when the results of *in vitro* studies to evaluate induction of CYP3A are negative, there is no need to conduct further clinical drug interactions studies for CYP3A or *in vitro*/clinical induction studies for CYP2C. When the results of the CYP3A induction studies are positive, induction of CYP2C should be further investigated in either *in vitro* or clinical studies. Because CYP1A2 and CYP2B6 are induced by nuclear receptors (AhR and CAR) other than PXR, the possibility of induction of CYP1A2 and CYP2B6 by the investigational drug should be investigated regardless of the results of the studies on CYP3A.

The concentration range of the investigational drug in the *in vitro* induction study varies according to their *in vivo* pharmacokinetics and should be set to include more than three levels of concentrations, including the maximum concentration predicted for hepatocytes *in vivo*, to determine

induction parameters such as the EC_{50} and E_{max}. Usually, in the case of drugs that exert influences on hepatic enzymes, the concentration range should be set to include a level at least 10-fold that of C_{max} (bound + unbound forms) obtained in the steady state after administration of the maximum therapeutic dose. In general, the mRNA level is compared with that of the control (vehicle), and enzyme induction in *in vitro* studies is regarded as positive when increases in the mRNA level after treatment with the investigational drug at the aforementioned concentrations are concentration-dependent, showing an over 100% increase. When the observed concentration-dependent increases in mRNA expression are less than 100%, the results can be regarded as negative as long as the increase corresponds to less than 20% of that in the reaction with a positive control.

4.2.1.6. Need for clinical studies to examine the possibility for effects of the investigational drug on concomitant drugs (induction and down-regulation of P450)

The R value is calculated to be compared with a cutoff criterion according to the following Formula 3, using the EC_{50} and E_{max} obtained from *in vitro* studies.*[note (8)] In addition to evaluation by the cutoff criteria, the MSPK model and PBPK model are also available for evaluation (see section 4.3).

Formula 3

$$R = 1/(1+d \cdot E_{max} \cdot [I]/(EC_{50}+[I]))$$

[I]: C_{max} (concentration of the bound form + unbound form of the drug)

EC_{50}: concentration that yields 50% of the maximum effect, E_{max}: maximum induction effect, d: conversion factor

For evaluation based on the cutoff criteria, $d = 1$ is used. If R is less than 0.9 ($R < 0.9$), the investigational drug in question is regarded as the enzyme-inducing drug.

4.2.2. Methods of drug interactions studies related to other drug metabolizing enzymes*[note (9)]

Phase I enzymes (enzymes involved in oxidation, reduction, hydrolysis, ring closure and ring-cleavage reactions) other than P450 that are involved in drug metabolism include monoamine oxidase, flavin monooxygenase, xanthine oxidase, aldehyde oxidase, alcohol dehydrogenase, and aldehyde dehydrogenase. When the investigational drug is the substrate of these phase I enzymes other than P450, identification of the enzymes/isozymes involved and determination of the degree of contribution, if their contribution to clearance is large, are also recommended. The possibility of the investigational drug serving as the substrate for these enzymes may be evaluable based on the previous findings available for the same therapeutic category drugs in some cases.

As for phase II enzymes, if the investigational drug is a substrate of UGT, the degree of contribution of UGT1A1, 1A3, 1A4, 1A6, 1A9, 2B7, 2B15, etc., in the elimination pathway should be investigated. In this case, examination of the inhibitory effect of the investigational drug is recommended not only on the UGT isozyme mainly involved in its metabolism but also on the UGT isozymes known to be involved in metabolism of a relatively large number of medical products (e.g., UGT1A1 and UGT2B7).

If the investigational drug or co-administered drug is mainly metabolized by enzymes other than those mentioned above, it is desirable to evaluate its inhibitory effect on such enzymes. Bearing in mind the examples of serious adverse effects observed in the concomitant use of sorivudine and 5-fluorouracil, if the degree of contribution of the enzymes other than P450 and UGT to the major elimination pathways of the drugs that are co-administered with the investigational drug is large, the inhibitory effects of the investigational drug and its metabolites on the corresponding enzymes should be examined. Evaluation of the need to implement clinical drug interaction studies based on the results of the above studies is in accordance with the case for P450.

4.3. Cutoff criteria and model analysis of metabolism-based drug interactions

To judge the need for clinical drug interaction studies, cutoff criteria should be employed in principle. However, cutoff criteria do not consider the characteristics of concomitant drugs, and the model-based analysis may provide additional values when clinical studies are planned*[note (10)] (see Figs. 4-2, 3). For such studies, the mechanism-based static pharmacokinetic (MSPK) model, and physiologically-based pharmacokinetic (PBPK) model, etc., are utilizable.

4.3.1. Cutoff criteria

Cutoff criteria are threshold values basically obtained from *in vitro* data, that are used for judging the risk of drug interactions during the clinical use of the investigational drug. Cutoff criteria are being set in a conservative manner to avoid false-negative cases and to not miss the possibility of drug interactions occurring in the clinical setting. Cutoff criteria indicate the intrinsic risk of interactions as an inhibitor or inducer, independent of the characteristics of a particular substrate drug that are used concomitantly.

4.3.2. Mechanism-based static pharmacokinetic model (MSPK model)*[note (11)]

The MSPK model considers the mechanisms of interactions, based on the CR of the metabolic pathway in, and separates the small intestine and liver as the sites of interactions. The MSPK model does not count changes in the drug concentrations over time for the sake of simplification, and easier

806 analysis is an advantage over the PBPK model. Examples of analysis of inducing interactions using the
807 MSPK model have been reported[19].

808 On the other hand, for analysis using the MSPK model, the prediction may tend to be excessive
809 because changes in the concentration over time are not taken into consideration. Since reversible
810 inhibition, TDI, and the enzyme-inducing effect are all incorporated in the formula of MSPK model
811 superficially, the model seemingly allows easy simultaneous assessment of multiple drug interaction
812 processes. Actually, the consequences of interactions involving multiple factors are complicated, and a
813 care should be taken for its application (refer to 4.2.1.4).

814

815 Formula 4

$$AUCR = \left(\frac{1}{[A_h \times B_h \times C_h] \times f_m + (1 - f_m)} \right) \times \left(\frac{1}{[A_g \times B_g \times C_g] \times (1 - F_g) + F_g} \right)$$

816

817 A, B, and C in the formula denote TDI, induction, and reversible inhibition, respectively, as described
818 in the following supplemental table. F_g is the fraction of the investigational drug reaching the portal
819 blood after absorption via the intestinal enterocytes, which is decreased when the investigational drug
820 is metabolized there. Also, fm denotes the fraction of intrinsic P-450 dependent metabolic clearance of
821 the substrate particular to the inhibition (or induction) relative to the whole metabolic clearance of the
822 liver.

823 Formula 4 (Supplemental table)

Time-dependent inhibition	$A_h = \frac{k_{deg,h}}{k_{deg,h} + \frac{[I]_h \times k_{inact}}{[I]_h + K_I}}$	$A_g = \frac{k_{deg,g}}{k_{deg,g} + \frac{[I]_g \times k_{inact}}{[I]_g + K_I}}$
Induction	$B_h = 1 + \frac{d \bullet E_{max} \bullet [I]_h}{[I]_h + EC_{50}}$	$B_g = 1 + \frac{d \bullet E_{max} \bullet [I]_g}{[I]_g + EC_{50}}$
Reversible inhibition	$C_h = \frac{1}{1 + \frac{[I]_h}{K_i}}$	$C_g = \frac{1}{1 + \frac{[I]_g}{K_i}}$

824

825 The subscripts “h” and “g” denote the liver and gastrointestinal tract, respectively, while $[I]_h$ and $[I]_g$
826 denote the concentration of the investigational drug in the liver hepatocytes and intestinal enterocytes,
827 respectively. In addition, “d” is the conversion factor obtained from the linear regression to the control
828 data set.

829

830 4.3.3. Dynamic physiologically-based pharmacokinetic (PBPK) model*[note (12)]

The PBPK model can explain changes in the drug concentrations over time in contrast to the MSPK model. With the PBPK model, evaluation of complicated interactions, including contributions of transporters and metabolites have been reported to be theoretically possible. In addition, the evaluation of the effects of the interacting drug on the overall pharmacokinetic profiles of the interacted drug is shown (see Fig. 4-2, 3). Parameters based on the physiological functions of humans and parameters peculiar to each drug are incorporated in the PBPK model.

When using the PBPK model for the prediction of interactions, it is necessary for the changes in the blood concentrations of the investigational drugs (particularly for an interacting drug) to be appropriately described from the parameters of the model. In general, it is difficult to accurately predict changes in the blood concentrations from *in vitro* data alone. Therefore, it should be noted that analysis of interactions using the PBPK model cannot provide sufficient accuracy until data on the pharmacokinetics of the affecting drugs are obtained from the clinical studies.

The accuracy of prediction based on the PBPK model should be checked after the clinical drug interaction studies were carried out. If there is marked discrepancy between the prediction and the results of clinical drug interaction studies, the obtained data should be carefully examined and reflected in the plans of subsequent *in vitro* and clinical drug interaction studies, as necessary. If the validity of the PBPK model analysis is confirmed, and the results of clinical drug interaction studies can be explained without contradictions, interactions with other drugs having the same mechanism may also be analyzed with modeling and simulations to consider necessity of clinical alerts.

4.4. Drug interactions with biotechnological/biological products (Therapeutic proteins)

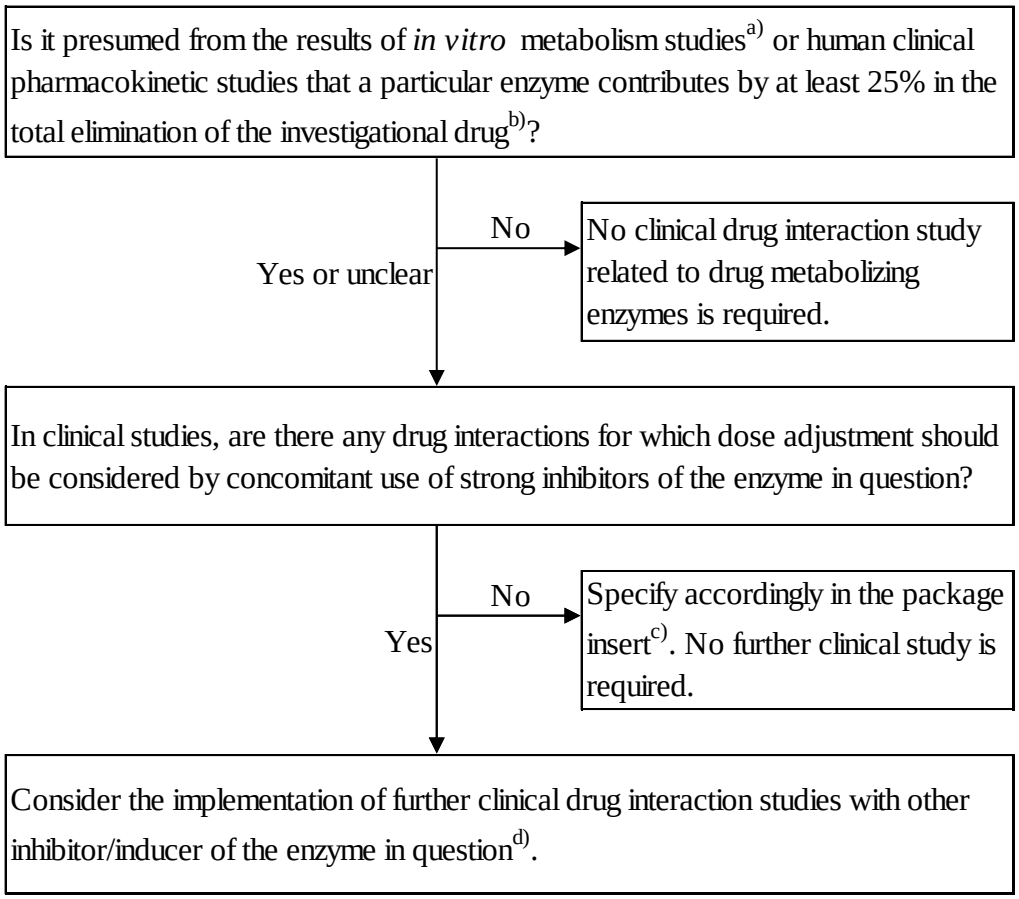
In general, biological products are eliminated through internalization and degradation in lysosomes of the target cells, after binding with specific receptors on the cell surface. Because the elimination pathway of biological products does not involve metabolism by P450 or other enzymes or transport by drug transporters, the possibility of interactions between the investigational drug and biological products is considered to be limited. If the investigational drug is related to cases for which interactions have been reported previously in the same class of drugs, or if the possibility of interactions is suggested in *in vitro* or other clinical studies, implementation of clinical drug interaction studies of the biological products and concomitant drugs should be considered.

If the investigational drug is a cytokine or a cytokine modifier, implementation of *in vitro* or clinical drug interaction studies to evaluate the effects of the investigational drug on P450 or transporter expressions should be considered if necessary, from the viewpoint of the efficacy and safety of the investigational drug and concomitant drugs.[note (13)] When the mechanisms of pharmacokinetic or pharmacodynamic interactions have been clarified for same class of drugs, with drug interactions

having been reported in clinical studies, appropriate *in vitro* studies or clinical drug interaction studies should be carried out to examine the possibility of the drug interactions in question. With regard to combination therapy prescribed by the dosage and administration, etc., biological products that are to be used concomitantly with other drugs (low-molecular-weight medical products or biological products) should be evaluated in clinical studies, if necessary, for the possibility of interactions between the concomitantly used drugs, and not only pharmacokinetic (PK) effects, but also pharmacodynamic (PD) effects should be evaluated, if necessary.

Figure 4-1

Evaluation of the possibility of the investigational drug as an affected drug (Identification of the enzymes involved in the metabolism of the investigational drug)



a) Targeted enzymes: CYP1A2, 2B6, 2C8, 2C9, 2C19, 2D6, 3A; UGT1A1, 1A3, 1A4, 1A6, 1A9, 2B7, 2B15; others

*When drug metabolizing enzymes other than P450 are mainly contributory, the feasibility of clinical drug interaction studies should be judged by the presence/absence of known inhibitors and inducers.

b) Major active metabolites of the investigational drug should also be evaluated in the same manner.

*Active metabolites: When the investigational drug is a pro-drug, or when the in vivo pharmacologic activity of metabolites accounts for 50% or more of the entire activity.

c) Information from clinical drug interaction studies, which is considered to be clinically useful, should be provided as appropriate in the package insert etc., regardless of the presence/absence of drug interactions.

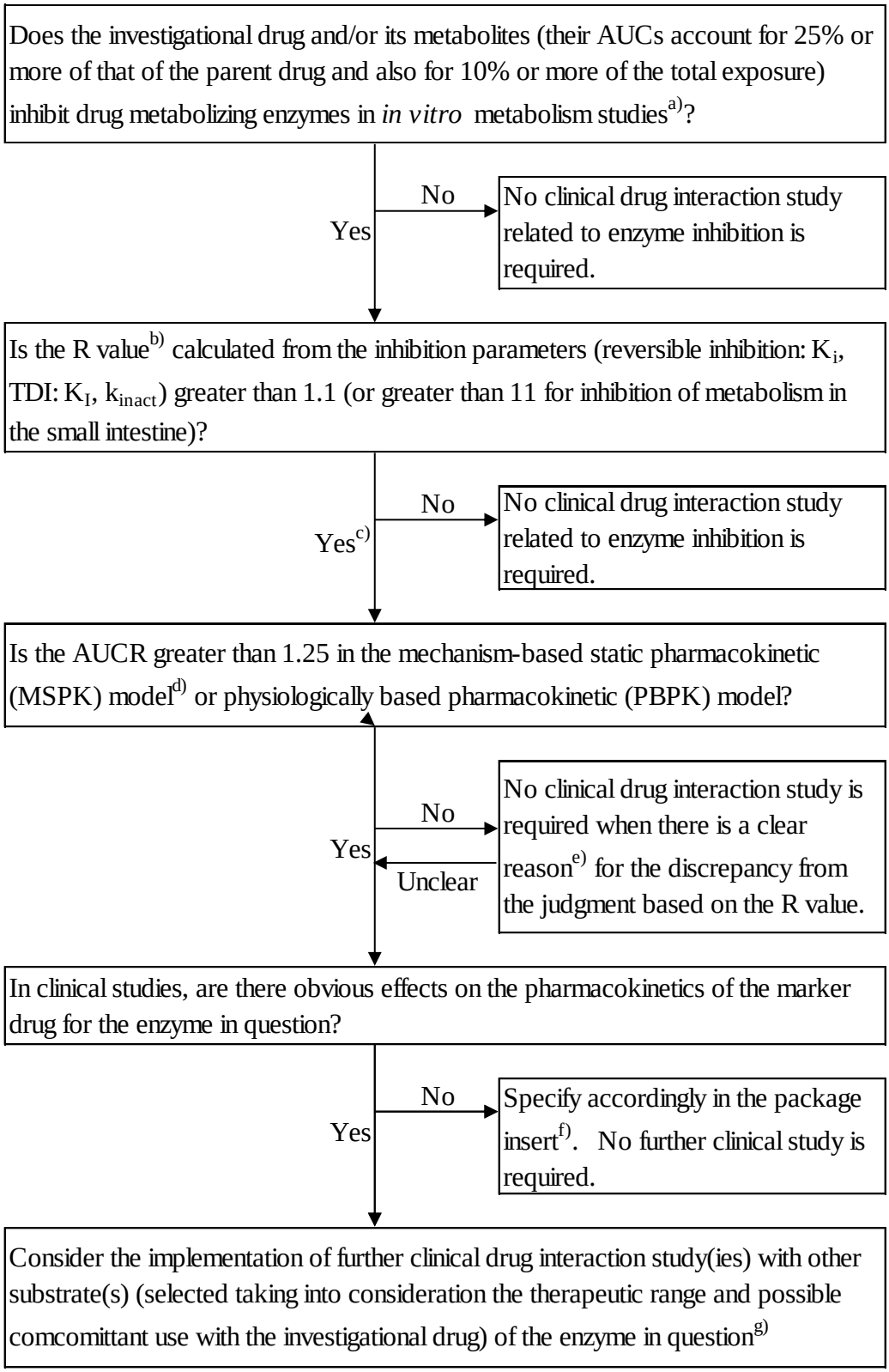
d) Inhibitor(s)/inducer(s) should be selected taking into consideration possible concomitant use with the investigational drug. A PBPK model can be used for evaluation if the validity of the model is confirmed and the results of clinical studies can be explained without contradiction. Clinical DI studies with inducer(s) are required when the risk of clinically significant drug interactions is inferred by simulations or other procedures based on clinical DI studies with inhibitor(s). In such cases, strong inducer(s) should be used in the study in principle.

879

880

881 **Figure 4-2**

882 **Evaluation of the possibility of the investigational drug inhibiting drug metabolizing enzymes**



883

a) Targeted enzymes: CYP1A2, 2B6, 2C8, 2C9, 2C19, 2D6, 3A; UGT1A1, 2B7; others

*Enzymes other than P450 are examined if they are involved in the major elimination pathway of the investigational drug.

*Concentration setting should include concentrations 10-fold higher than the C_{max} (bound + unbound forms).

* The effect of pre-incubation with the investigational drug should also be considered.

b) Reversible inhibition: $R=1+[I]/K_i$

TDI: $R=(k_{obs}+k_{deg})/k_{deg}$, $k_{obs}=k_{inact}\times[I]/(K_I+[I])$

[I]: C_{max} (bound + unbound forms), or Dose/250 mL (intestine)

c) May directly proceed to the clinical DI studies if the accuracy of the predictions is considered insufficient.

d) See Formula 4 (Section 4.3.2)

e) Clinical DI study is necessary in case of concurrent inhibition and induction.

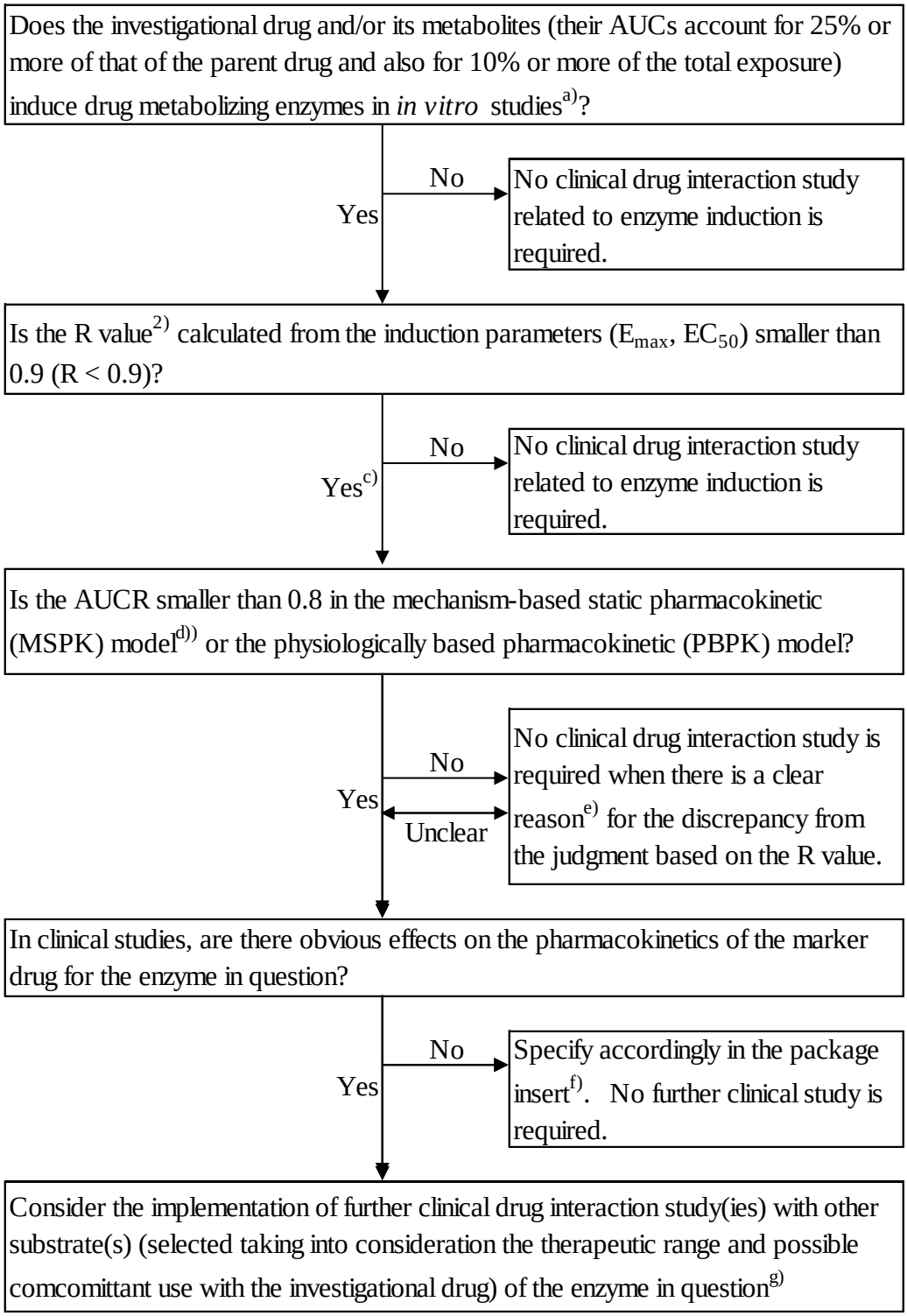
f) Information from clinical DI studies, which is considered to be clinically useful, should be provided as appropriate in the package insert etc., regardless of the presence/absence of drug interactions.

g) A PBPK model can be used for evaluation if the validity of the model is confirmed and the results of clinical studies can be explained without contradiction.

