INTRODUCTION

Purpose of toxicity study on pharmaceuticals is to characterize their toxic effects with respect to target organs, dose dependence, relationship to exposure, and potential reversibility by animal experiments or by in vitro toxicity studies. From these toxicity data we estimate the effects of drugs on human in relation to its clinical use. However, it is well known that there are possibilities of large differences in the toxicity of chemicals among species, strains, and individuals. For examples, LD50 of TCDD in guinea-pigs were about 1,000 times smaller than those in hamsters. Therefore, it is, sometimes, inappropriate to extrapolate animal data to human depending simply on the administered dose.

Physiological, pathological, environmental, pharmaceutical, experimental factors, may also affects toxic responses. Influences of these factors are represented mainly by the differences in drug concentration surrounding target tissues and by the differences in the sensitivity of target tissues to toxic insults. On the other hand, studies on the mechanism of toxicity and on drug metabolism made us understood that, in most cases, toxicity correlates better with the blood levels of the compounds than with their dose itself. Thus, we expected to overcome most of the species differences depending pharmacokinetics (PK) by comparing the toxicity based on blood levels.

Harmonization on Toxicokinetics (TK)

In the process of ICH, there was a proposal on the harmonization of the PK studies. However, it was difficult to harmonize all of PK issues. Thus, we tried to harmonize on two issues in PK. One was determination of blood levels in toxicity tests and the other was repeated dose tissue distribution studies. Harmonization of these issues were achieved in October, 1994 as a Note for guidance on toxicokinetics: The assessment of systemic exposure in toxicity studies and Pharmacokinetics: Guidance for repeated dose tissue distribution studies. Regulatory authorities of three region had taken each necessary steps to implement the agreements. In Japan, those were notified on July 2, 1996 and asked pharmaceutical companies to incorporate TK to the toxicity tests which start after January 1, 1997.

Definition and objectives of TK

TK in ICH guidelines as defined as the "generation of pharmacokinetic data, either as an integral component in the conduct of non-clinical toxicity studies or in specially designed supportive studies, to assess systemic exposure." This definition of TK is different from the other's. For example, OECD used the term, toxicokinetics, as study of the absorption, distribution, excretion, and metabolism of substances (OECD, 417). The only difference from PK seems to be the difference in the category of test substances. Thus, definition of TK by ICH is limited to the field of drug development for human use and it does not apply to the other chemicals like agricultural drugs and food additives, etc.

Primary objective of TK was defined "to describe the systemic exposure achieved in animals and its relationship to dose level and the time course of the toxicity study." Data on the linearity of the exposure levels depending on the dose is important for the drug evaluation. Data on the changes during repetitive administration by drug- or age-related

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induction or inhibition of ADME mechanism are also expected.

Secondary objectives were described as "to relate the exposure achieved in toxicity studies to toxicological findings and contribute to the assessment of the relevance of these findings to clinical safety, to support the choice of species and treatment regimen in non-clinical toxicity studies, and to provide information that, in conjunction with the toxicity findings, contributes to the design of subsequent non-clinical toxicity studies."

As an outcome of harmonization of TK, doses which cause maximum exposure are now accepted as a maximum dose in toxicity studies. In the case of carcinogenicity studies, exposure level which exceed about 25 times of clinical exposure is also considered enough as a maximum dose.

Toxicity studies that were covered by TK guidance were single dose toxicity study, repeated dose toxicity study, reproductive and developmental toxicity studies, mutagenicity study, and carcinogenicity study.

**Principles of TK studies**

Now, toxicity study for pharmaceuticals without any support by TK data is not acceptable. However, we do not require TK to incorporate into all of those toxicity studies. Necessity and range of TK studies should be determined by step by step approach and case by case decision-making, depending on the results of preceding toxicity studies, TK studies, PK studies, and clinical studies.

Systemic exposures are estimated mainly by the determination of the test substance in blood, plasma, or serum. However, determination of the metabolites or determination in other biological matrix, instead, can be acceptable in specific circumstances where it is appropriate. Toxicity studies on metabolites may be
considered in certain situations where human specific metabolites are indicated, where certain metabolites are formed significantly more in human than in experimental animals, or where pharmacologically or toxicologically significant metabolites are formed. It is said that there are situations where blood level achieved by the highest dose possible are lower than those in human after clinical dose. Those data are appreciated less but acceptable.

Matrices for TK studies are also obtained from those of satellite group or animals under circumstances closely mimicked with toxicity studies. However, it is better to get samples from animals under toxicity studies to relate the toxicity to TK data.

Sampling from control group is also needed to eliminate the factor other than the test substance. TK measurement for control group should be considered in case of endogenous substances. Clarification of basic PK parameters of the test substances is not intended. Number of animals used should be minimum needed. High level of precision in term of statistics is not necessarily needed.

Because TK data are considered essential for the evaluation of the toxicity findings, TK determinations are required to conduct under GLP.

Examples of TK studies

During these three years, I have reviewed 48 new drug applications. Among them, there were 5 applications with TK data. I will show you some examples.

In the case of compound A relationship of plasma maximum concentration (Cmax) to administered dose in human was similar to those in mice and quite different from those in dogs. This was quite unusual. Slight toxicity appeared by half of clinical dose in mice and 1/100 in dogs. Severe toxicity was observed by doses 15 time more than clinical dose in mice and about 1/3 in dogs. That is, there were big differences in the dose which cause toxicity. However, when we compared blood levels of this compound, the ratio was not so different between mice and dogs indicating that the toxicity related better to the blood levels of the compounds. Toxicities were appeared at the plasma concentration less than half of clinical level, which influenced on the evaluation of the drug.

In the case of compound B, the relationship in human was similar to dog, and the similarity to monkeys was the next. Significant toxicity was not observed in dog, but slight enlargement of the livers were observed in monkey and rat. The toxic doses were 10 to 20 times higher than clinical dose and the blood levels were one to 3 times higher than clinical level. Even though the blood level was also achieved in dog, any significant toxicity were not observed. It seemed that the toxicity depend more on the administered dose and less on the blood levels.

Data on compound C in human was similar to dog and marmoset. Toxicity appeared when the doses were 50-60 time higher than clinical. On the other hand, the blood levels (Cmax) were about 100 to 400 times higher than clinical. This compound showed drug interactions, by which blood levels increased to about 20 times. However, I considered that it does not cause significant impact on the use of these drug because only slight toxicity was observed by the blood level of 100 times.

Human data on Compound D was similar to dog. Slight toxicity in dogs appeared by the plasma concentration higher than about 30 times of clinical. Severe toxicity were observed when the concentration was about 100 times higher.

CONCLUSION

By the introduction of TK we will be able to get information on the relationships of plasma concentration to the beneficial effects and the toxicity of pharmaceuticals. These data are important to extrapolate data from animal experiments to human and to assess the safety margin depending on the blood levels. TK data are also useful to estimate the impact of drug interactions and genetical polymorphism of drug metabolizing enzymes to drug therapy.