INTRODUCTION

The main purpose of a toxicology program is to clarify toxicological profiles of test compounds in animals and to predict their safety in humans. For this purpose, a concept of safety margins based on the ratio of the highest non-toxic effective dose of the test compounds in animals to their clinical dose in humans has traditionally been used. However, it is well-known that there are sometimes non-linear relationship between the given doses and kinetics of the test compound and marked differences among animals and humans in rate of the absorption, distribution, metabolism and excretion (ADME). Since biological effects are considered to be better correlated with test compound (and/or its metabolites) concentrations in the blood (and/or tissues) than with dosages, the concept of safety margins calculated from dose levels alone is not really preferable for the prediction of drug safety for humans. These experiences show that toxicokinetics are of vital importance in evaluating the relation between given doses and toxicity symptoms by measuring systemic exposure (mainly plasma concentrations) in toxicology studies. Therefore, toxicokinetics is now spot-lighted and the pharmaceutical industry has routinely integrated toxicokinetics into the toxicology program.

OBJECTIVES

The primary objectives of toxicokinetics are: 1) to describe systemic exposure achieved in toxicology studies, 2) to relate systemic exposure with toxicological findings, 3) to support design of planning toxicology studies and 4) to define relationship between clinical doses and those used in toxicology studies. Toxicokinetic measurements of plasma or tissue samples from animals used in toxicology studies provide information on dose proportionality, potential of drug accumulation and species and sex differences in kinetics. Consequently, there is a possibility that problems of species differences which arise when the safety of the test compound in humans is predicted from the results of animal studies can be resolved by comparing the metabolic pathway in animals used in toxicology studies with that in humans and indexing the plasma concentrations.

CHARACTERISTICS OF TOXICOLOGY STUDIES

From a pharmacokinetic point of view, there are many specific characteristics in toxicology studies. Since very high doses and wide dose range are usually used in the toxicology studies, it must be noted that a capacity-limited process in the absorption and clearance may often occur, and mixed type of pharmacokinetics and metabolism may be established. Changes of plasma protein binding and distribution in the blood may influence tissue distribution of a compound. High doses and repeated dosing may give rise to induction or inhibition of drug metabolizing enzymes, which cause changes of toxicokinetics as well as the toxicity profile of a test compound. Aging may also affect the results of toxicology studies, especially in long-term studies. In addition, the toxic effects of the test compound may induce changes in toxicokinetics and metabolism of the test compound. For the evaluation of the test compound, therefore, the overall interpretation should be made on the basis of not only the assessment of toxic findings and the systemic exposure, but also the results of pharmacokinetics and metabolism studies which support safety assessment at each development stage.

BIOPHARMACEUTIC ASSESSMENT

In recent years, kinetics and metabolism studies as well as toxicology studies of a new candidate compound are integrated into the very early stage of the drug development process. The kinetic data obtained in the early stage can be powerful information for supporting the planning of early toxicology studies.
We usually conduct a biopharmaceutic assessment prior to the initial toxicology studies (Table 1).

In this stage, the physico-chemical properties of the test compounds are clarified, and then, in order to grasp the kinetic characteristics of the test compounds, the basic pharmacokinetics after a single dosing with non-toxic doses are investigated in the same animal species as those in toxicology studies.

It is important to determine the bioavailability of the parent compound and to elucidate the extent of the first-pass effect. Since test doses in toxicology studies are high, non-linear kinetics occur very often. Therefore, the information on the relationship of blood concentrations to test doses should be obtained before conducting toxicology studies. Effects of feeding on drug absorption should also be examined. When test compounds are insoluble or poorly absorbed from the gastrointestinal tract, an adequate dosing form of the test compounds should be prepared in order to optimize the mode of administration. In the example shown in Fig.1, there were no increases in the AUC or Cmax of Compound C in dogs after oral administration of 100 mg/kg as a PEG-400 solution. However, treatment with sucrose-fatty acid ester showed a marked improvement of the oral absorption of Compound C.