884

885 **Figure 4-3**

886 **Evaluation of the possibility of the investigational drug inducing drug metabolizing enzymes**



887

a) Targeted enzymes: CYP1A2, 2B6, 3A4

*Add CYP2C9 etc. if necessary.

*Under the concentration setting including concentrations 10-fold higher than the C_{max} (bound + unbound forms), the mRNA expression increases by at least

100% or by at least 20% of the value of the positive control.

b) $R=1/(1+d\times E_{max}\times[I]/(EC_{50}+[I]))$, defined as d=1

[I] : C_{max} (bound + unbound forms)

c) May directly proceed to the clinical DI studies if the accuracy of the predictions is considered insufficient.

d) See Formula 4 (Section 4.3.2)

e) Clinical DI study is necessary in case of concurrent inhibition and induction.

f) Information from clinical DI studies, which is considered to be clinically useful, should be provided as appropriate in the package insert etc., regardless of the presence/absence of drug interactions.

g) A PBPK model can be used for evaluation if the validity of the model is confirmed and the results of clinical studies can be explained without contradiction.

888

889

Table 4-1 Representative examples of *in vitro* P450 enzyme marker reactions[14, 20-22)]

Enzyme	Marker reaction
CYP1A2	Phenacetin O-deethylation, 7-Ethoxyresorufin-O-deethylation
CYP2B6	Efavirenz hydroxylation, Bupropion hydroxylation
CYP2C8	Paclitaxel 6α-hydroxylation, Amodiaquine N-deethylation
CYP2C9	S-Warfarin 7-hydroxylation, Diclofenac 4'-hydroxylation
CYP2C19	S-Mephenytoin 4'-hydroxylation
CYP2D6	Bufuralol 1'-hydroxylation, Dextromethorphan O-demethylation
CYP3A4*	Midazolam 1'-hydroxylation, Testosterone 6β-hydroxylation

*Both marker reactions should be used for the evaluation of CYP3A inhibition.

Table 4-2 Representative examples of *in vitro* P450 inhibitors[16, 20, 21, 23-25)]

Enzyme	Inhibitor
CYP1A2	Furafylline, α-Naphthoflavone
CYP2B6*	Ticlopidine, Thiotepa, Sertraline, Phencyclidine
CYP2C8	Montelukast, Quercetin, Phenelzine
CYP2C9	Sulfaphenazole, Tienilic acid
CYP2C19*	S-(+)-N-3-benzyl-nirvanol, Nootkatone, Ticlopidine
CYP2D6	Quinidine, Paroxetine
CYP3A4	Ketoconazole, Itraconazole, Troleandomycin, Verapamil, Azamulin

*At present, there is no known selective inhibitor that can be used *in vitro*. Although the inhibitors cited here are not selective, they can be used with other information or in a single enzyme system.

Table 4-3 Representative examples of *in vitro* P450 inducers[26-29)]

Enzyme	Inducer *
CYP1A2	Omeprazole, Lansoprazole
CYP2B6	Phenobarbital
CYP2C8	Rifampicin
CYP2C9	Rifampicin
CYP2C19	Rifampicin
CYP3A4	Rifampicin

*This table only provides examples, and does not provide an exhaustive list.

5. Drug interactions in transport and excretion

5.1. Drug interactions in urinary excretion

Many drugs are filtered by the renal glomeruli, and reabsorbed passively in the renal tubules. However, highly polar drugs tend not to be reabsorbed, but to be excreted in urine. Drugs highly reabsorbed (weakly acidic or weakly basic drugs) may exhibit drug interactions due to variations in the urinary excretion when administered with drugs that alter the urinary pH. Many of the highly polar drugs are actively secreted into the renal tubules via transporters, and may be reabsorbed actively from the tubules. Drug interactions may occur during this process, and thus caution is necessary. In patients with decreased urinary excretory function due to renal disease or aging, drugs mainly excreted into urine often show high blood concentrations. Therefore, particular caution is necessary to watch for enhancement of the drug efficacy or the occurrence of adverse effects associated with further increase in the blood concentrations resulting from interactions during the urinary excretion process.

Organic anion transporter (OAT) 1 and OAT3 are transporters expressed on the blood side of the proximal tubular epithelial cells and transport drugs from the blood into the proximal tubular epithelial cells, and P-gp, multidrug and toxin extrusion (MATE) 1, MATE-2K, and BCRP are transporters expressed on the ureteral side of the proximal tubular epithelial cells that mediate excretion of drugs from the proximal tubular epithelial cells into the urine. Blood concentrations of concomitant drugs are known to be elevated if these transporters are inhibited (see Table 6-1). In addition, if P-gp, MATEs and BCRP are inhibited, the concentrations of drugs in the proximal tubular epithelial cells may be increased. If OCT2 that transports drugs from the blood into the proximal tubular cells is inhibited, the blood concentrations of concomitant drugs may be increased. It should be judged whether the investigational drug serves as the substrate or inhibitor of these transporters, and whether clinical drug interaction studies are necessary (see Figs. 6-6, 6-7). As for other transporters that may contribute to urinary excretion, multidrug resistance-associated protein (MRP) 2 and MRP4 mediate excretion of drugs from the proximal tubular epithelial cells into the urine. Furthermore, in the case of MATEs involved in the renal excretion of endogenous substances, it is possible that their inhibition by drugs increases the blood and tissue concentration of endogenous substances such as creatinine*[note (14)]. For evaluation of drug interactions mediated by transporters contributing to the renal excretion, findings reported for drugs that are similar in chemical structure to the investigational drug may be of help. Because metabolites may cause interactions with concomitant drugs, evaluation of drug interactions between major metabolites and these transporters may also be considered, with reference to the description in section 4.1.

5.2. Drug interactions in biliary excretion

Many drugs are excreted in the form of conjugates, and some in the unchanged form, into bile. Because biliary excretion is usually mediated by transporters, drug interactions may occur in the presence of concomitant drugs. Blood concentrations of drugs are known to increase if organic anion transporting polypeptide (OATP) 1B1 and OATP1B3, which are transporters expressed on the blood side of the hepatocytes and take up drugs from the blood into the hepatocytes, are inhibited (see Table 6-1). It should be judged whether the investigational drug serves as the substrate or inhibitor of these transporters, and whether clinical drug interaction studies are needed (see Fig. 6-4, 5). In addition, As for transporters that mediate hepatic uptake and biliary excretion, organic cation transporter (OCT) 1 is expressed on the blood side of the hepatocytes and transports drugs from the blood into the hepatocytes and MRP2 is expressed on the apical side of the hepatocytes and mediates excretion of drugs from the hepatocytes into bile. Furthermore, in the case of OATPs, MRP2 and bile salt export pump (BSEP) involved in the biliary excretion of endogenous substances such as bile acids and bilirubin, it is possible that their inhibition by drugs increases the blood and tissue concentration of endogenous substances*[note (14)]. For evaluation of drug interactions mediated by transporters contributing to the hepatic uptake and biliary excretion, findings reported for drugs that are similar in chemical structure to the investigational drug may be of help. Conjugates such as glucuronic acid conjugate are often excreted into the bile, deconjugated by enteric bacteria, and then reabsorbed (enterohepatic circulation). Drug interactions occurring during biliary excretion of conjugates may affect the residence time and AUC of the unchanged drug in the plasma.

6. Methods of drug interaction studies related to transporters

6.1. General considerations in i*n vitro* studies

For evaluation of transport using *in vitro* test systems for transporters, assessments with the use of typical substrates and typical inhibitors (See Table 6-5) should also be carried out and it is necessary to conduct the evaluation of an investigational drug with a test system that would allow sufficient observation of the function of the subject transporter.

In the case of a study carried out to explore the possibility of an investigational drug serving as a substrate for a particular transporter, the study should be designed and conducted under conditions where the investigational drug is used at sufficiently low concentrations relative to the estimated K_m value, to avoid saturation of the transporter. When the exact K_m value is not known, for instance, and if a proportional relationship between investigational drug concentration and velocity of transport is ascertained by a test conducted using two or more different concentrations estimated to be sufficiently lower than the K_m value, saturation of the transporter in the test concentration range can be ruled out.

In the case of a study performed to explore the possibility of an investigational drug serving as an inhibitor of a particular transporter, on the other hand, the effects of different concentrations of the investigational drug on the transport of a typical substrate should be assessed, and the K_i value should be calculated, in principle. When a drug with a known K_m value is used as the substrate, $IC_{50}=K_i$ can be assumed when using the substrate at concentrations sufficiently lower than the K_m value. When use of a drug with an unknown K_m value is unavoidable as the substrate, it is acceptable to assume $IC_{50}=K_i$ if a proportional relationship between the substrate concentration and velocity of transport is ascertained by a test conducted using two or more different, sufficiently low substrate concentrations. For studies of efflux transporters such as P-gp, BCRP, MATE1, and MATE2-K in a cellular test system, it is feasible to use the apparent IC_{50} value based on the concentration in the assay medium in order to evaluate the inhibition potency of transporters. In such studies, sufficiently low substrate concentrations should be used comparing with the K_m value.

6.2. *In vitro* studies to examine drug interactions related to transporters involved in absorption

Both P-gp and BCRP are important transporters that are expressed in the GI tract and may affect variations in the oral bioavailability. Because they are also expressed in the liver, kidney and brain, they can affect the elimination and brain distribution of drugs. Because of this, the possibility of serving as the substrate of P-gp or BCRP should be evaluated for all investigational drugs by *in vitro* studies (see Figs. 6-1 and 6-2).

For *in vitro* evaluation, it is desirable to conduct bidirectional transcellular transport studies using Caco-2 cells or other cell lines overexpressing particular transporters. Although several types of transporters such as P-gp, BCRP and MRP2 are expressed in Caco-2 cells, involvement of each transporter can be evaluated if selective inhibitors for the respective transporters can be used. If the use of selective inhibitors is not feasible, cell lines overexpressing particular transporter genes can be used.

When investigating the involvement of efflux transporters such as P-gp and BCRP, permeability of the investigational drug from the apical side (A) to the basal side (B) should be compared with the permeability of the drug in the opposite direction, i.e., from B to A. If Caco-2 cells are used, the flux ratio (=B to A/A to B ratio) should be calculated from the ratio of the permeability from B to A to that from A to B. If transporter-expressing cell lines are used, correction using the flux ratio of non-expressing cells should be applied to calculate the net flux ratio [= (flux ratio of expressing cells)/(flux ratio of non-expressing cells)]. When the net flux ratio (or the flux ratio in the case of Caco-2 cells) exceeds 2, typical inhibitors of efflux transporters should be used concomitantly, and a significant decrease in the net flux ratio (or the flux ratio in the case of Caco-2 cells) should be confirmed.

If inhibition of P-gp and BCRP by the investigational drug is evaluated, evaluable concentrations should be set considering that the expected maximum concentration (maximum single dose/250 mL) of the investigational drug in the lumen side of the GI tract is at least 10-fold higher than the expected IC_{50} value. If the solubility of the investigational drug is low, evaluable concentrations should be set based on the maximum concentration achievable within the range of the pH in the GI tract. When the IC_{50} value is higher than 0.1 x dose/250 mL, i.e., when the dose/250 mL/IC_{50} is less than 10 (dose/250 mL/IC_{50} < 10), inhibition of transporters in the GI tract in the *in vivo* situation can be excluded (Fig. 6-3). In the calculation of the IC_{50} value, analysis should use the net flux ratio (or the flux ratio in the case of Caco-2 cells) as an index.

In examining the possibility of an investigational drug serving as a substrate for P-gp or BCRP, use of the investigational drug at an excessively high concentration may possibly saturate a high-affinity transporter; eventually, setting of the testing concentrations is important. Therefore, the investigational drug as a substrate should be used at concentrations sufficiently lower than the K_m value. If the K_m value of the investigational drug is unknown, the investigational drug concentration should be set at least taking into account the transporter expression site and the drug concentration at that site. In the case of a transporter involved in efflux transport in the intestine, kidneys, liver and blood-brain barrier, like P-gp, the assessment should be made using the investigational drug in a concentration range that would be physiologically meaningful to the respective organs.

If bidirectional transcellular transport studies are carried out, setting of the pH value of both solutions on the acceptor side and on the donor side at 7.4 is recommended. In order to ensure that the study is valid, determination of the recovery rates of the added drugs on the acceptor side and on the donor side is also recommended.

If these bidirectional transcellular transport studies are employed for evaluation of the P-gp and BCRP, typical substrates (see Table 6-5) should be used for the evaluation to ensure the validity of the study system. With regard to typical substrates, it should be confirmed that the net flux ratio (or the flux ratio in the case of Caco-2 cells) exceeds 2, and that the net flux ratio (or the flux ratio in the case of Caco-2 cells) is significantly decreased by the addition of a typical inhibitor to a degree that allows theoretical estimation from the concentration of the added inhibitor and the IC_{50} value. As for typical inhibitors, it should be confirmed that the net flux ratio is decreased to a degree that allows theoretical estimation from the concentration of the inhibitor used and the IC_{50} value of the inhibitor.

6.3. *In vitro* studies to examine drug interactions related to transporters in the liver

Investigational drugs which are mainly eliminated via hepatic metabolism or biliary excretion (i.e., clearance via either route accounting for not less than 25% of the total clearance) should be assessed

for the possibility of their serving as substrates for the hepatic uptake transporters OATP1B1 and 1B3 (Fig. 6-1). However, there may be instances where the need for *in vitro* studies can be judged based upon examination of the distribution to the liver, e.g., based on the results of animal experiments on tissue distribution (Fig. 6-4).

Test systems consisting of OATP1B1- and OATP1B3-expressing cell lines or human hepatocytes may be used for studies conducted to explore the possibility of an investigational drug serving as a substrate or an inhibitor of OATP1B1 and/or OATP1B3. In studies conducted using OATP1B1- and OATP1B3-expressing cell lines or human hepatocytes, an experiment using a typical substrate (see Table 6-5) should be also performed to ascertain that the transporter function of OATP1B1 and OATP1B3 is adequately manifested in the test system. The uptake ratio of typical substrates (uptake ratio of transporter-expressing cells to non-transporter-expressing cells) of acceptable OATP1B1- and OATP1B3-expressing cell lines is usually more than 2, and it should be verified that the uptake is significantly reduced by a typical inhibitor to a degree that would allow theoretical estimation from the K_i value and concentration of the added inhibitor. In studies carried out using human hepatocytes, it should be ascertained that the uptake ratio of typical substrates is usually more than 2, and that the uptake is significantly reduced by a typical inhibitor to a degree that would allow theoretical estimation from the K_i value and the concentration of the added inhibitor.

In exploring the possibility of an investigational drug serving as a substrate for OATP1B1 and OATP1B3 using a test system consisting of OATP1B1- and OATP1B3-expressing cell lines, the investigational drug is judged to serve as a substrate for OATP1B1 and OATP1B3, if its uptake into the transporter-expressing cells exceeds 2-fold of the uptake into the non-transporter-expressing cells and also if it is verified that the uptake is significantly inhibited by a typical inhibitor of a known target transporter to a degree that would allow theoretical estimation from the K_i value and the concentration of the added inhibitor (see Fig. 6-4). In the case where the investigational drug uptake into the transporter-expressing cells is significantly greater, but less than 2-fold as compared to the uptake into the non-transporter-expressing cells due to investigational drug adsorption or any other reason, the investigational drug can be judged to serve as a substrate by separately setting a threshold value of uptake ratio if it is verified that the uptake is significantly inhibited by a typical inhibitor to a degree that would allow theoretical estimation from the K_i value and the concentration of the added inhibitor. It is also feasible, furthermore, to examine the involvement of OATP1B1 or OATP1B3 in advance by using typical substrates of OATP1B1 and OATP1B3 in an uptake study using human hepatocytes with proven sufficient retention of transporter function. If investigational drug uptake into human hepatocytes is evident, the investigational drug is judged to serve as a substrate of OATP1B1 or OATP1B3 insofar as it is verified that the uptake is significantly inhibited by a typical inhibitor to a

degree that would allow theoretical estimation from the K_i value and the concentration of the added inhibitor.

For studies carried out to assess inhibition of OATP1B1 and OATP1B3 by an investigational drug, it is recommended that substrates for OATP1B1 and OATP1B3 be selected taking into account drugs that are concomitantly administered in the clinical situation. If such selection entails difficulty, typical substrates of OATP1B1 and OATP1B3 (Table 6-5) may be utilized. Such selection is carried out using the substrates at concentrations sufficiently lower than the K_m value. In addition, it should be ascertained by a separate experiment that the test system fully manifests the functions of OATP1B1 and OATP1B3 by transporting typical substrates (Table 6-5) and by verifying that the uptake of the typical substrate is significantly reduced by a typical inhibitor to a degree that would allow theoretical estimation from the K_i value and the concentration of the added inhibitor (Table 6-5). As for setting of concentrations in the inhibition test, it should be ensured that the concentration range allowing judgment of whether or not the K_i value of the investigational drug is ≥4-fold the maximum blood unbound concentration of the drug at the inlet to the liver ($[I]_{u,\ inlet,\ max}$) at the estimated clinical dose is covered. *In vivo* transporter inhibition in the liver can be excluded if the K_i value is greater than $4\times([I]_{u,\ inlet,\ max})$ ($[I]_{u,\ inlet,\ max}/K_i<0.25$) (Fig. 6-5). Additional points to consider for inhibition experiments on OATP1B1 and OATP1B3 are stated elsewhere [*note (14)].

6.4. *In vitro* studies to examine interactions related to transporters in the kidney

For investigational drugs whose major route of elimination is active renal secretion (renal secretion clearance accounting for 25% or more of the total clearance), the possibility of the drug serving as a substrate for OAT1, OAT3, OCT2, MATE1 or MATE2-K should be explored *in vitro* (Fig. 6-1).

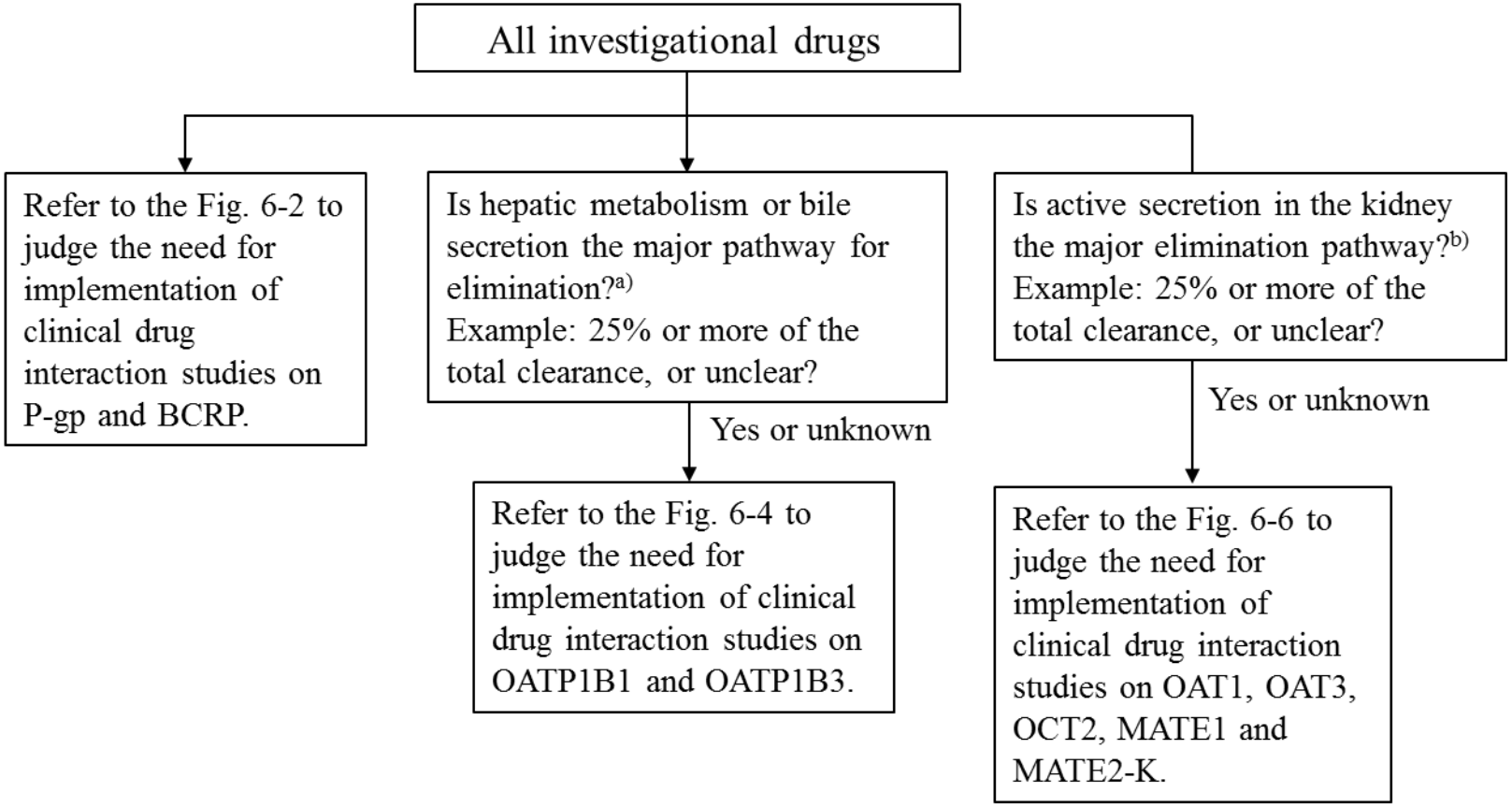
When the test is performed using a cell line expressing OAT1, OAT3, OCT2, MATE1 or MATE2-K, a parallel test should also be carried out with a typical substrate (see Table 5-5) to confirm that the test system adequately manifests the functions of these transporters. The intracellular uptake ratio of typical substrates (uptake ratio of transporter-expressing cells to non-expressing cells) of cell lines expressing OAT1, OAT3, OCT2, MATE1, or MATE2-K is usually more than 2. It should be verified that the uptake is significantly reduced by a typical inhibitor to a degree that would allow theoretical estimation from the K_i value (or the IC_{50} value in the case of MATE1 and MATE2-K only) and the concentration of the added inhibitor. Meanwhile, since the driving force is the proton gradient, hence reverse in direction, in the cases of MATE1 and MATE2-K, it is practicable to measure the transport activity based on the intracellular uptake after acidifying the intracellular milieu (e.g., preincubating MATE-expressing cells with ammonium chloride, or alkalifying the extracellular pH to approx. 8.4 in the uptake experiment)[30]. It is also practicable to use membrane vesicles prepared from MATE1- or

MATE2-K-expressing cells instead of MATE1- or MATE2-K-expressing cell lines[31]. In such instances, likewise, acidification of the intra-membrane vesicular milieu is necessary to gain transport-driving force.

