INVITED REVIEW

TOXICOKINETICS: ITS SIGNIFICANCE AND PRACTICAL PROBLEMS

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1. Opening Remarks

It is my great pleasure to give opening remarks for this Symposium on Toxicokinetics, because my studies on drug metabolism were started more than 30 years ago with the modification of strychnine toxicity by induction of a drug-metabolizing enzyme and by the sexual state of rats.

Since then I have emphasized the necessity of determination of plasma and tissue levels for the evaluation of efficacy and toxicity of drugs. Most of the sex- or species-related difference in drug efficacy and toxicity is related to the plasma level of drugs. However, there are some exceptional cases in which drug toxicity is related to the amount of a formed reactive intermediate but not the parent compound.

These results indicate the importance of drug-metabolism studies, especially determination of reactive intermediates, in toxicokinetics studies. These reactive intermediates play an important role in mutagenic and carcinogenic toxicities and immunological reaction-mediated cell toxicity.

The extrapolation of toxicological data from experimental animals to humans is most difficult, but an important science in drug-toxicity studies.
Although there are marked differences among individual drugs for the ratio of a non-toxic dose in rat or dog to a clinical dose, I observed a clear unimodal distribution of this ratio as shown in Fig. 1-1. This ratio might be called a safety index.

The median of the safety index for rat was 30 (n=183) and for dogs was 16 (n=168). The ratios of biological half-life ($t_{1/2}$) between experimental animals and humans are also unimodally distributed. The median of the $t_{1/2}$ ratio between human and rat, and between human and dog was 3.01 (n=142) and 1.90 (n=130), respectively.

The correlation coefficient between the $t_{1/2}$ ratio and safety index of individual drugs for rat and dog gave $r=0.492$, $p<0.001$ (n=142) and $r=0.553$, $p<0.001$ (n=132), respectively (Fig. 1-2). Therefore, taking into consideration the species differences in the rate of drug metabolism, we could adjust safety indexes by dividing with $t_{1/2}$ ratios. The new safety indexes were about 10 irrespective of rat and dog.

In a preliminary experiment, the same analysis using the AUC (area under drug-concentration curve) and safety index gave similar results. These results indicate that toxicokinetics offer promising data for the analysis of

![Fig. 1-1 Distribution frequency of ratio of non-toxic dose in animal study (repeated administration in rats and dogs) and therapeutic dose in human use.](image1)

![Fig. 1-2 Relationship between ratio of the maximum non-toxic dose in dog studies to clinical therapeutic dose and ratio of plasma half-life in humans to dogs.](image2)
species differences and extrapolation in drug toxicity study. In further studies, we need to collect and analyze the data on toxic serum levels of experimental animals and therapeutic and toxic serum levels in humans.

2. ICH-2 and Development of "Note for Guidance on Toxicokinetics"

presented by David E. Case

1) ICH

The six sponsors of the International Conferences on Harmonization (ICH) are:

CEC Commission of the European Communities
FDA US Food and Drug Administration
MHW Japanese Ministry of Health and Welfare
EFPIA European Federation of Pharmaceutical Industry Associations
PMA US Pharmaceutical Manufacturers Association
JPMA Japanese Pharmaceutical Manufacturers Association

These are sometimes referred to collectively as the 'Six-Pack' and their activities are coordinated by IFPMA-the International Federation of Pharmaceutical Manufacturers Associations.

The terms of reference for ICH, in summary, are:

To provide a basis for constructive dialogue between regulatory authorities and the pharmaceutical industry, to identify areas where modifications in technical requirements or greater mutual acceptance of procedures could lead to a more economical use of resources, and to make recommendations on practical ways to achieve greater harmonization.

The first Conference took place in Brussels in November 1991, and I am sure the various conclusions of this meeting are well known to you. The second Conference is to take place in Orlando in October 1993 and there are three 'Safety Topics' to be addressed: Carcinogenicity Studies, Genotoxicity and Toxicokinetics.

Topics to be covered at Orlando which have implications for 'kinetics' include 'Toxicokinetics', 'Repeated Dose Tissue Distribution Studies' and 'High Dose Selection in Carcinogenicity Studies', but I only intend to address the first and the last of these three.