The knowledge obtained from the studies mentioned above is useful for determining some factors in toxicology studies, such as dose, drug formulation, feeding time, time of observation for general signs, and so forth. More recently, in vitro metabolism studies in liver preparations from animals and humans are conducted in this stage for selection of appropriate animals as toxicology species.

### Table 1. Biopharmaceutic assessment.

- Physicochemical properties of a test compound
- Single dose kinetics
  - Pharmacological doses
    - Cmax, Tmax, t1/2, AUC
    - Bioavailability
    - First-pass effect
  - Toxicological doses
    - Linearity
    - Effect of food
- Formulations
  - Solution, suspension, powder
  - Investigation of solvents and solubility enhancers

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**Fig. 1.** Relationship between doses and Cmax or AUC of Compound C in dogs after single oral dosing.
IMPLEMENTATION OF TOXIKOKINETICS AND SUPPORT OF ADME

Toxicokinetic data are generated in each stage of compound development, from the first screening study (e.g., 2-week study) to the long-term chronic studies of up to 1-year duration.

Early toxicology studies are performed in the preclinical stage of the drug development program, where there is no information on the pharmacokinetics of the compound in humans. Therefore, toxicokinetics are vital at this stage for investigating how the administered dose relates to systemic exposure. Prior to and during pivotal toxicology studies considerably more information can be obtained from related ADME studies in animals and humans. This information may be useful for a) comparing the absorption, excretion and metabolism in animals with those in humans, b) making sure of the concentrations to be measured (free or total) in toxicokinetic studies, c) identifying the target organ(s)/tissue(s), and d) predicting the retention and/or accumulation of test compounds in the body.

In planning pivotal toxicology studies, therefore, in addition to toxicokinetic data and toxicological findings obtained in the preceding short-term toxicology studies, the information on ADME should be used in designing toxicology protocol.

In short-term toxicology studies, the monitoring of toxicokinetics is usually limited to the parent compound. In pivotal toxicology studies, however, it should be determined whether concentrations of metabolite(s) are also assessed. When a major and active metabolite common to animals and humans is found, an appropriate bioanalytical method needs to be developed and the determination of plasma concentrations of this metabolite should be performed in further toxicology studies.

To explain toxic symptoms or to elucidate their mechanism, retrospective studies are occasionally conducted. For instance, Fig.2 shows the relationship between plasma/liver concentrations of compound A and GOT/GPT values in the plasma after daily intravenous administration for 28 days. In this case, changes of biochemical values in the plasma can be better correlated with increase of plasma and liver concentrations of the compound.

The type of retrospective studies needed will depend upon the nature of the compound, objectives
of the safety evaluation studies and the information to be generated.

Toxicokinetic data is important both for interpreting preclinical toxicological data and for assisting proper planning of future toxicology studies.

However, the plasma concentrations of test compounds may not always reflect their concentrations at the target site. When there is a poor relationship between toxicological findings and plasma concentrations of a parent compound, the possible cause should be elucidated, e.g., the mechanism of the toxicity, implication of reactive metabolites, covalent binding, changes in endogenous substances or receptor composition, etc.

Toxicokinetic data are generated from the toxicology studies performed in each drug development stage.

The combination of toxicokinetics and toxicological findings with ADME data is considered to be useful for validating the toxicology studies and designing further toxicology studies. Therefore, it should always be kept in mind that the conducting of a toxicokinetic study should be based on a flexible step-by-step approach and case-by-case decisions.

CONCLUSION

Toxicokinetics is an important component of animal toxicology studies. When the results of toxicology studies are discussed, the overall evaluation should be made on the basis of not only the toxicological findings but also the results of toxicokinetic and ADME studies. Furthermore, the data on pharmacokinetics in humans and toxicokinetics in animals are of vital importance to establishing the relationship between clinical studies and toxicology studies. These data are combined with toxicological findings to provide safety margins based on systemic exposure of drugs. From this point of view, it is important to exchange information or ideas among researchers participating in the toxicological, pharmacokinetic and clinical studies.