In assessing the possibility of an investigational drug serving as a substrate of a transporter, the investigational drug is judged to be a substrate of the subject transporter if the investigational drug uptake into the transporter-expressing cells is ≥2-fold that into the non-transporter-expressing cells, and also if the uptake of a typical substrate is significantly inhibited by a typical inhibitor of a known target transporter to a degree that would allow theoretical estimation from the K_i value and the concentration of the added inhibitor (see Fig. 6-6). In case where the investigational drug uptake into the transporter-expressing cells is significantly greater but less than 2-fold as compared to that into the non-transporter-expressing cells due to investigational drug adsorption or any other reason, the investigational drug can be judged to serve as a substrate by separately setting a threshold value of uptake ratio if it is verified that the uptake is significantly reduced by a typical inhibitor to a degree that would allow theoretical estimation from the K_i value and the concentration of the added inhibitor.

For studies conducted to examine the inhibition of OAT1, OAT3, OCT2, MATE1, or MATE2-K by an investigational drug, it is recommended that a substrate for the transporter to be used in the study be selected taking into account the drugs that are concomitantly administered in the clinical situation. If the selection entails difficulty, a typical substrate for the transporter (Table 6-5) may be utilized. Such selection is carried out using the substrate at concentrations which are considered to be sufficiently lower than the K_m value. In addition, it should be ascertained that the test system adequately manifests the function of the transporter via performance of the test using a typical substrate (Table 6-5) and also by verifying that the uptake is significantly reduced by a typical inhibitor (Table 6-5) to a degree that would allow theoretical estimation from the K_i value and the concentration of the added inhibitor. As for setting of concentrations in the test for inhibition of OAT1, OAT3, OCT2, MATE1, or MATE2-K, it should be ensured that the concentration range allowing judgment of whether or not the K_i value of the investigational drug is ≥4-fold the unbound C_{max} of the drug at the estimated clinical dose is covered. *In vivo* transporter inhibition in the kidney can be excluded if the K_i value is greater than 4× unbound C_{max} of the drug (Fig. 6-7).

Figure 6-1 : Evaluation of the possibility of the investigational drug serving as a substrate for P-gp, BCRP, OATP1B1, OATP1B3, OAT1, OAT3, OCT2, MATE1, or MATE2-K

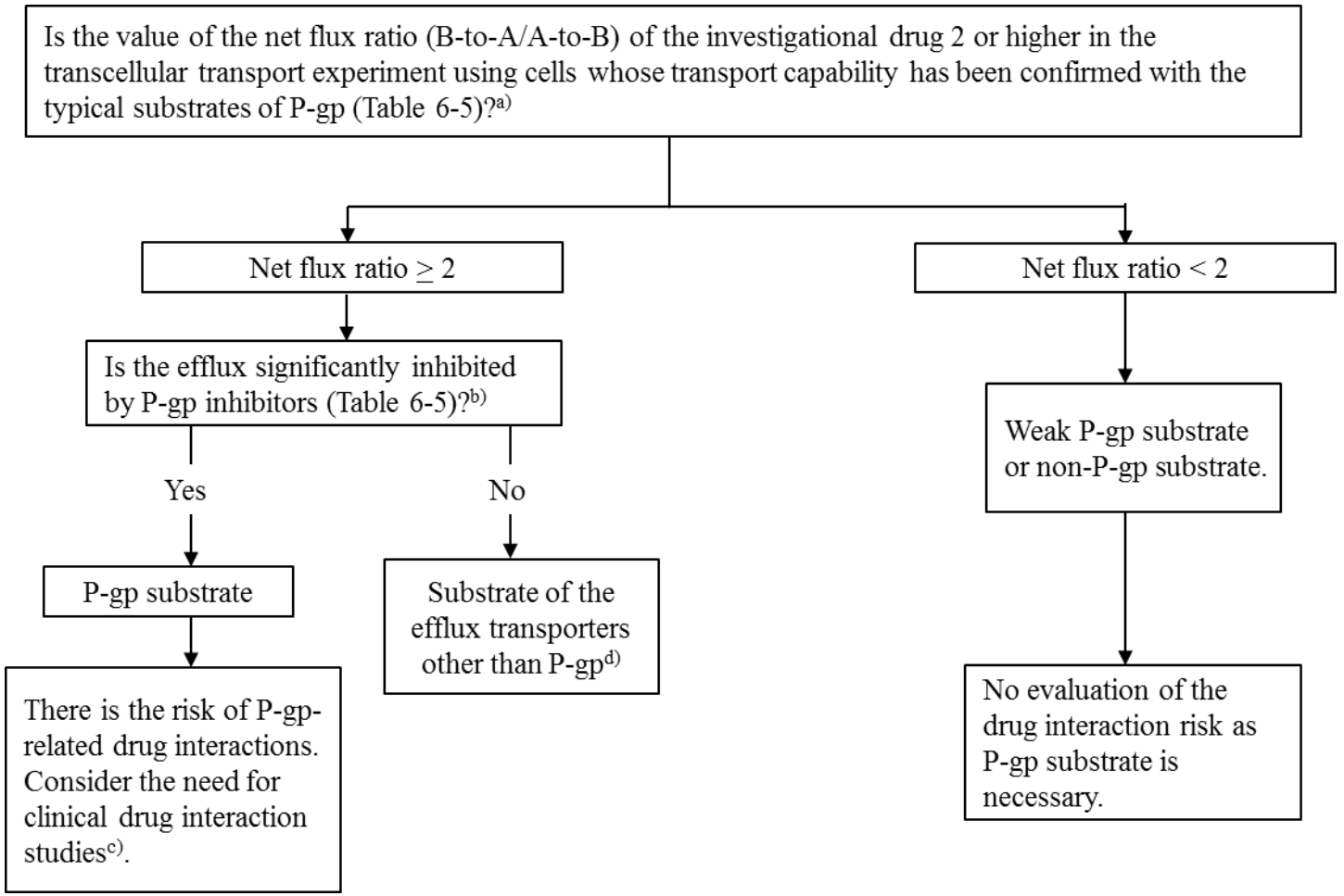


a) When an investigational drug for which the hepatic pathway is important (example: hepatic metabolism or biliary excretion accounts for 25% or more of the total clearance) is concerned, it should be examined whether the drug serves as a substrate of the hepatic uptake transporters

OATP1B1 and/or OATP1B3. Biliary excretion can be estimated from the preclinical data (*in vitro* hepatocytes uptake data or *in vivo* ADME study data with radiolabeled compounds) and non-renal clearance data.

b) When an investigational drug for which renal tubular secretion is important (secretion clearance accounts for 25% or more of the total clearance), it should be examined whether the drug is a substrate of OAT1, OAT3, OCT2, MATE1, and MATE2-K. The fraction of secretion clearance (%) should be estimated using the formula $(CL_r - f_u * GFR)/CL_{total}$, where f_u is the drug protein-unbound fraction in plasma.

Figure 6-2 : Evaluation of the possibility of the investigational drug serving as a substrate of P-gp or BCRP



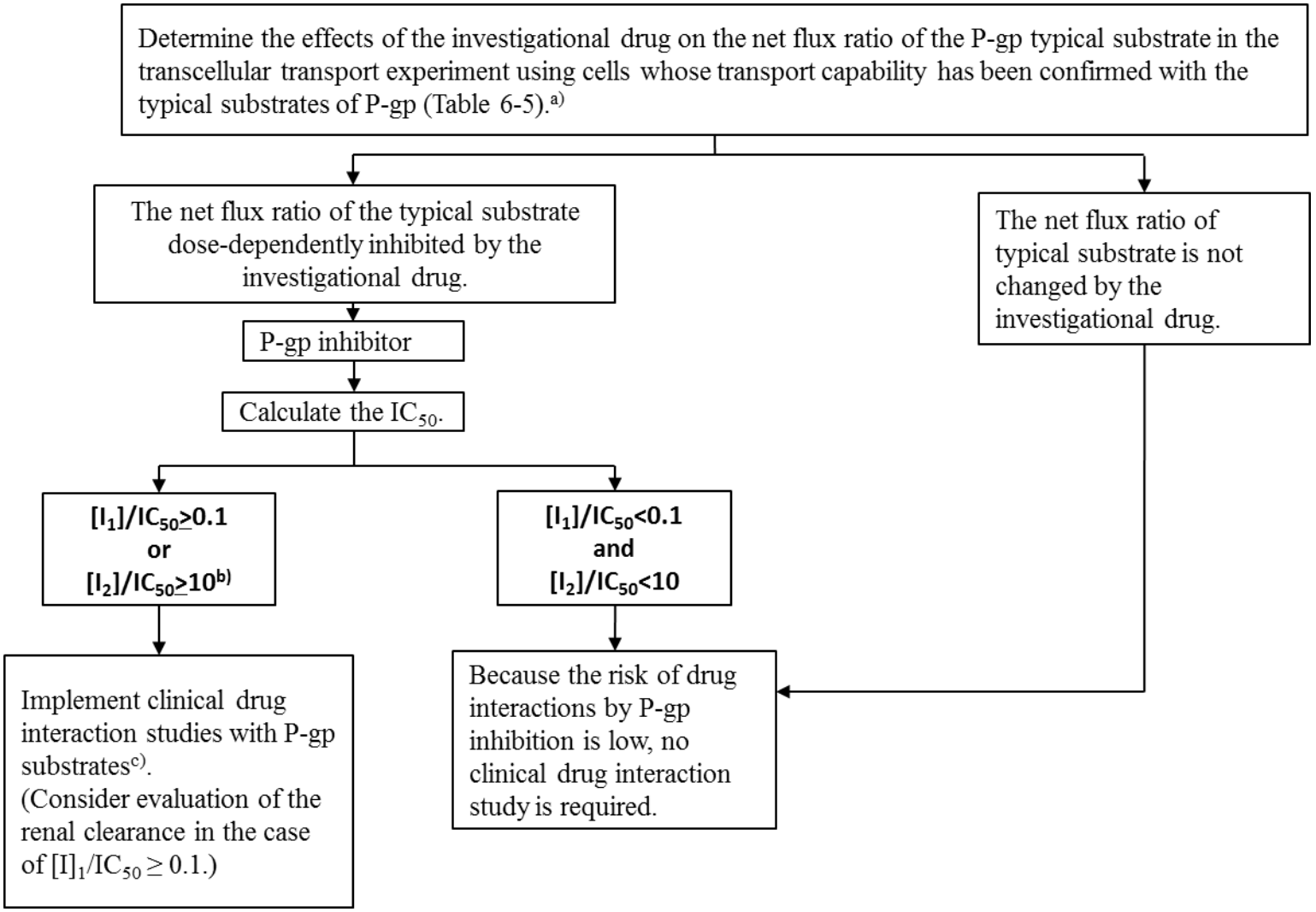
a) Transport capability should be confirmed with the net flux ratio of typical substrates (Table 6-5) in Caco-2 cells or an MDR1-expressing cell line as the index. If a net flux ratio of 2 does not seem to provide clear results based on previous experience with the cell lines used, a net flux ratio of more than 2 may be used as the cutoff value, or the relative ratio to the positive control may be used.

b) The net flux ratio decreases significantly, or approaches 1-fold.

c) Because P-gp is involved in GI absorption, renal tubular secretion and brain distribution, the need for drug interaction studies should be judged taking into consideration intestinal availability (F_aF_g), presence/absence of renal tubular secretion, and the risk of CNS toxicity. If the F_aF_g is more than 80%, it is presumed that 1.25-fold or higher exposure will not take place only by the P-gp inhibition in the GI tract. In the case of BCRP substrates, because genetic polymorphism in BCRP whose allele frequency is relatively high in Japanese population has been reported to decrease its function *in vivo*, which may cause individual variability in their pharmacokinetics, examination of whether the drug serves as a substrate of BCRP *in vitro* using this decision tree is recommended. The experimental method should be in accordance with that of P-gp substrate studies. Typical substrates and inhibitors are shown in Table 6-5. If the investigational drug is a BCRP substrate, it is currently difficult to design clinical drug interaction studies using *in vivo*-usable typical inhibitors (Table 6-4). Therefore, in this case, only a description that the drug is a BCRP substrate should be provided at the moment.

d) If the presence of large effects of transporters other than P-gp or BCRP on the absorption or efflux process in the GI tract is suggested by the known findings on the therapeutic class to which the investigational drug belongs, it may be considered that the transporters involved are identified, and the degree of their involvement is determined using Caco-2 cells or transporter-expressing cell lines. If necessary, implementation of clinical drug interaction studies may also be considered.

Figure 6-3：Evaluation of the possibility of the investigational drug being a P-gp or BCRP inhibitor

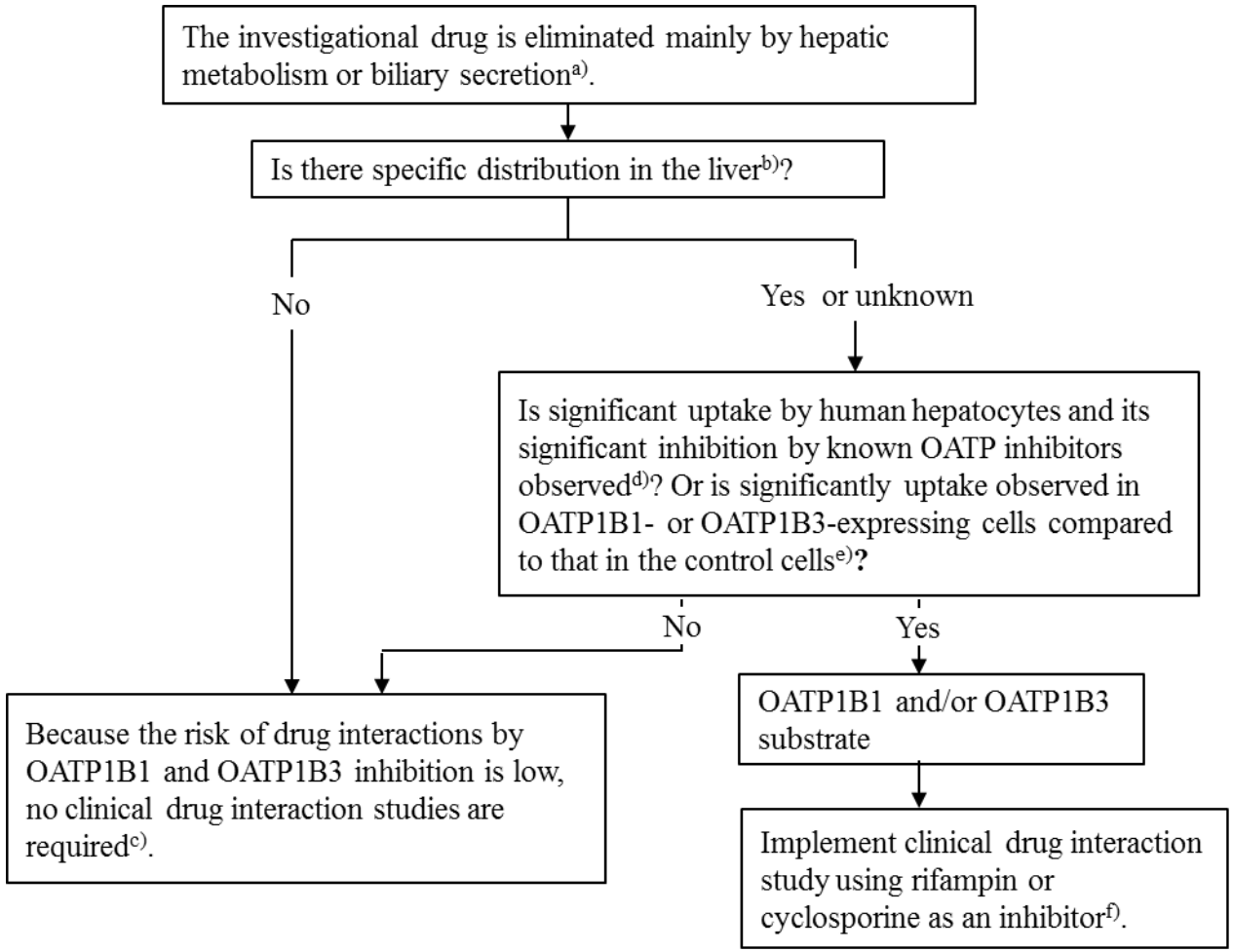


a) Transport capability should be confirmed with the net flux ratio of typical substrates (Table 6-5) (or the flux ratio in the case of Caco-2 cells) in Caco-2 cells or an MDR1-expressing cell line as an index. In addition, in the presence of an inhibitor, it should be confirmed that the net flux ratio (or the flux ratio in the case of Caco-2 cells) decreases significantly to the extent that can be theoretically estimated by the concentration of the added inhibitor and the K_i value. The concentration of the substrate drug should be sufficiently lower than its K_m value. If such setting is difficult, the concentration of the substrate drug should not be excessively high taking into consideration its concentration exposed to the P-gp (See text 6.2).

b) $[I]_1$ denotes the mean value of the total C_{max} (total concentration of the protein-unbound and protein-bound drug) in the steady state after the planned maximum clinical dose. $[I]_2$ = dose of the inhibitor/250 mL. In this case, the concentration of the typical substrate should be set a value sufficiently lower than the K_m value (Table 6-5).

c) The typical substrate to be used *in vivo* should be selected in reference to Table 6-4.

Figure 6-4 : Evaluation of the possibility of the investigational drug serving as a substrate of OATP1B1 or OATP1B3



a) See Fig. 6-1.

b) Judge from the results of tissue distribution studies in animals (autoradiography, etc.). Note that compounds that have a negative charge under physiological conditions or have low membrane permeability often serve as substrates of OATP transporters, although there are some exceptions. It is also possible that transporters are involved in the process of hepatic uptake of compounds that can be mainly eliminated by hepatic metabolism.

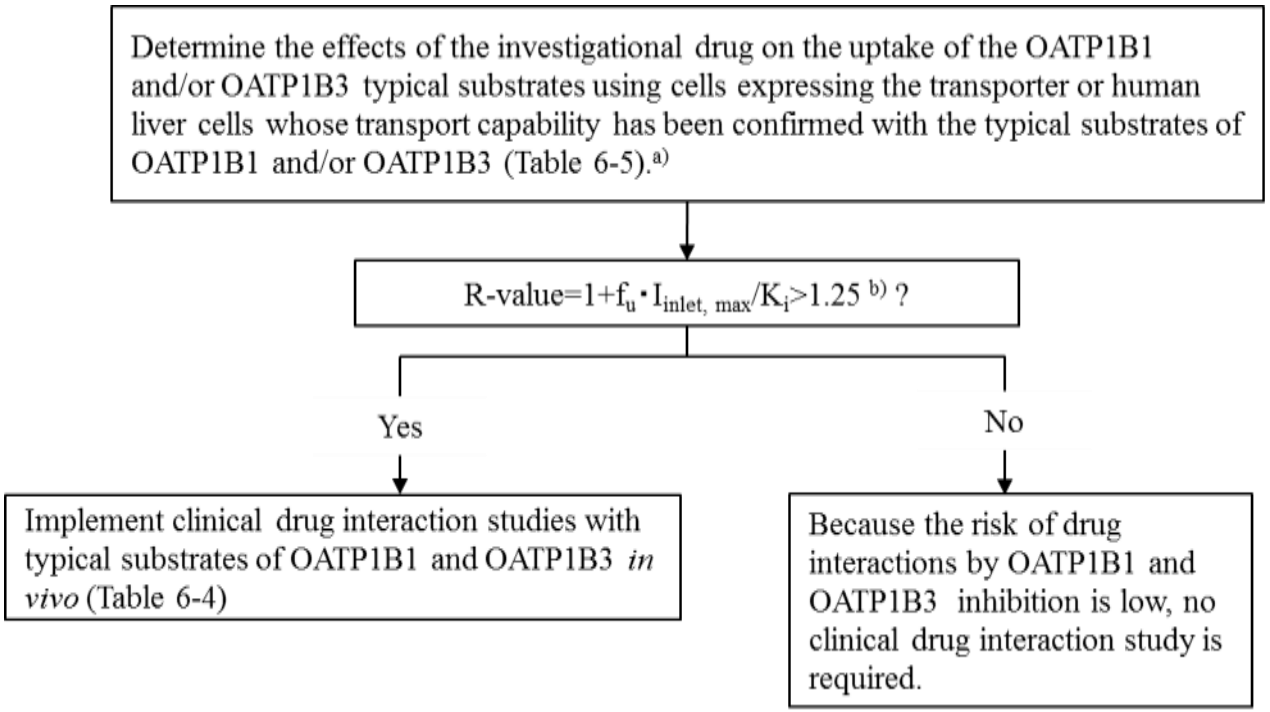
c) Cases where the involvement of passive diffusion is high, leading to masked transport by OATP1B1 and/or OATP1B3, are included.

d) Human hepatocytes whose OATP1B1 and/or OATP1B3 transport capability has been confirmed should be used. It should be confirmed that there is uptake of the typical substrates (Table 6-5) and significant inhibition by typical inhibitors (Table 6-5) to the extent that can be theoretically estimated by the concentration of the added inhibitor and the K_i value.

e) If an OATP1B1- or OATP1B3-expressing cell line is used, it should be confirmed that the uptake of the typical substrates (Table 6-5) is at least double that in the control cells, and that there is a significant inhibition by typical inhibitors (Table 6-5) to the extent that can be theoretically estimated by the concentration of the added inhibitor and the K_i value. However, if previous experience with the cell lines used suggest that an uptake ratio of 2 (ratio of uptake by the transporter-expressing cells to that by non-transporter-expressing cells) does not provide clear results, another threshold value of the uptake ratio may be used. In the case of highly lipophilic compounds, it should be borne in mind that the uptake may be difficult to detect in the cells expressing the transporter.

f) Since rifampin exerts the inducible effect after its repetitive administration, single administration of rifampin should be performed for the inhibition of OATPs.

Figure 6-5 : Evaluation of the possibility of the investigational drug being an OATP1B1 or OATP1B3 substrate

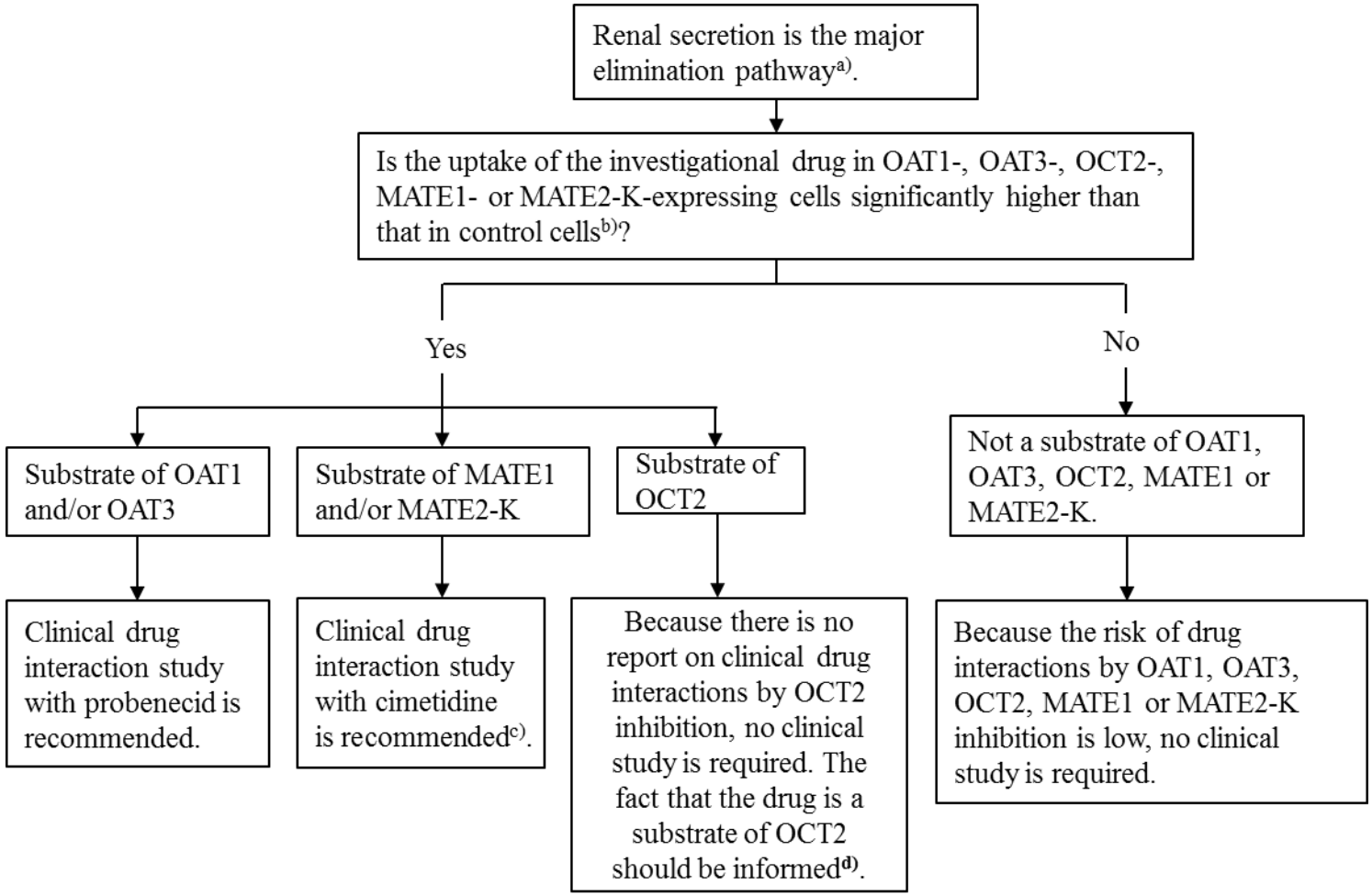


a) If hepatocytes are used, it should be confirmed that there is uptake of the typical substrates (Table 6-5) and that there is significant inhibition by typical inhibitors (Table 6-5) to the extent that can be theoretically estimated by the concentration of the added inhibitor and K_i value. If an OATP1B1- or OATP1B3-expressing cell line is used, it should be confirmed that the uptake of the typical substrates (Table 6-5) is at least double that in the control cells, and that there is a significant inhibition by typical inhibitors (Table 6-5) to the extent that can be theoretically estimated by the concentration of the added inhibitor and the K_i value. For the determination of K_i value of the investigational drug, typical substrates can be selected from Table 6-5 and their recommended concentration should be sufficiently lower than their K_m value (see Table 6-5). The

concentration range of the investigational drug for the inhibition study should be set taking into consideration its clinical concentration exposed to the OATP1B1 and OATP1B3 (blood concentration at the portal vein).

b) R value = $1 + (f_u \times I_{in,max}/K_i)$. In this formula, the $I_{in,max}$ is the estimated maximum concentration of the inhibitor at the inlet to the liver, and is equal to the $C_{max} + (k_a \times dose \times F_aF_g/Q_h)$. C_{max} is the maximum systemic plasma concentration of the inhibitor, dose is the dose of the inhibitor, F_aF_g is the intestinal availability of the inhibitor, k_a is the absorption rate constant of the inhibitor, and Q_h is the hepatic blood flow rate (example: 97 L/hr). If the F_aF_g and k_a values are unknown, 1 and 0.1 min^{-1} can be used as the values for the F_aF_g and k_a, respectively, because prediction of false-positive results can be avoided by the use of theoretical maximum values[32]. In the case of drugs whose f_u values are less than 0.01 or fractions bound to blood proteins are very high , resulting in inaccurate determination of the f_u value, it should be assumed that the f_u equals 0.01 to avoid false-negative results.

Figure 6-6: Evaluation of the possibility of the investigational drug serving as a substrate for OAT1, OAT3, OCT2, MATE1, or MATE2-K



a) See Fig. 6-1.

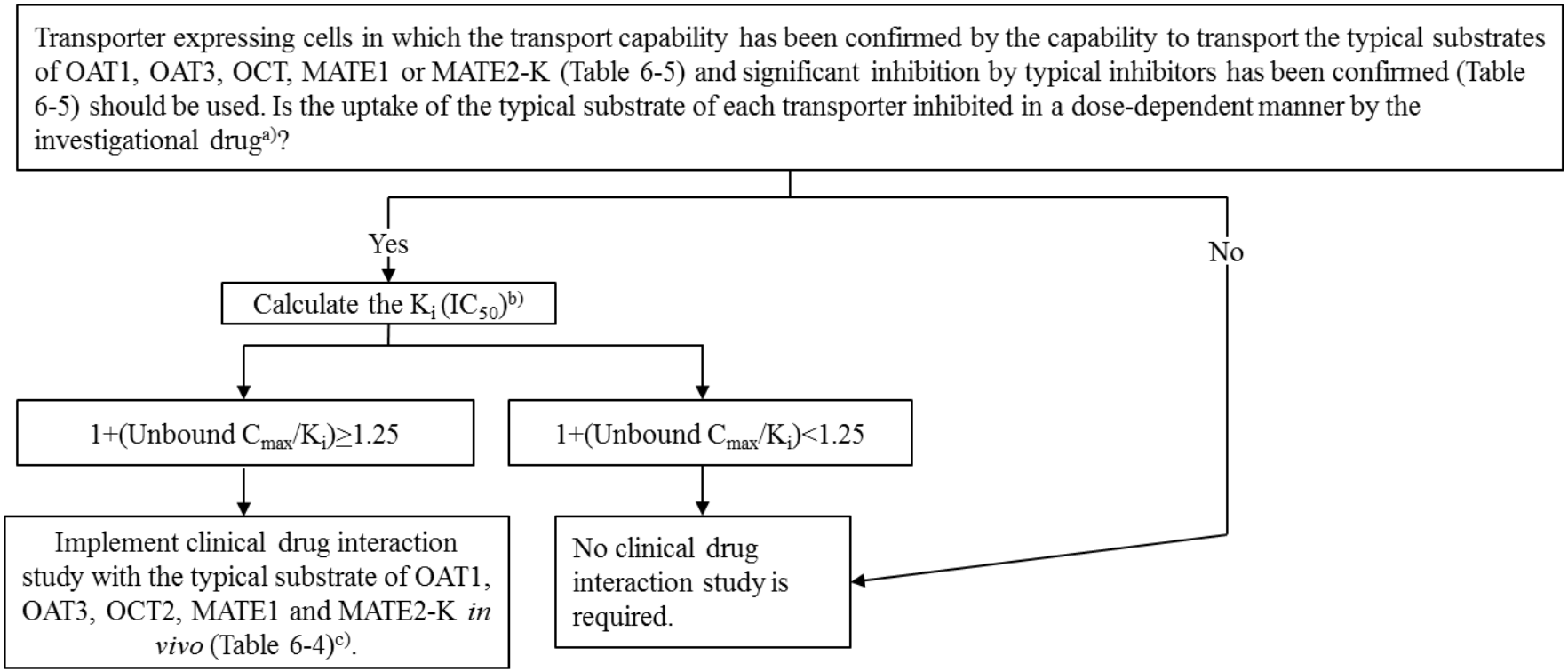
b) The uptake of the investigational drug should be determined using cells in which the ratio of the uptake in the typical substrate (Table 6-5) by transporter-expressing cell line to that by a non-expressing cell line (uptake ratio) has been confirmed to be not less than 2-fold, and there is significant inhibition by known inhibitors (Table 6-5) to the extent that can be theoretically estimated by the concentration of the added inhibitor and the K_i value.

If a transporter-expressing cell line is used, it should be confirmed that the uptake of the typical substrates (Table 6-5) is at least double that in the control cells, and that there is a significant inhibition by typical inhibitors (Table 6-5) to the extent that can be theoretically estimated by the concentration of the added inhibitor and the K_i value. However, if previous experience with the cell lines used suggest that an uptake ratio of 2 (ratio of uptake by the transporter-expressing cells to that by non-transporter-expressing cells) does not provide clear results, another threshold value of the uptake ratio may be used. In the case of highly lipophilic compounds, it should be borne in mind that the uptake may be difficult to detect in the cells expressing the transporter.

c) Since MATE1 and MATE2-K are involved in the renal excretion of drugs, it should be borne in mind that the kidney concentration may be increased even if the blood concentration remains unchanged.

d) If the investigational drug is an OCT2 substrate, it is currently difficult to design clinical drug interaction studies using *in vivo*-usable typical inhibitors (Table 6-4). Therefore, in this case, only a description that the drug is an OCT2 substrate should be provided at the moment.

Figure 6-7: Evaluation of the possibility of the investigational drug being an inhibitor of OAT1, OAT3, OCT2, MATE1 or MATE2-K



a) The uptake of the investigational drug should be determined using cells in which the ratio of the uptake in the typical substrate (Table 6-5) by transporter-expressing cell line to that by a non-expressing cell line (uptake ratio) has been confirmed to be not less than 2-fold, and there is significant inhibition by known inhibitors (Table 6-5) to the extent that can be theoretically estimated by the concentration of the added inhibitor and the K_i value. For the determination of K_i (or IC_{50}) value of the investigational drug, typical substrates can be selected from Table 6-5 and their recommended concentration should be sufficiently lower than their K_m value (see Table 6-5). The concentration range of the investigational

drug for the inhibition study should be set taking into consideration its clinical concentration exposed to the target transporters (unbound concentration in plasma).

b) Use IC_{50} based on the medium concentration instead of K_m may be used in the inhibition study of MATE1 and MATE2-K using cell system.

c) Inhibition of MATE1 and/or MATE2-K may cause an increase in the kidney concentration while causing no changes in the blood concentration.

Table 6-1 Examples of *in vivo* inhibitors that transporter-mediated drug interactions were clinically observed

Transporter	Gene	Inhibitor
P-gp	*ABCB1*	Lapatinib Dronedarone[a)] Cyclosporine Lopinavir/Ritonavir Quinidine Amiodarone Azithromycin Itraconazole Carvedilol Clarithromycin Quercetin[b)] Ranolazine[a)] Verapamil Diltiazem Darunavir/Ritonavir
BCRP	*ABCG2*	Curcumin[b)] Elacridar (GF120918)[a), c)] Elrombopag
OATP1B1, OATP1B3	*SLCO1B1*, *SLCO1B3*	Cyclosporin Rifampicin[d)] Atazanavir/Ritonavir Lopinavir/Ritonavir Clarithromycin Gemfibrozil[a)] Darunavir/Ritonavir
OAT1	*SLC22A6*	Probenecid
OAT3	*SLC22A8*	Probenecid
MATE1, MATE-2K	*SLC47A1*, *SLC47A2*	Cimetidine Pyrimethamine[a)]

a) Unapproved in Japan. ~~Dual inhibitors of P-gp and BCRP~~

b) Dietary supplements

c) Dual inhibitors of P-gp and BCRP

d) It should be noted that continuous administration enhances the inducing effect, leading to different consequences.

Table 6-2 Examples of *in vivo* inducers that transporter-mediated drug interactions were clinically observed

Transporter	Gene	Inducer
P-gp	*ABCB1*	Carbamazepine Phenytoin Rifampicin St. John's Wort[a)] Tipranavir/Ritonavir[b,c)]
OATP1B1, OATP1B3	*SLCO1B1*, *SLCO1B3*	Efavirenz Rifampicin

a) Dietary supplements

b) Unapproved in Japan

c) Note that the results of *in vitro* experiments indicate that ritonavir exerts inhibitory effect on P-gp, while tipranavir exerts both a weak inhibitory effect as well as strong inducing effect on P-gp. It should be noted that the apparent effects of these drugs arise from a combination of the inducing and inhibitory effects.

1272 **Table 6-3 Examples of *in vivo* substrates that transporter-mediated drug interactions**
1273 **clinically were observed**

Transporter	Gene	Substrates
P-gp	*ABCB1*	Aliskiren Ambrisentan[a)] Colchicine Dabigatran etexilate Digoxin Everolimus[a)] Fexofenadine Imatinib[a)] Lapatinib[a)] Maraviroc[a)] Nilotinib Ranolazine[a,b)] Saxagliptin[a)] Sirolimus[a)] Sitagliptin[a)] Talinolol [b)] Tolvaptan[a)] Topotecan[c)]
BCRP	*ABCG2*	Imatinib Rosuvastatin Sulfasalazine Diflomotecan [b)]

OATP1B1, OATP1B3	*SLCO1B1*, *SLCO1B3*	Atrasentan [b)] Atorvastatin Bosentan Ezetimibe Fluvastatin Fexofenadine Glibenclamide Nateglinide SN-38 (active metabolite of irinotecan) Rosuvastatin [d)] Simvastatin acid Pitavastatin[d)] Pravastatin Repaglinide Telmisartan[e)] Torsemide Valsartan Olmesartan
OCT2	*SLC22A2*	Metformin
MATE1, MATE2-K	*SLC47A1*, *SLC47A2*	Metformin Cisplatin Cephalexin
OAT1, OAT3	*SLC22A6*, *SLC22A8*	Adefovir[f)] Bumetanide[g)] Cefaclor Cidofovir[b,f)] Ciprofloxacin[g)] Famotidine[h)] Fexofenadine Furosemide Ganciclovir[f)] Methotrexate[g)] Penicillin G[g)] Zalcitabine [b)] Zidovudine

a) It should be noted that the apparent effects may be derived from inhibition of P-gp as well as inhibition of CYP3A4, because the compound is also a CYP3A4 substrate.

b) Unapproved in Japan

c) It should be noted that the apparent effects may be derived from inhibition of P-gp as well as inhibition of BCRP, because the compound is also a BCRP substrate.

d) The results of *in vitro* studies indicate that the contribution of OATP1B1 on the hepatic uptake is high.

e) The results of *in vitro* studies suggest that it is a selective substrate of OATP1B3 (vs. OATP1B1).

f) The results of *in vitro* studies suggest that it is a selective substrate of OAT1 (vs. OAT3).

g) The results of *in vitro* studies suggest that it is a selective substrate of OAT3 (vs. OAT1).

h) This is also a substrate of OCT2.

1274

1275

1276 **Table 6-4 Examples of typical substrates and inhibitors of transporters *in vivo***

Typical *in vivo* substrates

Transporter	**Gene**	**Substrate**
P-gp	*ABCB1*	Digoxin Fexofenadine[a)] Dabigatran etexilate
BCRP	*ABCG2*	Sulfasalazine Rosuvastatin[b)]
OATP1B1	*SLCO1B1*	Pitavastatin[c)] Pravastatin[d)] Rosuvastatin[b)]
OATP1B3	*SLCO1B3*	Telmisartan[e)]
OAT1	*SLC22A6*	Acyclovir Adefovir Ganciclovir Cidofovir [f)]
OAT3	*SLC22A8*	Benzylpenicillin Sitagliptin Ciprofloxacin Pravastatin[g)] Rosuvastatin[g)]
MATE1, MATE-2K, OCT2	*SLC47A1*, *SLC47A2*, *SLC22A2*	Metformin N-methylnicotinamide (NMN)[f, h)]

a) It is reported that OATP1B1, OATP1B3, MRP2, and MRP3 participate to hepatic clearance and OAT3, MATE1, and MATE2-K to renal clearance of fexofenadine.

b) It is reported that BCRP participates to intestinal absorption, OATP1B1, OATP1B3, and NTCP to hepatic uptake, and OAT3 to renal clearance, respectively, These drugs are also substrates for P-gp and MRP2 in *in vitro.*

c) Pitavastatin is also substrates of P-gp, MRP2, and BCRP in *in vitro*.

d) MRP2 and OAT3 are reported to participate to biliary 1277
and renal excretion, respectively.

e) Telmisartan is known to be conjugated by UGTs in intestine and liver.

f) Unapproved in Japan

g) Contribution of OAT3 is possible to estimate by elimination of the influence of renal clearance.

h) Because of the influence of endogenous substrates, foods, and timing of sampling, it is necessary to evaluate the role of transporter as a renal clearance.

1278

Typical inhibitors *in vivo* 1279

Transporter	**Gene**	**Inhibitor** 1280
P-gp	*ABCB1*	Quinidine Cyclosporin[a)] Ranolazine[b)] Amiodarone Itraconazole Clarithromycin[a)] Verapamil
BCRP	*ABCG2*	Curcumin b) Eltrombopag
OATP1B1, OATP1B3	*SLCO1B1,* *SLCO1B3*	Cyclosporin A[c)] Rifampicin[d)]
OAT1, OAT3	*SLC22A6,* *SLC22A8*	Probenecid
MATE1, MATE2-K	*SLC47A1,* *SLC47A2*	Cimetidine Pyrimethamine[b, e)]

a) It is reported that these drugs also inhibit OATP1B1 and OATP1B3 by clinical blood concentration.

b) Unapproved in Japan.

c) It is reported that the drug also inhibits intestinal P-gp by clinical blood concentration.

d) Single dose study are necessary to eliminate the influence of induction of the transporter by repeated administration.

e) Pyrimethamine is accepted to sale only as a combination drug with sulfadoxine in Japan.

1281 **Table 6-5 Examples of typical substrates and inhibitors *in vitro***

Typical *in vitro* substrates

Transporter	**Gene**	**Substrate**	**K_m value***
P-gp	*ABCB1*	Digoxin[a)]	73-177 μM
		Loperamide	(1.8-5.5 μM)
		Quinidine	1.69 μM
		Vinblastine[b)]	19-253 μM
		Talinolol[b)]	(72 μM)
		Fexofenadine[c)]	150 μM
BCRP	*ABCG2*	Estrone-3-sulfate[d)]	2.3-13 μM
		Dantrolene	
		Daidzein	
		Genistein	
		Coumestrol	
OATP1B1, OATP1B3	*SLCO1B1*, *SLCO1B3*	Estradiol-17β-glucuronide[e)]	2.5-8.3 μM (1B1), 15.8-24.6 μM (1B3)
		Estrone-3-sulfate[f)]	0.23-12.5 μM (1B1)
		Cholecystokinin octapeptide(CCK-8)[g)]	3.8-16.5 μM (1B3)
		Telmisartan[h)]	0.81 μM (1B3)
OAT1	*SLC22A6*	*p*-aminohippurate	4-20 μM
		Adefovir	23.8-30 μM
		Cidofovir	30-58 μM
OAT3	*SLC22A8*	Benzylpenicillin[i)]	52 μM
		Estrone-3-sulfate [j)]	2.2-75 μM
		Pravastatin[i)]	27.2 μM
MATE1, MATE-2K	*SLC47A1*, *SLC47A2*	Tetraethylammonium (TEA)[k)]	220-380 μM (MATE1), 760-830 μM (MATE-2K)
		Metformin[k)]	202-780 μM (MATE1), 1050-1980 μM (MATE-2K)
		1-methyl-4-phenylpyridinium (MPP+)[k)]	100 μM (MATE1), 110 μM (MATE-2K)
OCT2	*SLC22A2*	Tetraethylammonium (TEA) [k)]	33.8-76 μM

		Metformin[k)]	680-3356 μM
		1-methyl-4-phenylpyridinium (MPP+)[k)]	1.2-22.2 μM

* Figures in parentheses represent the K_i or IC_{50} values.

a) Substrates of OATP1B3.

b) Substrates of MRP2.

c) Substrates of OATPs, MRP2, and MRP3.

d) Substrates of OATPs, and NTCP.

e) Inhibition study indicates smaller K_i value. This substance has an appropriate characteristics of a marker drug.

f) Selective substrate of OATP1B1 (vs. OATP1B3) It is reported that inhibition study tends to indicates larger K_i value.

g) Selective substrate of OATP1B3 (vs. OATP1B1)

h) Selective substrate of OATP1B3 (vs. OATP1B1). Consider addition of albumin into test system to decrease the effects of nonspecific absorption.

i) Substrates of OATPs and MRP2.j) Substrate of OATP1B1 and BCRP.

k) Substrates of OCTs and MATEs.

1282

Typical *in vitro* inhibitors

Transporter	**Gene**	**Substrate**	**K_i or IC_{50} value***
P-gp	*ABCB1*	Cyclosporin A[a)]	0.5-2.2 μM
		Ketoconazole[b)]	1.2-6.3 μM
		Zosuquidar (LY335979)	0.024-0.07 μM
		Quinidine[c)]	3.2-51.7 μM
		Ritonavir[d)]	3.8-28 μM
		Tacrolimus[d)]	0.74 μM
		Valspodar (PSC833)[e)]	0.11 μM
		Verapamil[c)]	2.1-33.5 μM
		Elacridar (GF120918)[f)]	0.027-0.44 μM
		Reserpine[e)]	1.4-11.5 μM
BCRP	*ABCG2*	Sulfasalazine	0.73 μM
		Elacridar (GF120918)[g)]	0.31 μM
		Fumitremorgin C	0.25-0.55 μM
		Ko143	0.01 μM

		Ko134	0.07 μM
		noboviocin	0.063 - 0.095 μM
OATP1B1, OATP1B3	*SLCO1B1*, *SLCO1B3*	Estradiol-17□-glucuronide[e,f)]	2.5-8.3 μM (1B1), 15.8-24.6 μM (1B3)
		Estrone-3-sulfate[b,f)]	0.2-0.79 μM (1B1), 97.1 μM (1B3)
		Rifampicin	0.48-17 μM (1B1), 0.8-5 μM (1B3)
		Rifamycin SV	0.17-2 μM (1B1), 3 μM (1B3)
		Cyclosporin A[b, e, g)]	0.24-3.5 μM (1B1), 0.06-0.8 μM (1B3)
OAT1, OAT3	*SLC22A6*, *SLC22A8*	Probenecid[d)]	3.9-26 μM (OAT1), 1.3-9 μM (OAT3)
		Benzylpenicillin	1700 μM (OAT1), 52 μM (OAT3)
MATE1, MATE-2K	*SLC47A1*, *SLC47A2*	Pyrimethamine	77 nM (MATE1), 46 nM (MATE-2K)
		Cimetidine[c)]	1.1-3.8 μM (MATE1), 2.1-7.3 μM (MATE-2K)
OCT2	*SLC22A2*	Tetraethylammonium (TEA)[h)]	144 μM
		1-methyl-4-phenylpyridinium (MPP+)[h)]	(1.2-22.2 μM)

* Figures in parentheses represent the K_m values.

a) Substrates of MRP2, BCRP, NTCP, and OATPs.

b) Substrates of NTCP.

c) Substrates of OCTs and MATEs.

d) Substrates of OATPs.

e) Substrates of MRP2.

f) Substrates of BCRP.

g) P-gp inhibitor.

h) MATEs inhibitor.

The above table was prepared based on the reference[33)] and values obtained from relevant databases.

TP-search (http://www.TP-Search.jp/)

UCSF-FDA Transportal (http://bts.ucsf.edu/fdatransportal/)

1283

7. Evaluation of drug interactions by clinical studies

7.1. Need for clinical studies

Clinical drug interaction studies should be carried out ethically and scientifically. It is important to obtain sufficient information from *in vitro* studies using human tissue-derived specimens and expression systems, and to secure the safety of subjects, before clinical drug interaction studies. For predicting the drug interactions in humans based on the results of *in vitro* studies and etc., it would be useful to refer to the modeling analyses and simulation techniques which were applied for drugs of the Pharmacologically similar drugs having the same mechanism of drug interactions. For clinical drug interaction studies, it is necessary to prepare the study plan considering the safety of the subjects primarily, bearing in mind the adverse effects which are caused by the drug interactions.

7.2. Timing of clinical studies

It is recommended that, in principle, clinical drug interaction study(s) is conducted mainly in healthy volunteers, prior to phase III studies in principle, with the clinical dose of investigational drug (or marker drug, inducer drug, inhibitor drug if appropriate), when possibility of drug interactions has been suggested for the investigational drug. If significant drug interaction is observed, the drug interaction(s) with other drugs frequently co-administered with the investigational drug should be investigated as needed considering their characteristics and significance of the drug interaction. If the investigational drug is developed for the objective of being used concomitantly with other drugs, as in the cases of development of combination products, and development related to indications for combination therapy, etc., clinical drug interaction studies between the drugs in question should be carried out in principle.

The results of clinical drug interaction studies are utilized for the protocols of later clinical studies for considering acceptable concomitant drugs based on the observed drug interaction. In some cases, information obtained from PBPK model analysis and simulations helpful. If *in vitro* drug interaction studies suggest the possibility of clinically significant interactions with a concomitant drug, use of the concomitant drug in question should be contraindicated, until its safety is demonstrated by a clinical drug interaction study.

When the influences of drug interactions are to be examined in phase II or III studies, it is useful to obtain information on interactions with concomitantly used drugs through population pharmacokinetic analysis, for prediction of the pharmacokinetics taking into account inter-individual variations and evaluating the pharmacokinetics, efficacy, and safety of the investigational drug. If an occurrence of drug interactions is reported after the drug approval, post-approval clinical drug interaction studies should be considered as needed.

1318

1319 7.3. Relevant indices of drug interactions and judgment of the results

1320 To consider the mechanisms of drug interactions and evaluate the interactions quantitatively,
1321 pharmacokinetic parameters appropriate as indices of drug interactions should be selected.
1322 Pharmacokinetic parameters of the investigational drug and concomitant drugs, such as the AUC, C_{max},
1323 trough concentration, time to reach C_{max} (t_{max}), clearance, distribution volume and half-life, may be
1324 used according to need. Evaluation of the drug efficacy and adverse effects provide useful indices for
1325 drug interactions, depending on combination with the concomitant drugs.

1326 The presence/absence of drug interactions should be judged based on the results of clinical drug
1327 interaction studies, in terms of the 90% confidence interval of the geometric mean ratio of
1328 pharmacokinetic parameters obtained with and without the concomitant use of affecting drugs. The use
1329 of 90% confidence interval allows us to estimate distribution of the ratios for the pharmacokinetic
1330 parameters with and without concomitant drugs, and thereby to judge the intensity of the influences of
1331 drug interactions. When the 90% confidence interval of the geometric mean ratio falls in the range of
1332 80-125%, it is generally judged that there are no pharmacokinetic interactions between the drugs in
1333 question. It should be noted that there may be no clinically important drug interactions even when the
1334 90% confidence interval of the geometric mean ratio is outside the above range. On the contrary, there
1335 may be clinically significant drug interactions even when the 90% confidence interval of the geometric
1336 mean ratio is within the above range. Judgments based on the statistical test alone is not necessarily
1337 appropriate because the drug interaction may not be clinically important even if statistically significant
1338 difference ($p < 0.05$) was observed.

1339 If there is the possibility of clinically important drug interactions, it is appropriate to make a
1340 judgment of information and alerts on drug interactions taking into consideration the safety margin and
1341 the efficacious range of the investigational drug or concomitant drugs, considering their plasma drug
1342 concentration-response relationships.

1343

1344 7.4. Design of clinical studies

1345 Clinical drug interaction studies are carried out using study designs of randomized cross-over,
1346 add-on, parallel-group designs, and etc. The parallel-group design is not recommended usually because
1347 inter-individual differences become confounding factors, and it is acceptable only when the cross-over
1348 design and the add-on design are not feasible. Comparison with a control group in the separate study
1349 (historical controls) is unjustifiable in principle.

Drug interaction studies can be conducted in an open-label manner, except in cases where it is important to evaluate pharmacodynamic endopoints, including adverse events that are susceptible to biases, such as in evaluation by blood pressure or observation of symptoms.

Subjects who have ingested ethical drugs, over-the-counter drugs, supplements, health food products, tobacco, or alcohol prior to the registration should be considered to be excluded from the clinical drug interaction studies because these substances may alter the activities of drug metabolizing enzymes and transporters.

If the clearance of the investigational drug is considered to be strongly influenced by drug metabolizing enzymes or transporters whose activities are altered by genetic polymorphisms (CYP2D6, CYP2C9, CYP2C19, OATP1B1, etc.), the degree of drug interactions may vary according to the gene polymorphism. In this case, a study design using stratification by genotype may be useful (see section 6.10.5.1.).

7.5. Dose and route of administration

The doses of inhibitors or inducers used in the study should be those that maximize the possibility of drug interactions, the planned or approved maximum doses and minimum intervals of administration should be used. For substrate drugs, any dose in the linear range can be used. If the substrate drug has dose-dependent pharmacokinetics, the dose used should be determined with consideration of the therapeutic dose. A reduced dose of the substrate drug(s) may need to be considered for safety concerns, and influences of alterations in the dosage and administration on the evaluation of drug interactions, e.g. with the object of detection sensitivity of analysis method, should be discussed and described in the clinical study protocol and the clinical study report.

The route of administration chosen for a metabolic drug-drug interaction study is important. For an investigational agent, the route of administration generally should be the one planned for clinical use. When multiple routes are being developed, it should be considered whether the need for metabolic drug-drug interaction studies by each route depends on the expected mechanisms of interaction and the degree of changes in the AUC of the investigational drug and metabolites. If only oral dosage forms will be marketed, studies with an intravenous formulation are not usually recommended,

7.6. Duration and timing of administration

In metabolic drug-drug interaction studies, it is desirable to examine drug interactions in the steady state at multiple dose for the investigational drug, if the investigational drug is the interacting drug, in consideration of the possibility of mutual effects from unknown metabolites. In particular, a loading dose could be used at least several days for drugs that may cause enzyme induction or have

shown TDI in *in vitro* studies. In this case, it is considered that the dose and dosing interval may be adjusted and target steady state concentrations could be reached earlier. It is recommended that a possibility of recovery for CYP enzyme activity is evaluated, e.g., when substrate drug is administered continuously the day after administration of investigational drug. However, when the interacting drug neither shows the possibility of TDI nor enzyme induction, etc., or when the investigational drug is expected to be used in a single-dose therapy in clinical practice, implementation of single-dose studies is a possible option. In general, drug interaction studies using a single dose design can be applied to investigational drugs that are substrate drugs. If the expected drug interactions may cause prolonged variations the enzyme activities due to TDI or induction, etc., it is recommended to set a third cross-over period in which the interacting drug is removed to evaluate enzyme activity to be returned to normal. If the absorption of the interacting drug is influenced by other factors (e.g., intragastric pH), it is useful to identify the cause of the interactions by, for instance, confirming interactions that occur under suppressed gastric acid secretion.

Attention should also be paid to the influences of the timing of administration of the substrate and the interacting drug on interactions between these drugs. In clinical drug interaction studies, the administration interval between the two drugs should be defined in advance, and usually, the two drugs should be administered simultaneously. If the drug interactions occur for the most part during the first pass, the strength of drug interactions may be decreased by allowing an interval between the administrations of the two drugs. However, it is also possible that distinct drug interactions occur when the two drugs are administered at different time points.*[note (15)]

7. 7. Selection of inhibitors for drug metabolizing enzymes and transporters

7.7.1. Studies using inhibitors of P450

For evaluation of the possibility that an pharmacokinetics of investigational drug is affected by P450 inhibition, a clinical drug interaction study is conducted with inhibitors of the enzymes involved in the metabolic pathway of the investigational drug, selected on the basis of the results of *in vitro* studies and clinical pharmacology studies. In case of oral coadministration of interacting drug and substrate in clinical drug interaction study, strength of inhibition is considered following; a interacting drug is considered a *strong inhibitors* if a interacting drug increase the AUC of sensitive substrates by 5-fold or higher (or decrease the CL/F to less than 1/5), a interacting drug is considered *moderate inhibitors* if a interacting drug increase the AUC of sensitive substrates by 2-fold or higher but less than 5-fold (or decrease the CL/F to less than 1/2 but more than 1/5), and a interacting drug is considered *weak inhibitors* if a interacting drug increase the AUC of sensitive substrates by 1.25-fold or higher but less than 2-fold (or decrease the CL/F to less than 1/1.25 but more than 1/2). (see Table 7-

1 and section 7.9 for sensitive substrates). In selecting inhibitors to be used in clinical drug interaction studies, it should be used strong inhibitors of enzymes involved in the elimination of the investigational drug, however safety of the subject should be considered as much as possible (see Section 4.2.1.2. and Table 7-1). In the event that it is difficult from the safety concern to conduct clinical interaction studies using strong inhibitors, such as when such drugs may be contraindicated for coadministration with other drugs in the clinical practice, the effects of moderate or weak inhibitors should be evaluated through clinical drug interaction studies while paying attention to the safety of the subjects. When the necessity for consideration of dose adjustment is suggested from results of interaction studies using strong inhibitors, in consideration of the possibility of coadministration in clinical practice, the effects of other inhibitors on the same drug metabolizing enzyme should also be evaluated in clinical study. Inhibitors other than those which have been evaluated in clinical drug interaction studies may be evaluated, as required, via phase II or phase III clinical trials or via model analysis.

For major drug metabolizing enzymes of the investigational drug not listed in Table 7-1, the inhibitory effects on the specific enzyme would be examined using drugs that are used concomitantly in the clinical practice, while considering the safety at blood concentrations of the investigational drug over the therapeutic dose and the contribution of the specific metabolic pathway to the overall elimination of the investigational drug.

7.7.2. Studies using inhibitors of drug metabolizing enzymes and other than P450 and transporters

If there is the risk that the investigational drug is metabolized by enzymes other than P450 or is transported by transporters and causes drug interactions by inhibition in clinical practice, it is recommended to consider the feasibility of clinical drug interaction studies, considering whether known inhibitors are presence of the specific enzymes or specific transporters. When clinical drug interaction studies are conducted, the interactions should be evaluated according to the same procedures as those for the drugs metabolized by P450.

7. 8. Selection of inducers for drug metabolizing enzymes

In investigating the effects of P450 inducers on the pharmacokinetics of an investigational drug, inducers for P450s involved in the metabolic pathways of the investigational drug should be selected. When interacting drug effects on the AUC of oral sensitive substrates, strength of induction is considered following; a interacting drug is considered a *strong inducers* if a interacting drug decrease the AUC of sensitive substrates to 1/5 or less (or increase the CL/F by higher than 5-fold), a interacting drug is considered *moderate inducers* if a interacting drug decrease the AUC of sensitive substrates to 1/2 or less but more than 1/5 (or increase the CL/F by ≥2- to <5-fold), and a interacting drug is

considered *weak inducers* if a interacting drug decrease the AUC of sensitive substrates to 1/1.25 or less but more than 1/2 (or increase the CL/F by ≥1.25- to <2-fold). (see Table 7-2). When inducers to be used in clinical drug interaction studies is selected, it is desirable that strong inducers of P450 involved in the elimination of the investigational drug be used, however safety of the subject should be considered as much as possible. (see Section 4.2.1.2. and Table 7-2). In the case of an investigational drug whose elimination proceeds mainly via metabolism by CYP3A, it is assumed that the likelihood of drug interactions with strong inducers such as rifampicin is extremely high, so that coadministration with these strong inducers will eventually be contraindicated. Therefore, the effects of such investigational drugs should be assessed through clinical drug interaction studies using moderate or weak inducers. In the case of an investigational drug which may have to be used concomitantly with a specific enzyme inducer from the viewpoints of indications and dosage, it is recommended that a clinical drug interaction study with the specific inducer be conducted with consideration for safety of subject as much as possible, in order to determine an appropriate treatment method (see Section 4.2.1.2.).

7. 9. Selection of substrates for drug metabolizing enzymes and transporters

Generally, in the case of interacting drugs and affected drugs to be coadministered orally, the contribution ratio (CR) of a specific P450 enzyme to elimination of an investigational drug whose AUC increases by 5-fold or higher (or a decrease in the CL/F ratio to < 1/5) when coadministered with a "*strong inhibitor*" of the specific P450 enzyme is considered to be roughly ≥80%, and it is considered *a sensitive substrate* of the enzyme (see Table 7-3). The CR of a specific P450 enzyme to elimination of investigational drug whose AUC increases by ≥2- to <5-fold (or a decrease in the CL/F ratio to ≥1/5 but < 1/2) is considered to be roughly between 50% and <80%, and it is considered a *moderate sensitive substrate* of the enzyme (see Table 7-3). For assessing by clinical studies whether an investigational drug inhibits or induces a drug metabolizing enzyme (or a transporter) or not, a drug interaction study should be performed using a marker drug (or a typical substrate drug for transporters, Table 6-4) which a substantive drug metabolizing enzyme (or transporter) contribution to total elimination is high (sensitive substrate) and whose pharmacokinetics are markedly altered by the co-administration of known specific pathway of the enzyme system. Examples of *in vivo* marker drugs, the following may be mentioned: (1) midazolam for CYP3A; (2) theophylline for CYP1A2; (3) bupropione and efavirenz for CYP2B6; (4) repaglinide for CYP2C8; (5) S-warfarin and tolbutamide for CYP2C9; (6) omeprazole for CYP2C19; and (7) metoprolol for CYP2D6 (see Table 7-3). In case of that it has been demonstrated in clinical drug interaction studies that an investigational drug inhibits or induces metabolism of a marker drug or sensitive substrate, it should be considered whether additional

clinical drug interaction studies is added using a substrate drug for the specific enzyme which is very likely to be coadministered post-marketing (see Figs. 4-2 and -3, and Sections 4.2.1.4. and 4.2.1.6.)* note (16).

7.10. Other considerations for evaluation by clinical studies

7.10.1. Drugs metabolized by a single enzyme and multiple enzymes

The concentrations of drugs in the body that are metabolized by a single enzyme (drugs with a single metabolizing enzyme) become markedly high if the enzyme involved is inhibited. On the other hand, for drugs that are metabolized by multiple enzymes (drugs with multiple metabolizing enzymes), the elevation of concentrations in the body would be less, because the investigational drug is metabolized by other enzymes (alternate enzymes) even if the principal enzyme is inhibited. Likewise in the case of enzyme induction, concentrations in the body are lowered markedly if the investigational drug is metabolized by the induced enzyme, although the decrease in concentrations of the investigational drug would be relatively slight if there are other enzymes that are involved in the metabolism of the investigational drug. For predicting the degree of these interactions, the investigations with modeling and simulation to the analyzed results of appropriately designed drug interaction studies are considered to be useful.

7.10.2. Drug interactions involving both drug metabolizing enzymes and transporters

Multiple mechanisms may be involved in drug interactions due to overlapping of the substrate specificities for drug metabolizing enzyme and transporter (complex drug-drug interactions)[34]. Overlapping of the substrate specificities for CYP3A and P-gp is a representative example. These drug interactions can be evaluated with the use of inhibitors that exert a strong inhibitory action against both P-gp and CYP3A such as itraconazole. However, caution is necessary in the interpretation of the study results, because it is impossible to identify the mechanism responsible for the change in AUC even if the interaction is evident.

It is also possible that the investigational drug causes interactions by inhibiting (or inducing) multiple enzymes and transporters, or by inhibiting a certain enzyme (or transporter) and inducing another enzyme or transporter simultaneously. In addition, if both metabolizing enzymes and transporters are inhibited by concomitant use of multiple drugs, the resultant influences may be even more complex and serious.*note (17)

7.10.3. Cocktail studies

Cocktail studies can be used for evaluating the actions of the investigational drug on several different enzymes and transporters in a single clinical drug interaction study[34]. If designed appropriately, cocktail studies allow examination of both inhibition (competitive inhibition and TDI) and induction. When the results of appropriately implemented cocktail studies are negative, there is no need to carry out further evaluation of the enzymes or transporters in question. However, if the results are positive, further clinical drug interaction studies with a sensitive substrates (marker or typical substrates, see Tables 7-3, 6-4) are necessary. The substrates used in cocktail studies should consist of sensitive substrates susceptible to interactions for each target enzyme (and transporter).*note (18) The effects of the investigational drug on the CL/F or AUC should be obtained for each marker or substrate drug used.

7.10.4. Evaluation by population pharmacokinetic analysis

Drug interactions that have not been evaluated in independent drug interaction studies may be investigated if the study plan is such as to allow evaluation of drug interactions in population pharmacokinetic analyses in phase II or III studies by collecting the information of concomitant drugs. On the other hand, it may not be possible to evaluate interactions of the investigational drug to other drugs because the blood concentrations of concomitant drugs are generally not measured in phase II or III studies. Handling of assay samples, timing of collection, and etc., should be determined appropriately for the evaluation of drug interactions.

7.10.5. Considerations for special populations

7.10.5.1. Evaluation of drug interactions related to genetic polymorphisms

The degree of drug interactions (inhibition or induction) of the investigational drug on a certain target enzyme or transporter may vary according to the genotype of the subject. In subjects in whom the major elimination pathway (drug metabolizing enzymes or transporters) is lacking or functioning poorly, the drug concentrations are generally high. If the alternative pathway of metabolism or excretion of the investigational drug for these subjects is inhibited by concomitant use of an inhibitor, the drug concentrations rise further, possibly causing safety issues.

Molecular species of metabolizing enzymes and transporters with pharmacokinetics that is greatly affected by the genetic polymorphism include CYP2D6, CYP2C9, CYP2C19, and OATP1B1 (SLCO1B1)[36]. For drugs whose major elimination pathway is mediated by these metabolizing enzymes or transporters, the study design using stratification by genotypes may be useful in clinical drug interaction studies.

It is also necessary to give consideration to racial or ethnic differences in the type and frequency of genetic polymorphisms. In particular, ethnic differences should be borne in mind when implementing clinical studies of investigational drugs whose major elimination pathway is mediated by CYP2C19 and CYP2D6; genetic polymorphisms that cause the defective activity of the former enzyme and marked reduction of the activity of the latter enzyme are frequent among East Asians.*[note (19)]

7.10.5.2. Investigational drugs mainly applied to special populations or patient populations with specific diseases

If the investigational drug is mainly administered to pediatrics, geriatrics or patient populations with renal dysfunction or hepatic impairment, drug interactions of the investigational drug can be evaluated also by population pharmacokinetic analyses appropriately designed, or by using PBPK models. To predict drug interactions in the clinical setting, it is important to make an appropriate prediction of the relative CR of the enzyme to the overall elimination, and attention should be paid not to miss clinically significant drug interactions in these populations, e.g., by conducting examinations under the assumption of the worst case scenario in model prediction.

7.10.5.3. Studies in patients

Clinical drug interaction studies are usually carried out in healthy volunteers, and drug interactions are often extrapolated to patient populations based on the results of studies in healthy volunteers. For some drugs such as anticancer drugs, because of safety issues, studies are implemented in patient populations in which these drugs are indicated, and these studies may have limitations of the study design in terms of the study duration, doses, and schedule of blood collection. When investigating drug interactions in these populations, it may be useful to supplement information accordingly by applying modeling and simulation, as well as giving adequate consideration to inter-individual variability in patient populations.

1577 **Table 7-1 Examples of the *in vivo* inhibitors of P450**

CYP molecular species	Strong inhibitors ≥5-fold increase in the AUC (decrease in the CL/F ratio to less than 1/5)	Moderate inhibitors ≥2- to 5-fold increase in the AUC (decrease in the CL/F ratio to less than 1/2 but more than 1/5)	Weak inhibitors ≥1.25- to <2-fold increase in the AUC (decrease in the CL/F to less than 1/1.25 but more than 1/2) **t**
CYP3A	5~~10 fold (less than 1/10): itraconazole, voriconazole, ritonavir, telaprevir, indinavir, cobicistat 10 fold or highr (1/10 or higher）: clarithromycin, grapefruit juice, erythromycin, nelfinavir, saquinavir Unapproved, 5~10 fold (less than 1/10）: ketoconazole, posaconazole, troleandomycin, conivaptan Unapproved, 10 fold (1/10 or higher ）: boceprevir, nefazodone	diltiazem, verapamil, fluconazole, miconazole, aprepitant, imatinib, crizotinib, ciprofloxacin, tofisopam, atazanavir, fosamprenavir, cyclosporine, istradefylline, amprenavir Unapproved: casopitant, almorexant, dronedarone, schisandra sphenanthera extract	1.25~1.5 fold (less than 1/1.5）: fluvoxamine, cimetidine, ranitidine, chlorzoxazone, cilostazol, tacrolimus, fosaprepitant, goldenseal (hydrastis canadensis) 1.5~2 fold (1/1.5 or higher）: bicalutamide, nilotinib, pazopanib, azithromycin, berberine, cranberry juice, everolimus, propiverine, roxithromycin, amlodipine, gemfibrozil, linagliptin, valsartan, oral contraceptives, isoniazid, tolvaptan, alprazolam, amiodarone, ginkgo (herbal product), lapatinib, atorvastatin Unapproved, 1.25~1.5 fold (less than 1/1.5）: clotrimazole, ivacaftor, tabimorelin, almorexant, lomitapide, ranolazine, ticagrelor Unapproved, 1.5~2 fold (1/1.5 or higher）: lurasidone ,fluoxetine, zileuton, lacidipine,
CYP2D6	5~10 fold (less than 1/10）: quinidine, cinacalcet 10 fold (1/10 or higher ）: paroxetine, terbinafine Unapproved, 5~10 fold (less than 1/10）: bupropion, fluoxetine Unapproved,10 fold (1/10 or higher ）: dacomitinib	duloxetine, mirabegron, celecoxib, escitalopram Unapproved : moclobemide	1.25~1.5 fold (less than 1/1.5）: cimetidine, sertraline, clobazam, ritonavir, labetalol, amiodarone 1.5~2 fold (1/1.5 or higher ）: echinacea (herb) Unapproved, 1.25~1.5 fold (less than 1/1.5）: desvenlafaxine, cobicistat, deramciclane, abiraterone, lorcaserin Unapproved,1.5~2 fold (1/1.5 or higher ）: vemurafenib
CYP2C9	fluorouracil derivatives (TS-1, UFT, tegafur, fluorouracil, doxifluridine, capecitabine), miconazole Unapproved: sulfaphenazole, carmofur	amiodarone, fluconazole, miconazole, cyclosporine, bucorol Unapproved: tienilic acid	1.25~1.5 fold (less than 1/1.5): voriconazole 1.5~2 fold (1/1.5 or higher): fluvastatin, fluvoxamine, cimetidine, disulfiram Unapproved,1.25~1.5 fold (less than 1/1.5): diosmin

CYP2C19	10 fold (1/10 or higher): fluconazole, fluvoxamine, ticlopidine, voriconazole	clarithromycin Unapproved: fluoxetine, moclobemide, ketoconazole	1.25~1.5 fold ((less than 1/1.5): allicin, etravirine, ticlopidine, clarithromycin, omeprazole, ritonavir 1.5~2 fold (1/1.5 or higher): oral contraceptives, clopidogrel, roxithromycin, Unapproved: 1.25~1.5 fold (less than 1/1.5): ketoconazole, troleandomycin Unapproved:1.5~2 fold (1/1.5 or higher): armodafinil, grapefruit juice
CYP2C8	Unapproved:10 fold (1/10 or higher): gemfibrozil	cyclosporine, deferasirox Unapproved: gemfibrozil, teriflunomide	1.25~1.5 fold (less than 1/1.5): trimethoprim 1.5~2 fold (1/1.5 or higher): clarithromycin, itraconazole Unapproved: 1.25~1.5 fold (less than 1/1.5): gemfibrozil, telithromycin
CYP1A2	10 fold (1/10 or higher): ciprofloxacin, fluvoxamine, zafirlukast, rofecoxib	methoxsalen, oral contraceptives, mexiletine Unapproved: enoxacin, clinafloxacin, etintidine, idrocilamide	1.25~1.5 fold (less than 1/1.5): cimetidine, ciprofloxacin, mexiletine 1.5~2 fold (1/1.5 or higher) : acyclovir, allopurinol, fluvoxamine, peginterferon alpha-2a (sc) Unapproved:1.25~1.5 fold (less than 1/1.5): zileuton, enoxacin, grepafloxacin, piperine, pefloxacin, rofecoxib, pipemidic acid Unapproved: 1.5~2 fold (1/1.5 or higher): antofloxacin, viloxazine, daidzein,
CYP2B6	-	-	1.25~1.5 fold (less than 1/1.5): ticlopidine, tenofovir 1.5~2 fold (1/1.5 or higher): clopidogrel, voriconazole

*Refer to Table 7-3 and section 7.9 (Selection of substrates for drug metabolizing enzymes)

This table was prepared based on the package insert, literature and other published documents, information from the drug interaction database of the University of Washington, etc, and will be updated based on new information.

Drugs for external use and combination drugs are not included.

Fosamprenavir (potassium hydrate), which is a prodrug of amprenavir, a moderate inhibitor of CYP3A, has been approved.

Table 7-2 Examples of *in vivo* inducers of P450

CYP molecular species	Strong inducers decrease in the AUC to 1/5th or less (increase of the CL/F ratio by ≥5-fold)	Moderate inducers decrease in the AUC to 1/2 or less but more than 1/5 (increase in the CL/F ratio by ≥2- to <5-fold)	Weak inducers decrease in the AUC to 1/1.25 or less but more than 1/2 (increase in the CL/F ratio by ≥1.25- to <2-fold-)
CYP3A	rifampicin, rifabutin, phenobalbital, phenytoin, carbamazepine	etravirine, efavirenz, St. John's wort, modafinil, bosentan	rufinamide, Unapproved : armodafinil
CYP2C9	-	rifampicin, phenobalbital, carbamazepine, aprepitant	-
CYP2C19	rifampicin, ritnavir	rifabutin	-
CYP2C8	-	rifampicin	-
CYP1A2	-	phenytoin, smoking	Montelukast Unapproved : moricizine
CYP2B6	-	efavirenz	rifampicin, nevirapine

*Refer to Table 7-3 and section 7.9 (Selection of substrates for drug metabolizing enzymes)

This table was prepared based on the package insert, literature and other published documents, information from the drug interaction database of the University of Washington, etc. and will be updated based on new information.

Table 7-3 Examples of substrate drugs susceptible to pharmacokinetic drug interactions by P450 inhibition or induction

CYP molecular species	Sensitive substrates susceptible to pharmacokinetic drug interactions AUC increases by ≥5-fold (or a decrease in the CL/F ratio to < 1/5) when coadministered with a strong inhibitor of P450	Moderate sensitive substrates susceptible to pharmacokinetic drug interactions AUC increases by ≥2- to <5-fold (or a decrease in the CL/F ratio to ≥1/5 but < 1/2) when coadministered with a strong inhibitor of P450
CYP3A	alprazolam, aprepitant, azelnidipine, blonanserin, budesonide, buspirone, eleptriptan, eplerenone, colchicine, conivaptan, darifenacin, darunavir, dasatinib, evelolimus, felodipine, fluticasone, indinavir, lopinavir, lovastatin, maraviroc, midazolam, nisoldipine, quetiapine, rosuvastatin, saquinavir, sildenafil, simvastatin, sirolimus, tadalafil, tolvaptan, triazolam, vardenafil Unapproved: alfentanil, dronedarone, lurasidone, tipranavir, ticagrelor	atorvastatin, pimozide, rilpivirine, rivaroxaban, tacrolimus
CYP2D6	atomoxetine, desipramine, dextromethorphan, nortriptyline, maprotiline, metoprolol, paroxetine, perphenazine, propafenone, tamoxifen, tolterodine, tramadol, trimipramine, tropisetron, venlafaxine Unapproved: doxepin, encainide, nebivolol	amitriptyline, clomipramine, flecainide, imipramine, timolol, propranolol
CYP2C9	celecoxib, diclofenac, glimepiride, tolbutamide, warfarin	glibenclamide, nateglinide, phenytoin, ibuprofen, fluvastatin
CYP2C19	clobazam, omeprazole*, lansoprazole, S-mephenytoin, voriconazole	clopidogrel, diazepam, escitalopram, esomeprazole, etizolam, rabeprazole, sertraline,
CYP2C8	montelukast, repaglinide*	pioglitazone
CYP1A2	caffeine, duloxetine, pirfenidone, ramelteon, tizanidine Unapproved: alosetron, melatonin, tacrine	clozapine, olanzapine, ropinirole, ramosetron, theophylline
CYP2B6	efavirenz Unapproved: bupropion	

Refer to section 7.9 for marker drug recommended to clinical drug interaction studies

This table was prepared based on the package insert, literature and other published documents, information from the drug interaction database of the University of Washington, etc. and will be updated based on new information.

This table is prepared to provide examples of drugs that are associated with great involvement of P450 enzymes in the metabolism, and is not based on an exhaustive survey.

1607 It should be noted that the degree of pharmacokinetic drug interaction is often inconsistent with significance of the clinical actions need to be taken for such interaction

1608 * Refer to note(16)

1609

8. Labeling recommendations

Information obtained from drug interaction studies during the drug development process appears to be valuable for proper use of drugs, when provided appropriately to professionals involved in patient care in actual medical practice through descriptions in package inserts, etc.

The basic ideas for reflection of information on pharmacokinetic drug interactions in package inserts are as follows. When judging the contents of the information and alerts, attention should be focused on whether or not pharmacokinetic changes might affect the therapeutic efficacy or adverse drug reactions.

8.1. Description in precautions for use

When concomitant use of other drugs may cause enhancement or attenuation of the pharmacological actions, known adverse drug reactions, occurrence of new adverse drug reactions of the investigational drug or of the concomitant drugs, or aggravation of the primary disease, and if clinical precautions are necessary for these cases, it should be determined whether the warning should be “Contraindication (Do not use concomitantly)” or “Precaution (Be careful about concomitant use)” according to the degree of reduction in the efficacy resulting from the drug interactions, type and grade of the adverse drug reactions, and the degree of variations in the pharmacokinetic parameters (AUC and C_{max}). When the serious adverse drug reactions are anticipated realistically, and the consequence would be more clinically important than expected therapeutic effects, regardless of pharmacokinetic changes, the combination should be contraindicated in principle. When the therapeutic effect is clinically important, however, the predicted exposure of the investigational drug may exceeds the exposure expected for approval dosage and administrations due to pharmacokinetic changes, and in situations when a management would be required to avoid serious clinical risk and concerns of patients, the combination should be precaution or contraindication in accord with the seriousness of the risk.

In the section of “Drug Interactions,” at the beginning of the section, pharmacokinetic properties of the investigational drug related to drug interactions, such as enzyme molecular species involved in the metabolism, approximate CR of the enzyme, inhibition and induction potentials, and mechanisms of drug transport in absorption, distribution and excretion, should be described briefly based in principle on the clinical pharmacokinetic information. If an

investigational drug affects the pharmacokinetics of other drugs via P450 (inhibitors or inducers: interacting drugs), the potency of interaction should also be stated (see Sections 7.8 to 9, Tables 7-1 and -2). When an alert is raised for concomitant use with other drugs, the caution should be expressed in an easily understandable manner using tables, etc. The names of the concomitant drugs and the details of the drug interactions (clinical symptoms, treatment, mechanisms, risk factors, etc.) should be provided with appropriate classifications based on the type of interactions (mechanism, etc.). In the case of pharmacodynamic interactions, the therapeutic class should be given in the drug name column in addition to the names of the drugs. All contraindicated drugs should be identified by both non-proprietary names and representative brand names. Contraindicated drugs should also be described briefly in the section of "Contraindications."

Precautions to avoid the influences of drug interactions should be described under "Clinical Symptoms and Treatment". Further, the mechanisms underlying drug interactions and risk factors that may potentially raise a safety concern due to concomitantly administered medication should be described in the "Mechanisms and Risk Factors". If the mechanisms underlying interactions are unclear, the mechanisms should be stated as unknown.

If adjustment of the dosage regimen of the investigational drug is necessary to compensate the drug interactions, the adjustment should be described comprehensively in the "Precautions for Dosage and Administration" based on the quantitative information secured from conducted clinical interaction studies, etc. If the precaution is critical from the viewpoint of risk management, it should be described in the "Important Basic Precautions" section.

If the investigational drug interactions with drugs used for diagnostic (testing) purposes, and the consequence is apparent variations in clinical laboratory test which does not related to the clinical symptoms or functional disorders, cautions should be described under "Influences on Clinical Laboratory Test Results." Drug incompatibilities should be described under "Precautions for Application" or "Precautions for Handling." Important interactions with biotechnical and biological products or foods and beverages should also be described in the same manner.

When a clinically significant adverse drug reaction had been caused unambiguously by pharmacokinetics drug interactions in pharmacologically similar drugs, and similar degree of pharmacokinetic interactions of the same mechanism are suggested for the investigational drug appropriately by simulations or by a model analysis, alerts should be described in the

labeling considering possibility of the concomitant use in clinical practice. Although the alerts is determined based on pharmacokinetic changes as index in this case, the grade and contents of the alerts should be described in consideration of the clinical significance including changes in the efficacy and safety, and how the drug interaction should be managed. In the description of alerts, the use of model analysis and simulations should be clearly specified.

8.2. Description in the pharmacokinetics section

In regard to the descriptions provided in the "Pharmacokinetics" section, matters related to the mechanisms of interactions (descriptions of quantitative information on the major elimination pathway, enzymes involved in that pathway, and the degree of their contribution, inhibition and induction of drug metabolizing enzymes, mechanisms of drug transport in absorption, distribution and excretion, and corroborating *in vivo* or *in vitro* study results) should be described in the pertinent sections on metabolism, excretion, etc. When providing information concerning data, it should be specified whether the data were derived from *in vitro* studies or clinical drug interaction studies, whether they were obtained by actual measurement or represent estimates obtained from simulation, etc., in a clear distinctive manner. Regardless of whether the interactions are significant or not, information from the clinical drug interaction studies, which is considered to be clinically useful, should be provided in the "Pharmacokinetics" section appropriately. If the pharmacokinetic change(s) may exert influence upon the therapeutic responses or risk of occurrence of adverse drug reactions, the observed pharmacokinetic changes, as well as the dosage and administration, etc., used in the studies should be provided. Study results should be illustrated as a quantitative, simple outline of the changes in the AUC, C_{max}, etc., using narrative text, tables and/or figures (e.g., forest plot). Further information on the detailed study design and data should be provided using materials other than the package inserts. In any such instance of provision of information in the package insert, the evidences should be clearly stated, e.g., by citing the literature.

8.2.1. Drugs whose pharmacokinetics are affected by other drugs (substrates: affected drug)

For drugs whose pharmacokinetics are affected by other drugs, the pharmacokinetic mechanisms underlying the interactions and the magnitude of the influence should be exerted, and the information should be provided quantitatively. Information on this issue can be

obtained usually by the clinical drug interaction studies using selective and strong inhibitors of the pathway in question (see Sections 7-7, -8 and Tables 7-1 and -2 for inhibitor and inducer drugs). On the other hand, if a particular clearance pathway is not the major elimination pathway for the investigational drug, it is sufficient merely to provide supporting information obtained from *in vitro* studies.

8.2.2. Drugs that affect pharmacokinetics of other drugs (inhibitors, inducers: interacting drugs)

Investigational drugs that affect pharmacokinetics of other drugs should be described by providing the pharmacokinetic mechanisms underlying the interactions and the magnitude of the influence exerted quantitatively. This information is generally obtained from clinical drug interaction studies carried out with a sensitive substrate drug to the pathway involved in the interaction (see Section 7.9. and Table 7-3). If the elimination pathway in which substrate drugs are affected by concomitant use of the investigational drug is metabolism, the generalized inhibitory or inductive potency of inhibitors or inducers should be described as needed (see Sections 7.8 to 9, Tables 7-1 and -2). As for transporters, the observed degree of inhibitory or inductive effect exerted on a typical substrate drug(s) (see Table 6-4) should be described, because it is currently not possible to define these generalized criteria.

8.3. Description of affected drugs and interacting drugs

All drugs contraindicated for coadministration, whether interacting drugs or interacted drugs, should be described by both non-proprietary names and representative brand names.

For calling attention to “Precautions of Concomitant Use”, drugs in this category should be described by the non-proprietary names. It is impracticable, nevertheless, to describe drug interactions involving CYP3A for all combinations of the drug in question with concomitant drugs in package inserts, inasmuch as there are a large number of drugs requiring caution in concomitant use and as the degree of caution required varies not only with the efficacy, but also with the pharmacokinetic characteristics of the concomitantly administered drugs

In regard to drug interactions involving CYP3A, a number of “strong” or “moderate” CYP3A inhibitors and inducers that are very likely to cause drug interactions that would substantially affect the efficacy and safety, and should be alerted in the "Precaution of Concomitant Use". Therefore, descriptions of names of individual CYP3A substrate drugs,

inhibitors, and inducers in the precautions for coadministration column in the “Drug Interactions” section may be condensed as follows; in the case of CYP3A inhibitors and inducers, by stating that the package inserts of concomitant drugs need to be checked, and by identifying classification of the strength of interaction, and in the case of CYP3A substrate drugs, by stating the drug is metabolized mainly by CYP3A at the outset in the “Drug Interactions” section. Even in such instances, names of approximately three representative concomitant drugs should be listed taking into account the frequency of coadministration in the clinical setting.

In alerts of drug interactions with CYP molecular species other than CYP3A, with drug metabolizing enzymes other than CYP, or with transporters, the individual names of concomitant drugs should be stated by the non-proprietary names as described above.

9. Relevant guidelines and documents

This guideline shows general principles of investigational methods of study and alerting (labeling recommendations?) related to drug interactions. Although previously issued guidelines, guiding principles, etc., include descriptions of studies of drug interactions, the present guideline provides the integrated contents of such guidelines in which current new findings and concepts have been incorporated. It is desirable to refer to information described in other relevant guidelines, guidances and other regulatory documents when considering individual investigational drugs.

1) Clinical Safety Data Management: Definitions and Standards for Expedited Reporting (ICH E2A Guideline), Notification No. 227 of the Evaluation and Licensing Division, PMSB, dated March 20, 1995.

2) Post-approval Safety Data Management: Definitions and Standards for Expedited Reporting (ICH E2D Guideline), Notification No. 0328007 of the Safety Division, PFSB, dated March 28, 2005.

3) Pharmacovigilance Planning (ICH E2E Guideline), Notification No. 0916001 of the Evaluation and Licensing Division, PMSB, dated September 16, 2005.

4) Structure and Content of Clinical Study Reports (ICH E3 Guideline), Notification No. 335 of the Evaluation and Licensing Division, PMSB, dated May 1, 1996. Questions & Answers (R1), Office Memo, dated October 18, 2012.

5) Dose-Response Information to Support Drug Registration (ICH E4 Guideline), Notification No. 494 of the Evaluation and Licensing Division, PMSB, dated July 25, 1994.

6) Ethnic Factors in the Acceptability of Foreign Clinical Data (ICH E5 Guideline), Notification No. 672 of the Evaluation and Licensing Division, PMSB, dated August 11, 1998. Questions and Answers, Office Memo, February 25, 2004 and October 5, 2006.

7) Guideline for Good Clinical Practices (GCP) (ICH E6 Guideline), March 27, 1997.

8) Studies in Support of Special Populations: Geriatrics (ICH E7 Guideline), Notification No. 104 of the New Drugs Division, PAB dated December 2, 1993. Questions & Answers, Office Memo, dated September 17, 2010.

9) General Considerations for Clinical Trials (ICH E8 Guideline), Notification No. 380 of the Evaluation and Licensing Division, PMSB, dated April 21, 1998.

10) Clinical Investigation of Medicinal Products in the Pediatric Population (ICH E11 Guideline), Notification No. 1334 of the Evaluation and Licensing Division, PMSB, dated December 15, 2000. Questions & Answers, Office Memo, dated June 22, 2001.

11) Definitions for Genomic Biomarkers, Pharmacogenomics, Pharmacogenetics, Genomic Data and Sample Coding Categories (ICH E15 Guideline), Notification No. 0109013 of the Evaluation and Licensing Division, PMSB, Notification No. 0109002 of the Safety Division, PFSB, dated January 9, 2008.

12) Biomarkers Related to Drug or Biotechnology Product Development: Context, Structure and Format of Qualification Submissions (ICH E16 Guideline), Notification No. 0120-(1) of the Evaluation and Licensing Division, PMSB, Notification No. 0120-(1) of the Safety Division, PFSB, dated January 20, 2011.

13) Guidance on Nonclinical Safety Studies for the Conduct of Human Clinical Trials and Marketing Authorization for Pharmaceuticals (ICH M3 (R2) Guideline), Notification No. 0219-(4) of the Evaluation and Licensing Division, PMSB, dated February 19, 2010. Questions & Answers, Office Memo, dated August 16, 2012.

14) Guidelines for the Design and Evaluation of Oral Prolonged Release Dosage Forms, Notification No. 5 of the First Evaluation and Registration Division, PAB, dated March 11, 1988.

15) Guidelines on Nonclinical Pharmacokinetic Studies, Notification No. 496 of the Evaluation and Licensing Division, PMSB, dated June 26, 1998.

16) Clinical Pharmacokinetic Studies on Pharmaceuticals, Notification No. 796 of the Evaluation and Licensing Division, PMSB, dated June 1, 2001.

17) Partial revision of Guidelines for Bioequivalence Studies of Generic Products, Notification No. 0229-(10) of the Evaluation and Licensing Division, PMSB, dated February 29, 2012. Questions & Answers, Office Memo, dated February 29, 2012.

18) Guidance for Conducting Microdose Clinical Studies, Notification No. 0603001 of the Evaluation and Licensing Division, PMSB, dated June 3, 2008.

19) Studies utilizing Pharmacogenomics, Notification No. 0930007 of the Evaluation and Licensing Division, PMSB, dated September 30, 2008.

20) Joint MHLW/EMA Reflection Paper on the Development of Block Copolymer Micelle Medicinal Products, Notification No. 0110-1 of the Evaluation and Licensing division, PNSB, dated January 10,2014.

21) Revision of Ethical Guidelines for Human Genome and Genetic Sequencing Research, Notification No. 24-593 of the Research Promotion Bureau, MEXT, Notification No. 0208-(1) of the Health Sciences Division, MHLW, Notification No. 20130206-(1) of the Manufacturing Industries Bureau, METI, dated February 8, 2013
http://www.mhlw.go.jp/stf/houdou/2r9852000002uz1d.html
22) Guidelines for Package Inserts for Prescription Drugs, Notification No. 606 of the PAB, Notification No. 59 of the Safety Division, PAB, dated April 25, 1997.
23) Guidelines for Precautions for Prescription Drugs, Notification No. 607 of the PAB, dated April 25, 1997. Questions & Answers, Office Memo, dated April 25, 1997. Writing Precautions for Prescription Drugs, Office Memo, dated December 25, 2000.

The following overseas guidelines and domestic instruction manuals can be used as references.

1) FDA: Guidance for Industry Drug Interaction Studies - Study Design, Data Analysis, Implications for Dosing, and Labeling Recommendations DRAFT GUIDANCE (2012,2)
2) EMA: Guideline on the investigation of drug interactions (2013,1)
3) FDA: Guidance for Industry Clinical Pharmacogenomics: Premarket Evaluation in Early-Phase Clinical Studies and Recommendations for Labeling (2013,1)
4) EMA: Guideline on the use of pharmacogenetic methodologies in the pharmacokinetic evaluation of medicinal products (2012,8)
5) Commentary on “Population Pharmacokinetic Studies”

10. Notes

(1) Example of drug interaction via P-gp inhibition at blood-brain barrier

In blood-brain barrier, several transporters such as P-gp and BCRP are expressed on the blood side and limit transport of drugs into brain. Therefore, inhibition of these transporters may increase the transport of affected drugs into the brain. Although reports on interactions with transporters in the brain distribution of drugs are currently limited, a reported example is that transfer of the P-gp substrate verapamil into the brain is increased by combined use of the P-gp inhibitor cyclosporine[37)].

(2) Calculation of the contribution ratio (CR) by means of metabolic activity

In general, CR can be evaluated directly from the value of fraction metabolized (fm) measured using human hepatic microsomes when the drug in question is an oral drug metabolized mainly by P450 in liver and contributions of the intestinal metabolism and other excretion clearances, such as biliary and urinary excretions are minor. In addition, precisely speaking, this is restricted to cases where the degree of drug interactions can be calculated simply by the phase I metabolic reaction. If there are also changes in transport such as hepatic uptake, more advanced methods using a PBPK model, etc., are necessary. When the investigational drug in question is an intravenous drug (injection drug), the CR to total clearance (CL_{total}) rather than the CL/F has to be evaluated.

If the investigational drug is metabolized by enzymes that show genetic polymorphism (e.g., CYP2D6, CYP2C9, CYP2C19, or UGT1A1), changes in the CL in subjects who genetically lack activity (poor metabolizers, PM) may be assumed to be equivalent to that in the case of concomitant use of inhibitors that inhibit the enzymes almost completely. Therefore, a comparison of the CL in PM and subjects with the wild-type enzyme (extensive metabolizer, EM) may allow us to estimate the CRs of the enzymes in question to the overall elimination of the investigational drug. Likewise, importance of particular transporter in the clearance pathways of the drug can be evaluated by comparing pharmacokinetics between subjects having different genotype of the transporter in question (ex. OATP1B1(SLC01B1) c.521 T>C).

(3) Precautions for identification of drug metabolizing enzymes in *in vitro* metabolic studies

If using correlation studies comparing the particular enzymatic activity (metabolism of the marker substrate) and the metabolism of the investigational drug using liver microsomes prepared from multiple individuals to identify enzymes with a large contribution to metabolism, it should be borne in mind that the activities of various enzymes may be correlated with one another in each individual. If correlation studies are carried out by necessity, e.g., in cases where no highly selective enzyme inhibitor is available, other procedures should be combined to make a proper judgment. Another method available is the relative activity factor (RAF) method by which the metabolic activity of the microsomal fraction prepared from recombinant cells of various P450 enzymes is corrected by the content of respective P450 enzymes in the liver to evaluate their contribution. However, in general, because sufficient verification is required to confirm the propriety of using the RAF method, judgment may still require a combination of other procedures.

It should also be noted that the enzymes by which a drug is metabolized as a substrate are not necessarily consistent with the enzymes that the drug inhibits. For instance, quinidine is metabolized mainly by CYP3A, but strongly inhibits CYP2D6[38, 39]. In addition, when metabolites are confirmed *in vivo* despite no or hardly observable metabolism *in vitro*, an attempt to find an *in vitro* study system that would allow us to identify the contributory enzyme should be made, through use of the chemical structure and previously reported data.

(4) Confirmation of the propriety of enzyme induction studies using hepatocytes

Because cultured human hepatocytes show large variations among different individuals and interlot variations, it is desirable to use hepatocytes derived from at least 3 donors. In addition, when the cellular viability rate is lower than 80% at the beginning of the culture, or when the cellular viability rate is markedly decreased at the end of culture, hepatocytes derived from other donors should be used. In this type of studies, cells are exposed continuously to the investigational drug, usually by changing the culture medium containing the investigational drug once daily. Although the culture period is typically 2 to 3 days, an appropriate period should be adopted by reference to literature reports, etc. It is necessary to confirm that the induction response has not been affected by cytotoxicity by appropriately evaluating the cell morphology and cell viability before and at the end of culture. When toxicity or decreased cell viability has been found, their influences on the study results should be considered carefully. If a marked decrease in the concentration of the

investigational drug due to metabolism or degradation of the investigational drug or protein binding in the medium is expected under the culture conditions, determination of the actual drug concentrations by measuring the concentration and the protein binding of the investigational drug in the medium at several time points, including the last day of culture, is recommended.

(5) Precaution about the genetic polymorphism of P450

When P450 enzymes that lack activity because of genetic polymorphism (e.g., CYP2C19 and CYP2D6) are closely involved in the metabolic pathway of the investigational drug, important elimination pathways should be judged taking into account that the CR of the enzymes may vary greatly in particular populations, such as persons with defective activity. See also Notes (2) and (19).

(6) Examples of time-dependent inhibition (TDI) and its evaluation

Representative examples include TDI of CYP3A by the HIV protease inhibitors ritonavir and saquinavir, macrolide antibiotics erythromycin and clarithromycin, and the calcium channel blockers verapamil and diltiazem. In the case of diltiazem, both unchanged diltiazem and its major metabolite N-desmethyldiltiazem inhibit CYP3A in a time-dependent manner[40]. An example of TDI of CYP2D6 is that caused by paroxetine[41]. The TDI is maximized when the enzyme subjected to the action reaches a new steady state, similar to the case for enzyme induction. Although this situation depends on the degradation rate constant (k_{deg}) and the inactivation rate constant (k_{inact}) of the enzyme, repeated administration of the inhibitor may cause enhancement of the inhibition over time, often resulting in inhibition persisting for a prolonged period of time after discontinuation of inhibitor treatment. For example, inhibition of CYP3A activity in humans reached a maximum after 4 days of repeated administration of erythromycin at a daily dose of 800 mg (the AUC determined at 2, 4, and 7 days of oral administration of midazolam, an marker substrate of CYP3A, was increased by 2.3-fold, 3.4-fold and 3.4-fold, respectively)[42]. Reference values of the respective P450 degradation rate constants can be obtained from the scientific literature based on *in vitro* and *in vivo* data[43]. In addition, it should be noted that enzymes that are present in both the intestine and the liver, like CYP3A, have different degradation rate constants depending on the tissue[44]. However, because there are variations in these values, it is

recommended to carry out sensitivity analysis to clarify the influences of the variability of k_{deg} on the estimated results.

(7) Evaluation of down-regulation of drug metabolizing enzymes

Although an algorithm of evaluation using *in vitro* data and several approaches for quantification have been proposed in regard to enzyme induction[19, 45-48], down-regulation has not been verified as yet. As an example of drug-induced down-regulation, decreased clearance of phenytoin and warfarin, presumably due to decreased activity of CYP2C9 caused by fluorinated pyrimidines has been reported, although the detailed mechanism still remains unclear[49]. Thus, reports of drug-induced down-regulation and the mechanism of such down-regulation are currently very limited. Therefore, implementation of clinical drug interaction studies is recommended when concentration-dependent down-regulation has been observed in an *in vitro* environment.

(8) Determination based on the cutoff criteria in enzyme induction studies

Although it is possible to set arbitrary cutoff criteria to determine the need of clinical studies for evaluating enzyme induction, the criteria should be set on the basis of experience with inducing drugs and non-inducing drugs supported by sufficient clinical evidence[48]. When the results of evaluation using hepatocytes derived from one or more donors exceed the predefined criteria, the investigational drug in question is regarded as an inducing drug, thus requiring additional evaluation. If the concentration of the investigational drug in *in vitro* studies cannot be set at a high level because of solubility, cytotoxicity, etc., of the investigational drug, it may become impossible to draw a conclusion in these evaluation studies because of the difficulty in calculating the EC_{50} and E_{max}. In such cases, the determination of enzyme induction should be examined by clinical drug interaction studies.

(9) Need for drug interaction studies related to drug metabolizing enzymes other than P450

Case examples of drug interactions based on inhibition or induction of enzymes other than P450 are limited, and it is usually difficult to predict such drug interactions. Reports of clinically significant drug interactions are also limited with regard to UGT, the second major drug metabolizing enzyme following P450. The most prominent example is increased metabolic clearance of glucuronidation of valproic acid in the case of combined use of

carbapenem. The mechanism of this phenomenon is inhibition of an enzyme that catalyzes the backward reaction of glucuronide to valproic acid[50].

When the major drug metabolizing enzymes are those usually not included in screening for enzyme inhibition of the investigational drug, there is hardly any information about strong or moderate inhibitors of the enzyme in question. In this case, drugs that are used concomitantly at high frequencies should also be surveyed, or conducted *in vitro* inhibition studies, for the determination of inhibitory actions, in addition to evaluation of the intensity of inhibition of the enzyme in question by the investigational drug itself as appropriate. The need for such studies varies according to the safety of the investigational drug at exposure doses exceeding the therapeutic range and the degree of involvement of the catalytic pathway in drug elimination.

(10) Points to remember in model analysis

When model analysis is carried out, explanation of the structure of the model, rationale for setting system-based parameters and parameters peculiar to the investigational drug, type of error model, output of the model, and sensitivity analysis taking into consideration the confidence interval should be included in the application materials. Model analysis has to be reproducible in an objective manner. Disclosure or provision in electronic media of the final model equation and the parameters used should be considered. When models predetermined by commercially available software (structural model and error model) are used, the version of the software and modifications from the predetermined model in the analysis of the investigational drug should be specified clearly.

(11) Formula of mechanism-based static pharmacokinetic (MSPK) model and precautions

In the MSPK model, the prediction results vary widely according to the pharmacokinetic properties of the concomitant drugs. Therefore, in the above Formula 4, fm should be set as 1 when the maximum interaction on a particular drug metabolizing enzyme is estimated for the investigational drug that affects other drugs. If there is extrahepatic clearance of concomitant drugs, such as urinary excretion, calculation of the AUCR should take into consideration such clearance. However, in Formula 4, it is assumed that there is no contribution of such clearance to estimate the maximum interaction. On the other hand, if influences on particular medical products are to be estimated, parameters particular to each

drug require corroboration by scientific literature. In the formula, the induction part (B_h and B_g) can be used after evaluation of the eligibility of the lot of hepatocytes used. In the evaluation of eligibility, the EC_{50} and E_{max} of multiple control inducers with different levels of induction potency should be measured for hepatocytes of the particular lot used in the *in vitro* study system, and the *in vivo* inductive action of control inducers for the marker substrate drug (e.g., midazolam) should be predicted using *in vitro* induction parameters for the control inducers. The d value is calculated from a comparison of the predicted inductive action and the inductive action imposed on the marker substrate drug in the clinical setting. The AUCR should be calculated from the d value and is used to measure the EC_{50} and E_{max} values of the investigational drug. At this point, it is recommended that the parameters to be input be selected in a conservative manner. In the case of irreversible inhibition and induction in the small intestine, it should be noted that experience of analysis using the MSPK model is limited.

(ii) Concentrations of the investigational drug in the liver cells and intestinal cells

For the concentrations of the investigational drug in the pharmacokinetic models such as the MSPK model, the concentration of the unbound form in the portal blood and the maximum concentration in the intestinal wall should be used as the concentrations of the investigational drug at sites where enzymes that are inhibited or induced are mainly localized (in the heptacytes and enterocytes). $[I]_h$ is the maximum concentration of the unbound inhibitor/inducer in portal blood ($[I]_{u,inlet,max}$), and can be estimated by a conservative method using the equation $[I]_h = f_{u,b} \times ([I]_{max,b} + F_a \times F_g \times ka \times Dose/Q_H)$[51], where F_a is the ratio of absorption from the GI tract (more specifically, the fraction of the drug transported from the lumen of the GI tract into the GI tract wall cell), Ka is the absorption ratio constant, Q_H is the total hepatic blood flow (97 L/h)[52], $f_{u,b}$ is the unbound ratio in blood, and $[I]_{max,b}$ is the maximum total blood concentration (unbound + bound forms) of the inhibitor in the steady state. When the protein binding ratio in blood is high (99% or higher), making measurement difficult, $f_{u,b} = 0.01$ should be used. $[I]_g$ may also be estimated with the equation $[I]_g = F_a \times k_a \times Dose/Q_{en}$, using the hypothetical blood flow volume into the intestinal cells (Q_{en}, 18 L/h)[53, 54]. Although it is desirable to actually measure the ka, the ka value may be set at 0.1/min as the maximum estimated value. The propriety of the methods used for estimation of the ka and

F_g should be demonstrated. If there is uncertainty about their propriety, sensitivity analysis should be implemented.

(12) Precautions in the use of the physiologically-based pharmacokinetic (PBPK) model

Although the use of the PBPK model is recommended, it should be noted that, at present, the degree of improvement over the MSPK model is not necessarily clear in the risk assessment of clinical drug interactions. The PBPK model is complex. Particularly in the case of drug interactions due to inhibition of drug metabolizing enzymes, it is important to predict changes in intrinsic clearance from *in vitro* data. Other factors such as protein binding and changes in blood flow volume often have no influence on the degree of drug interactions. Although it is theoretically possible for the PBPK model to predict variations in the PK of a particular subject population, it should be considered carefully whether *in vitro* experimental data can be extrapolated to *in vivo* situations, because a number of factors can cause variations of *in vitro* experimental data.

(13) Cases of interactions with biotechnological/biological products (Therapeutic proteins)

The following cases have been reported as cases of interactions between chemical drugs and biotechnological/biological products.

- Modified metabolism of P450 substrates due to changes in the expression levels of P450 proteins: Cytokines such as IL-6 and IFNα-2b cause decreases in the transcription levels of various P450 isoforms, leading to decrease in their enzymatic activities. This causes elevation of the blood concentration of the substrate drug metabolized by these P450 molecular species[55)].
- "Normalization" of the P450 activity by inhibition of cytokine-mediated decreases of the enzymatic activities of P450 isoforms: Tocilizumab administration in patients with rheumatism causes a decrease in the AUC of simvastatin[56)].
- Mechanism other than regulation of transporters or P450 expressions: The immunosuppressive action of methotrexate causes the decreased clearance of concomitant drugs (therapeutic proteins) by reduced anti-drug antibody formation[57, 58)].

(14) Points of concern in the evaluation of transporter-mediated drug interactions

(i) Special cases in inhibition of OATPs

Time-dependent inhibition may occur with organic anion transporting polypeptide (OATP) transporters. In the case of such inhibition, if an inhibitor is preincubated for a certain period with cells expressing transporters (expression system, human hepatocytes, etc.) before inhibition experiments, the apparent K_i value may be estimated at a lower level than the K_i value obtained by the usual inhibition experiments without preincubation[59,60]. It should be noted that this apparent K_i value may sometimes more accurately reflect the intensity of *in vivo* drug interactions. In addition, because it has been reported that the K_i value of an inhibitor varies according to the substrate[61], it is useful to use drugs that are expected to be used concomitantly in the clinical setting as substrate drugs for the inhibition experiments. Furthermore, there are reports of some cases in which the inhibition potency cannot be explained unless inhibition by the drug of the protein-bound form is taken into account, sometimes necessitating consideration based on the total drug concentration, including the concentration of the protein-bound form of the drug[62].

(ii) Variations in the level of endogenous substances relating to the transporter function

Some transporters are involved in the transport of endogenous substances. Such transporters include sodium-taurocholate cotransporting polypeptide (NTCP) and BSEP that contribute to hepatic transport of bile acids, OATPs and MRP2 that contribute to hepatic transport of bilirubin or its glucuronide, and MATEs that partially contribute to renal secretion of creatinine and N-methylnicotinamide. Inhibition of these transporters may cause increases in the blood concentrations of endogenous substances or their accumulation in the tissues. When there are variations in the laboratory test results for endogenous substances, it should be noted that not only hepatotoxicity and nephrotoxicity, but also inhibition of transporters can cause such variations. On the other hand, a recent report has called attention to the trend of a high risk in the manifestation of hepatotoxicity in the clinical setting when medical products causing strong inhibition of BSEP are used[63].

(15) Importance of the duration and timing of administration

As represented by ritonavir, which is an inhibitor of CYP3A and also an inducer of CYP2C9, drugs that serve as both inhibitors and inducers of drug metabolizing enzymes may be associated with different apparent interaction potencies according to the timing of use in combination[64, 65]. In this case, it is recommended to set a sufficient duration of administration

that would allow the level of expression of drug metabolizing enzymes to reach another steady state. It is also recommended to implement clinical drug interaction studies with varying timings of administration of the investigational drug and concomitant drugs and to interpret their influences in a cautious manner.

Rifampicin is known as a strong inducer of CYP3A and other drug metabolizing enzymes, but it is also an inhibitor of transporters such as OATP1B1[66, 67]. Therefore, when combined administration studies are carried out for the purpose of investigating the inhibitory effect of rifampicin on transporters, it is optimal that sampling for determining the concentration of the investigational drug should be conducted just after a single administration of rifampicin. On the other hand, in studies aimed at estimation of the potencies of other inducers by clarifying the effects of rifampicin as a strong enzyme inducer, the enzyme induction activity may be underestimated because of OATP1B1 inhibition by rifampicin. Therefore, it is optimal to conduct sampling of the investigational drug one day after the final administration of rifampicin.

(16) Choice of the substrate drugs of drug metabolizing enzymes

Some marker substrates used in clinical drug interaction studies may be substrates of 2 or more kinds of P450 species or transporters. Thus, it should be borne in mind that they are not specific substrates. For instance, omeprazole is a substrate of CYP2C19, but is also metabolized by CYP3A. When using omeprazole as a substrate for evaluating CYP2C19 inhibition (induction), measurement of both the unchanged form of the drug and metabolites (CYP2C19-mediated hydroxy omeprazole and CYP3A-mediated omeprazole sulfone) is recommended[68].

Particular caution is necessary when drugs used concomitantly with the investigational drug include substrates with a narrow therapeutic range. It has been suggested that substrates with a narrow therapeutic range may cause effects of serious safety concern (e.g., torsade de pointes) when the exposure dose is increased even only slightly by concomitant use of P450 inhibitors. Typical examples of substrates with a narrow therapeutic range include warfarin (even a slight increase in its concentration may cause significant bleeding), drugs that are likely to induce torsade de pointes, almost all cytotoxic antineoplastic drugs, and aminoglycoside antibiotics, and so on. When combined use of these substrates with a narrow therapeutic range is expected, the need for clinical drug interaction studies and the dose and

duration of the substrate drugs should be investigated from the consideration of safety concern.

(17) Cases of drug interactions involving both drug metabolizing enzymes and transporters

Itraconazole, which inhibits both CYP3A and P-gp, and rifampicin, which induces these two proteins are cited as examples of drugs that cause inhibition or induction of multiple enzymes/transporters simultaneously. In these cases, the inhibitory and inductive potencies are not necessarily equivalent. In fact, the strong CYP3A inhibitor voriconazole also inhibits P-gp, so that the exposure doses of P-gp substrates such as digoxin and fexofenadine are not significantly increased. In addition, some relatively strong P-gp inhibitors such as amiodarone and quinidine (that cause 1.5-fold or greater changes in the AUC of digoxin and fexofenadine) are weak CYP3A inhibitors. When choosing inhibitors for drug interaction studies of an investigational drugs that serve as substrates of CYP3A, P-gp, or both CYP3A and P-gp, the difference in the potency of inhibition between CYP3A and P-gp should be taken into consideration[34]. It should also be noted that rifampicin is a known inducer of multiple P450 enzymes and transporters, and is an inhibitor of the uptake transporter OATP1B1 [see "note (11)"].

As an example of the complex effects of inhibition of both drug metabolizing enzymes and transporters during concomitant use of several drugs, the substantial change in the AUC of repaglinide after simultaneous administration of itraconazole and gemfibrozil has been reported. This seems to be attributable to the overall effects of the inhibitory action of itraconazole on CYP3A4 (enzyme) and the inhibitory action of gemfibrozil and its metabolites on the OATP1B1 (transporter) and CYP2C8 (enzyme)[69].

(18) Use of cocktail substrate studies

Similar to general clinical drug interaction studies, cocktail studies are usually conducted to verify the actions of an investigational drug demonstrated in *in vitro* studies. Cocktail studies, however, may be carried out instead of *in vitro* studies in order to evaluate the inhibitory and inductive potency of various metabolites for enzymes (and transporters).

It is necessary for the specificity of substrates to be used in cocktail studies to have been demonstrated in drug interaction studies or pharmacogenetic studies that use selective inhibitors against particular enzymes (and transporters). Although it is desirable for the

propriety of the used doses to be shown by clinical evidence of the lack of interactions among substrates, it can be assumed that there are no interactions among substrates when the K_m values of target enzymes (and transporters) are sufficiently low in comparison with the C_{max} and the estimated concentrations in the GI tract.

(19) Evaluation of drug interactions in consideration of genetic polymorphism

Defective CYP2C19 activity is frequent among East Asians. Although defective CYP2D6 activity is rare, the frequency of *CYP2D6*10*, the genetic polymorphism that causes substantially decreased enzymatic activity, is high in this population[36)]. When the results of studies in East Asians are compared with those in subjects other than East Asians in regard to investigational drugs for which these P450 isoforms mediate the major pathways for its clearance, particular attention to genetic polymorphism is required. In particular, the degree of drug interactions may be expected to be greater in the subjects with defective CYP2C19 activity. If there is a possibility of clinical important effects, it is useful to add clinical studies aimed at investigation of drug interactions taking genetic polymorphism into account. When implementing clinical studies with genetic polymorphism taken into account, maximum consideration should be given to the safety of the subjects, because the blood concentrations in persons with defective activity are expected to become high. It is also useful to preliminarily examine the possibility of influences on drug interactions by means of model analyses and simulations.

The following are examples of drug interactions that require consideration of genetic polymorphisms.

Systemic exposure of voriconazole, which is mainly metabolized by CYP2C19, is markedly increased in subjects with defective CYP2C19 activity when an inhibitor for the alternative metabolic pathway by CYP3A is used concomitantly[70)]. Systemic exposure to tolterodine, which is mainly metabolized by CYP2D6, is markedly increased in subjects with defective CYP2D6 activity when an inhibitor for the alternative metabolic pathway by CYP3A is used concomitantly[71)].

It is known that clearance is influenced by the genetic polymorphisms of some drug metabolizing enzymes and transporters such as CYP3A5, UGT1A1, P-gp, and BCRP[36, 72, 73)]. In Japanese, attention should be paid for reduced activity alleles *UGT1A1*6* and *UGT1A1*28*, decreased transporting activity allele BCRP (*ABCG2*) c.421C>A, because their frequencies

are high. *CYP3A5*3* is a common polymorphism of CYP3A5, and is known to cause deficit enzyme expression. Although CYP3A5 generally resembles CYP3A4 in its substrate recognition capability, some inhibitors are reported to exhibit different inhibition constants between CYP3A4 and CYP3A5. Therefore, it should be borne in mind that clearance of CYP3A substrates is markedly reduced in the subjects with *CYP3A5*3*, in the case when a concomitant drug is a strong inhibitor for CYP3A4 but weak for CYP3A5..

10. Glossary

1) Substrate: In this guideline, a drug that is subject to metabolism or transport by transporters.
2) Concomitant drug: When two or more drugs are used, each drug is called a concomitant drug, in a broad sense. In the narrow sense, a concomitant drug is a drug that is added to the basic drug treatment.
3) Interacting drug (perpetrator drug): A drug that affects the *in vivo* pharmacokinetics of other drugs when administered concomitantly. For instance, in the case of metabolism, the affecting drug may inhibit or induce drug metabolizing enzymes.
4) Affected drug (victim drug): A drug whose *in vivo* pharmacokinetics is affected by a concomitant drug. For instance, in the case of metabolism, the metabolism of an affected drug may be decreased by inhibition of the drug metabolizing enzymes or increased by induction of the drug metabolizing enzymes by the affecting drug.
5) Investigational drug: In this guideline, a medicinal product or a drug under development that is investigated as to its potential to act as an affecting drug or an affected drug.
6) Marker drug: A drug that has been demonstrated in plural clinical studies to have a high specificity for enzymes, transporters or plasma proteins that are involved in the pharmacokinetics serves as an index of pharmacokinetic variations. Marker drugs need to be easy to quantify, and to be shown in their high safety if they are to be used in clinical studies.
7) Drug metabolized by a single enzyme: A drug metabolized mainly by a single drug metabolizing enzyme. The total metabolic clearance of this drug is markedly influenced by the activity change of the drug metabolizing enzyme by drug interaction, and thus drug interactions are likely to occur.
8) Drug metabolized by multiple enzymes: A drug metabolized by multiple drug metabolizing enzymes. The total metabolic clearance of this drug is less susceptible to the activity changes of drug metabolizing enzymes caused by drug interactions, and therefore this kind of drug shows lower risk compared to the drug metabolized by a single enzyme.
9) Transporter: A carrier that transports drugs across biological membranes (i.e., into and out of cells).
10) Selective inhibitor, selective substrate: A drug that inhibit relatively selective to specific drug metabolizing enzyme or transporter. A drug that is metabolized or transported by a relatively specific drug metabolizing enzyme or transporter.

11) Typical inhibitor, typical substrate (Table 6-4, Table 6-5): A drug that is frequently used for inhibition of a specific transporter, but inhibits other drug metabolizing enzymes and/or transporters and thus is not a selective inhibitor. A drug that is frequently used as a substrate for a specific transporter, but is metabolized and/or transported by other enzymes and/or transporters thus is not a selective substrate.

12) Strong inhibitor, moderate inhibitor, weak inhibitor: When a drug which increases the AUC of "a substrate drug susceptible to drug interactions" by ≥5-fold (or reduces the CL/F ratio to less than 1/5), the drug is termed as a "strong inhibitor". Likewise, a drug that causes an increase in the AUC by ≥2-fold but <5-fold (or a decrease in the CL/F to <1/2 but ≥1/5) is termed as a "moderate inhibitor", and a drug that causes an increase in the AUC by ≥1.25-fold but <2-fold (or a decrease in the CL/F to <1/1.25 but ≥1/2) is termed as a "weak inhibitor" (see description in Section 7.7.).

13) Strong inducer, moderate inducer, weak inducer: A drug that reduces the AUC of "a substrate drug susceptible to drug interactions" to ≤1/5 (or increases the CL/F ratio by ≥5-fold) is termed as a "strong inducer". Likewise, a drug that causes a decrease in the AUC to ≤1/2 but >1/5 (or an increase of the CL/F by ≥2-fold but <5-fold) is termed as a "moderate inducer", and a drug that reduces the AUC to ≤1/1.25 but >1/2 (or increases the CL/F by ≥1.25-fold but <2-fold) is termed as a "weak inducer" (see description in Section 7.8.).

14) Substrate drug susceptible to drug interactions, substrate drug moderately susceptible to drug interactions: A substrate drug whose AUC increases by ≥5-fold (or whose CL/F ratio decreases to <1/5) when co-administered with a "strong inhibitor". Likewise, a substrate drug whose AUC increases by ≥2-fold but <5-fold (or whose CL/F decreases to <1/2 but ≥1/5) when co-administered with a "strong inhibitor". (see description in Section 7.9.).

12. References

1) Murakami T, Takano M.: Intestinal efflux transporters and drug absorption. Expert Opin Drug Metab Toxicol. 2008;4:923-39.

2) Glaeser H.: Handb Exp Pharmacol. 2011;201:285-97.

3) Hilgeroth A, Hemmer M, Coburger C.: The impact of the induction of multidrug resistance transporters in therapies by used drugs: recent studies. Mini Rev Med Chem. 2012;12:1127-34.

4) Chen X-W, Sneed KB, Pan S-Y, Cao C, Kanwar JR, Chew H, Zhou S-F.: Herb-drug interactions and mechanistic and clinical considerations. Curr Drug Metab. 2012;13:640-51.

5) Dolton MJ, Roufogalis BD, McLachlan AJ.: Fruit juices as perpetrators of drug interactions: the role of organic anion-transporting polypeptides. Clin Pharmacol Ther. 2012;92:622-30.

6) Shirasaka Y, Shichiri M, Murata Y, Mori T, Nakanishi T, Tamai I.: Long-lasting inhibitory effect of apple and orange juices, but not grapefruit juice, on OATP2B1-mediated drug absorption. Drug Metab Dispos. 2013;41:615-21.

7) Hanley MJ, Cancalon P, Widmer WW, Greenblatt DJ.: The effect of grapefruit juice on drug disposition. Expert Opin Drug Metab Toxicol. 2011;7:267-86.

8) Mattson RH, Cramer JA, Williamson PD, Novelly RA.: Valproic acid in epilepsy: clinical and pharmacological effects. Ann Neurol. 1978;3:20-5.

9) Williams JA, Hyland R, Jones BC, Smith DA, Hurst S, Goosen TC, Peterkin V, Koup JR, and Ball SE.: Drug-drug interactions for UDP-glucuronosyltransferase substrates: a pharmacokinetic explanation for typically observed low exposure (AUCi/AUC) ratios. Drug Metab Dispos. 2004;32:1201-8.

10) Hisaka A, Ohno Y, Yamamoto T, Suzuki H.: Theoretical considerations on quantitative prediction of drug-drug interactions. Drug Metab Pharmacokinet. 2010;25:48-61.

11) Yasumori T, Nagata K, Yang SK, Chen LS, Murayama N, Yamazoe Y, Kato R.: Cytochrome P450 mediated metabolism of diazepam in human and rat:

involvement of human CYP2C in N-demethylation in the substrate concentration-dependent manner. Pharmacogenetics. 1993;3:291-301.

12) Kato R, Yamazoe Y.: The importance of substrate concentration in determining cytochromes P450 therapeutically relevant in vivo. Pharmacogenetics. 1994;4: 359-62.

13) Iwatsubo T, Hirota N, Ooie T, Suzuki H, Shimada N, Chiba K, Ishizaki T, Green CE, Tyson CA, Sugiyama Y.: Prediction of in vivo drug metabolism in the human liver from in vitro metabolism data. Pharmacol Ther. 1997;73:147-71.

14) Yuan R, Madani S, Wei X, Reynolds K, Huang S-M.: Evaluation of cytochrome P450 probe substrates commonly used by the pharmaceutical industry to study in vitro drug interactions. Drug Metab Dispos. 2002;30:1311-9.

15) Austin RP, Barton P, Cockroft SL, Wenlock MC, Riley RJ.: The influence of nonspecific microsomal binding on apparent intrinsic clearance, and its prediction from physicochemical properties. Drug Metab Dispos. 2002;30:1497-503.

16) Grimm SW, Einolf HJ, Hall SD, He K, Lim H-K, Ling KJ, Lu C, Nomeir AA, Seibert E, Skordos KW, Tonn GR, Van Horn R, Wang RW, Wong YN, Yang TJ, Obach RS.: The conduct of in vitro studies to address time-dependent inhibition of drug-metabolizing enzymes: a perspective of the Pharmaceutical Research and Manufacturers of America. Drug Metab Dispos. 2009;37:1355-70.

17) Vieira M, Kirby B, Ragueneau-Majlessi I, Galetin A, Chien J, Einolf HJ, Fahmi OA, Fischer V, Fretland A, Grime K, Hall SD, Higgs R, Plowchalk D, Riley R, Seibert E, Skordos K, Snoeys J, Venkatakrishnan K, Waterhouse T, Obach RS, Berglund EG, Zhang L, Zhao P, Reynolds K, Huang S-M.: Evaluation of various static in vitro-in vivo extrapolation models for risk assessment of CYP3A inhibition potential of an investigational drug. Clin Pharmacol Ther. 2013 Sep 18. doi: 10.1038/clpt.2013.187.

18) Huang S-M, Temple R, Throckmorton DD, Lesko LJ: Drug interaction studies: study design, data analysis, and implications for dosing and labeling. Clin Pharmacol Ther. 2007;81:298–304.

19) Fahmi OA, Ripp SL.: Evaluation of models for predicting drug-drug interactions due to induction. Expert Opin Drug Metab Toxicol. 2010;6:1399-416.

20) Tucker GT, Houston JB, Huang S-M.: Optimizing drug development: strategies to assess drug metabolism/transporter interaction potential—toward a consensus. Pharm Res. 2001;18:1071-80.

21) Walsky RL, Obach RS.: Validated assays for human cytochrome P450 activities. Drug Metab Dispos. 2004;32:647-60.

22) Ward BA, Gorski JC, Jones DR, Hall SD, Flockhart DA, Desta Z.: The cytochrome P450 2B6 (CYP2B6) is the main catalyst of efavirenz primary and secondary metabolism: implication for HIV/AIDS therapy and utility of efavirenz as a substrate marker of CYP2B6 catalytic activity. J Pharmacol Exp Ther. 2003;306:287-300.

23) Fontana E, Dansette PM, Poli SM.: Cytochrome P450 enzymes mechanism based inhibitors: Common sub-structures and reactivity. Curr Drug Metab. 2005;6:413-54.

24) Walsky RL, Obach RS, Gaman EA, Gleeson J-PR, Proctor WR.: Selective inhibition of human cytochrome P4502C8 by montelukast. Drug Metab Dispos. 2005;33:413-8.

25) Ishigami M, Uchiyama M, Kondo T, Iwabuchi H, Inoue S, Takasaki W, Ikeda T, Komai T, Ito K, Sugiyama Y.: Inhibition of in vitro metabolism of simvastatin by itraconazole in humans and prediction of in vivo drug-drug interactions. Pharm Res. 2001;18:622-31.

26) Bjornsson TD, Callaghan JT, Einolf HJ, Fischer V, Gan L, Grimm S, Kao J, King SP, Miwa G, Ni L, Kumar G, McLeod J, Obach RS, Roberts S, Roe A, Shah A, Snikeris F, Sullivan JT, Tweedie D, Vega JM, Walsh J, Wrighton SA.: The conduct of in vitro and in vivo drug-drug interaction studies, A PhRMA perspective. J Clin Pharmacol. 2003;43:443-469.

27) Roymans D, Looveren CV, Leone A, Parker JB, McMillian M, Johnson MD, Koganti A, Gilissen R, Silber P, Mannens G, Meuldermans W.: Determination

of cytochrome P450 1A2 and cytochrome P450 3A4 induction in cryopreserved human hepatocytes. Biochem Pharmacol. 2004;67:427-37.

28) Madan A, Graham RA, Carroll KM, Mudra DR, Burton LA, Krueger LA, Downey AD, Czerwinski M, Forster J, Ribadeneira MD, Gan L-S, Lecluyse EL, Zech K, Robertson P Jr, Koch P, Antonian L, Wagner G, Yu L, Parkinson A.: Effects of Prototypical microsomal enzyme inducers on cytochrome P450 expression in cultured human hepatocytes. Drug Metab Dispos. 2003;31:421-31.

29) Raucy JL, Mueller L, Duan K, Allen SW, Strom S, Lasker JM.: Expression and induction of CYP2C P450 enzymes in primary cultures of human hepatocytes. J Pharmacol Exp Ther. 2002;302:475-82.

30) Tanihara Y, Masuda S, Sato T, Katsura T, Ogawa O, Inui K.: Substrate specificity of MATE1 and MATE2-K, human multidrug and toxin extrusions/H(+)-organic cation antiporters. Biochem Pharmacol. 2007;74:359-71.

31) Tsuda M, Terada T, Asaka J, Ueba M, Katsura T, Inui K.: Oppositely directed H+ gradient functions as a driving force of rat H+/organic cation antiporter MATE1. Am J Physiol Renal Physiol. 2007;292:F593-8.

32) Ito K, Iwatsubo T, Kanamitsu S, Ueda K, Suzuki H, Sugiyama Y.: Prediction of pharmacokinetic alterations caused by drug-drug interactions: metabolic interaction in the liver. Pharmacol Rev. 1998;50:387-412.

33) Yoshida K, Maeda K and Sugiyama Y: Transporter-mediated drug--drug interactions involving OATP substrates: predictions based on in vitro inhibition studies. Clin Pharmacol Ther. 2012; 91: 1053-64.

34) Zhang L, Zhang Y, Huang S-M.: Scientific and regulatory perspectives on metabolizing enzyme-transporter interplay and its role in drug interactions - challenges in predicting drug interaction. Mol Pharmaceut. 2009;6:1766–74.

35) Inui N, Akamatsu T, Uchida S, Tanaka S, Namiki N, Karayama M, Chida K, Watanabe H.: Chronological effects of rifampicin Discontinuation on cytochrome P450 activity in healthy Japanese volunteers, using the cocktail method. Clin Pharmacol Ther. 2013;94:702-708.

36) Kurose K, Sugiyama E, Saito Y.: Population differences in major functional polymorphisms of pharmacokinetics/pharmacodynamics-related genes in Eastern Asians and Europeans: implications in the clinical trials for novel drug development. Drug Metab Pharmacokinet. 2012;27:9-54.

37) Muzi M, Mankoff DA, Link JM, Shoner S, Collier AC, Sasongko L, Unadkat JD.: Imaging of cyclosporine inhibition of P-glycoprotein activity using 11C-verapamil in the brain: studies of healthy humans. J Nucl Med. 2009;50:1267-75.

38） Nielsen TL, Rasmussen BB, Flinois JP, Beaune P, Brosen K.: In vitro metabolism of quinidine: the (3S)-3-hydroxylation of quinidine is a specific marker reaction for cytochrome P-4503A4 activity in human liver microsomes. J Pharmacol Exp Ther. 1999;289:31-7.

39） von Moltke LL, Greenblatt DJ, Duan SX, Daily JP, Harmatz JS, Shader RI.: Inhibition of desipramine hydroxylation (cytochrome P450-2D6) in vitro by quinidine and by viral protease inhibitors: relation to drug interactions in vivo. J Pharm Sci. 1998;87:1184-9.

40) Zhao P, Lee CA, Kunze KL.: Sequential metabolism is responsible for diltiazem-induced time-dependent loss of CYP3A. Drug Metab Dispos. 2007;35:704-12.

41) Bertelsen KM, Venkatakrishnan K, Von Moltke LL, Obach RS, Greenblatt DJ.: Apparent mechanism-based inhibition of human CYP2D6 in vitro by paroxetine: comparison with fluoxetine and quinidine. Drug Metab Dispos. 2003;31:289-93.

42) Okudaira T, Kotegawa T, Imai H, Tsutsumi K, Nakano S, Ohashi K.: Effect of the treatment period with erythromycin on cytochrome P450 3A activity in humans. J Clin Pharmacol. 2007;47:871-76.

43) Yang J, Liao M, Shou M, Jamei M, Yeo KR, Tucker GT, Rostami-Hodjegan A.: Cytochrome P450 turnover: regulation of synthesis and degradation, methods for determining rates, and implications for the prediction of drug interactions. Curr Drug Metab. 2008;9:384-93.

44) Obach RS, Walsky RL, Venkatakrishnan K.: Mechanism-based inactivation of human cytochrome p450 enzymes and the prediction of drug-drug interactions. Drug Metab Dispos. 2007;35:246-55.

45) Shou M, Hayashi M, Pan Y, Xu Y, Morrissey K, Xu L, Skiles GL. Modeling, prediction, and in vitro in vivo correlation of CYP3A4 induction. Drug Metab Dispos. 2008;36:2355-70.

46) Almond LM, Yang J, Jamei M, Tucker GT, Rostami-Hodjegan A.: Towards a quantitative framework for the prediction of DDIs arising from cytochrome P450 induction. Curr Drug Metab. 2009;10:420-32.

47) Fahmi OA, Hurst S, Plowchalk D, Cook J, Guo F, Youdim K, Dickins M, Phipps A, Darekar A, Hyland R, Obach RS.: Comparison of different algorithms for predicting clinical drug-drug interactions, based on the use of CYP3A4 in vitro data: predictions of compounds as precipitants of interaction. Drug Metab Dispos. 2009;37:1658-66.

48) Fahmi OA, Kish M, Boldt S, Obach RS.: Cytochrome P450 3A4 mRNA is a more reliable marker than CYP3A4 activity for detecting pregnane X receptor-activated induction of drug metabolizing enzymes. Drug Metab Dispos. 2010;38:1605-11.

49) Gilbar PJ, Brodribb TR.: Phenytoin and fluorouracil interaction. Ann Pharmacother. 2001;35:1367-70.

50) Suzuki E, Nakai D, Yamamura N, Kobayashi N, Okazaki O, Izumi T.: Inhibition mechanism of carbapenem antibiotics on acylpeptide hydrolase, a key enzyme in the interaction with valproic acid. Xenobiotica 2011;41:958-63.

51) Ito K, Chiba K, Horikawa M, Ishigami M, Mizuno N, Aoki J, Gotoh Y, Iwatsubo T, Kanamitsu S, Kato M, Kawahara I, Niinuma K, Nishino A, Sato N, Tsukamoto Y, Ueda K, Itoh T, Sugiyama Y.: Which concentration of the inhibitor should be used to predict in vivo drug interactions from in vitro data? AAPS PharmSci. 2002;4:53-60.

52) Yang J, Jamei M, Yeo KR, Rostami-Hodjegan A, Tucker GT.: Misuse of the well-stirred model of hepatic drug clearance. Drug Metab Dispos. 2007;35:501-2.

53) Yang J, Jamei M, Yeo KR, Tucker GT, Rostami-Hodjegan A: Prediction of intestinal first-pass drug metabolism. Curr Drug Metab. 2007;8:676-84.

54) Rostami-Hodjegan A, Tucker GT.: 'In silico' simulations to assess the 'in vivo' consequences of 'in vitro' metabolic drug-drug interactions. Drug Discov Today: Technol. 2004;1:441–8.

55) Islam M, Frye RF, Richards TJ, Sbeitan I, Donnelly SS, Glue P, Agarwala SS, Kirkwood JM.: Differential effect of IFN -2b on the cytochrome P450 enzyme system: a potential basis of IFN toxicity and its modulation by other drugs. Clin Cancer Res. 2002;8:2480-7.

56) Schmitt C, Kuhn B, Zhang X, Kivitz AJ, Grange S.: Disease-drug-drug interaction involving tocilizumab and simvastatin in patients with rheumatoid arthritis. Clin Pharmacol Ther. 2011;89:735-40.

57) Maini RN, Breedveld FC, Kalden JR, Smolen JS, Davis D, Macfarlane JD, Antoni C, Leeb B, Elliott MJ, Woody JN, Schaible TF, Feldmann M.: Therapeutic efficacy of multiple intravenous infusions of anti-tumor necrosis factor alpha monoclonal antibody combined with low-dose weekly methotrexate in rheumatoid arthritis. Arthritis Rheum. 1998;41:1552-63.

58) Seitz K, Zhou H.: Pharmacokinetic drug-drug interaction potentials for therapeutic monoclonal antibodies: reality check. J Clin Pharmacol. 2007;47:1104-18.

59) Suzuki K, Shitara Y, Fukuda K, Horie T.: Long-lasting inhibition of the intestinal absorption of fexofenadine by cyclosporin A in rats. J Pharm Sci. 2012;101:2606-15.

60) Amundsen R, Christensen H, Zabihyan B, Asberg A.: Cyclosporine A, but not tacrolimus, shows relevant inhibition of organic anion-transporting protein 1B1-mediated transport of atorvastatin. Drug Metab Dispos. 2010;38:1499-504.

61) Izumi S, Nozaki Y, Komori T, Maeda K, Takenaka O, Kusano K, Yoshimura T, Kusuhara H, Sugiyama Y.: Substrate-dependent inhibition of organic anion transporting polypeptide 1B1: comparative analysis with prototypical probe substrates estradiol-17β-glucuronide, estrone-3-Sulfate, and sulfobromophthalein. Drug Metab Dispos. 2013;41:1859-66.

62) Zhu Q, Liao M, Chuang BC, Balani SK, Xia C.: Effects of Protein Binding on Transporter Inhibitions", Abstract for 17th North American Regional ISSX Meeting (Oct 16-20, 2011), P324.

63) Dawson S, Stahl S, Paul N, Barber J, Kenna JG.: In vitro inhibition of the bile salt export pump correlates with risk of cholestatic drug-induced liver injury in humans. Drug Metab Dispos. 2012;40:130-8.

64) Foisy MM, Yakiwchuk EM, Hughes CA.: Induction effects of ritonavir: implications for drug interactions. Ann Pharmacother. 2008;42:1048-59.

65) Kirby BJ, Collier AC, Kharasch ED, Dixit V, Desai P, Whittington D, Thummel KE, Unadkat JD.: Complex drug interactions of HIV protease inhibitors 2: in vivo induction and in vitro to in vivo correlation of induction of cytochrome P450 1A2, 2B6, and 2C9 by ritonavir or nelfinavir. Drug Metab Dispos. 2011;39:2329-37.

66) van Giersbergen PL, Treiber A, Schneiter R, Dietrich H, Dingemanse J.: Inhibitory and inductive effects of rifampin on the pharmacokinetics of bosentan in healthy subjects. Clin Pharmacol Ther. 2007;81:414-9.

67) Reitman ML, Chu X, Cai X, Yabut J, Venkatasubramanian R, Zajic S, Stone JA, Ding Y, Witter R, Gibson C, Roupe K, Evers R, Wagner JA, Stoch A.: Rifampin's acute inhibitory and chronic inductive drug interactions: experimental and model-based approaches to drug-drug interaction trial design. Clin Pharmacol Ther. 2011;89:234-42.

68) Michaud V, Ogburn E, Thong N, Aregbe AO, Quigg TC, Flockhart DA, Desta Z.: Induction of CYP2C19 and CYP3A activity following repeated administration of efavirenz in healthy volunteers. Clin Pharmacol Ther. 2012;91:475-82.

69) Kudo T, Hisaka A, Sugiyama Y, Ito K.: Analysis of the repaglinide concentration increase produced by gemfibrozil and itraconazole based on the inhibition of the hepatic uptake transporter and metabolic enzymes. Drug Metab Dispos. 2013;41:362-71.

70) Shi HY, Yan J, Zhu WH, Yang GP, Tan ZR, Wu WH, Zhou G, Chen XP, Ouyang DS.: Effects of erythromycin on voriconazole pharmacokinetics and association with CYP2C19 polymorphism. Eur J Clin Pharmacol. 2010;66:1131-6.

71) Brynne N, Forslund C, Hallén B, Gustafsson LL, Bertilsson L.: Ketoconazole inhibits the metabolism of tolterodine in subjects with deficient CYP2D6 activity. Br J Clin Pharmacol. 1999;48:564-72.

72) Ieiri I.: Functional significance of genetic polymorphisms in P-glycoprotein (MDR1, ABCB1) and breast cancer resistance protein (BCRP, ABCG2). Drug Metab Pharmacokinet. 2012;27:85-105.

73) Shirasaka Y, Chang SY, Grubb MF, Peng CC, Thummel KE, Isoherranen N, Rodrigues AD.: Effect of CYP3A5 expression on the inhibition of CYP3A-catalyzed drug metabolism: impact on CYP3A-mediated drug-drug interactions. Drug Metab Dispos. 2013;41:1566-74.