2) Definition of Toxicokinetics

The word 'toxicokinetics' is a comparatively new word—although toxicokinetics as we now know it was being practised in our laboratories over twenty years ago. If one looks at Biological Abstracts to track the use of this word as a 'keyword', one finds an explosion in its use after about 1983 and no reference to it at all before 1972. The word was apparently first used in the title of a scientific paper by a Russian-VA Filov in 1973, in a paper entitled "Mathematical Aspects of Pharmacokinetics and Toxicokinetics". Twenty years ago the word seems to have been used to describe all aspects of the pharmacokinetics and metabolism of toxic substances—but over the last two decades it has come to be used by the kineticist as a term to describe the pharmacokinetics of substances undergoing toxicological investigation. So, whereas it was originally used by the toxicologist to describe a supporting activity, it has been adopted by the kineticist to describe one specific area of activity as part of the overall drug development process.

In 1989 toxicokinetics was defined as:
"The application of pharmacokinetics principles to the investigation of toxicity and other adverse effects of drugs"

A year later, Smith and his colleagues defined toxicokinetics as:
"Pharmacokinetics performed during actual toxicity tests or in conditions closely mimicking them".
In 1992 Chasseaud\textsuperscript{4} defined toxicokinetics as:
"... the application of pharmacokinetic techniques to concentration-time data generated at the higher dose levels that are customary in toxicity studies"

In preparation for the ICH2 discussions the EFPIA working group decided that before starting to identify the issues in this area and before trying to draw the various topics together, it would be essential to agree first on a definition of the word 'Toxicokinetics' – as this was needed in the first place in order to delineate the scope of the subject to be discussed.

After much discussion, the definition adopted by EFPIA was as follows:
"Toxicokinetics is the generation of pharmacokinetic data as an integral component in the conduct of preclinical toxicity studies and the use of these data in the interpretation of toxicological findings and their relevance to clinical safety issues"

This definition was sent to our opposite numbers in PMA and JPMA and, as there was no criticism of this definition, it has been used in this form ever since. The definition will undoubtedly be modified slightly in the course of our ongoing discussions – but these are the words that currently appear in Draft 10 of the Note for Guidance on Toxicokinetics which is being prepared for ICH2.

It should be noted that toxicokinetics constitutes just one small part of the total package of data which is generated during the course of the development of a new medicinal product. Many other kinds of pharmacokinetic and metabolic studies may contribute to an understanding of the kinetics of a new drug substance – but toxicokinetics focuses on the actual behaviour of the substance under the conditions of the toxicity studies themselves.

In preparation for an ISSX meeting at San Diego last year a survey was carried out of normal practice in nonclinical pharmacokinetics in pharmaceutical companies in Japan, the USA and in Europe; 110 companies participated – 45 from Japan, 40 from Europe and 25 from the US. One part of the questionnaire concerned the practice of toxicokinetics, and it is clear from the responses that there are notable differences in practice between the territories.

Thus, whereas 100\% of European and American companies claimed to be generating toxicokinetic data from their 1-month rodent and nonrodent toxicity studies, this was apparently done in only about 45\% of Japanese companies. A similar set of data was obtained with respect to the 6-month toxicity studies. At the present time, toxicokinetics as defined above therefore seems to be practised much more as a routine in the Western world than in Japan.

3) Development of ICH Guidance

As mentioned before, a ‘Note for Guidance on Toxicokinetics’ was initially drafted by the European working group starting in early 1992. Draft 7 of this document was discussed at an ICH ‘Six-Pack’ meeting in Tokyo in September 1992, and this draft was circulated widely for comment in the three territories. Draft 9 was reviewed at a further meeting held in Brussels in March 1993 and Draft 10 was also circulated widely for comment. In just the last few days, the comments received on this latest draft have been discussed here in Tokyo.

This document describes what is essentially the state of the art in this subject and embodies normal working practices; it is an attempt to come to an agreed position for the industry and the authorities.

For your information I shall now just summarize some of the main elements in this ‘Note for Guidance’. It will undoubtedly undergo further redrafting – but this is how it looks at present:

The Note for Guidance contains Sections entitled:
- Introduction
- The Objectives of Toxicokinetics
- Strategy in developing toxicokinetic testing
- General principles to be considered
- Toxicokinetics in the various areas of toxicity testing
- Supplementary Notes
- References

In the Introduction it is emphasized that this document only concerns pharmaceutical products. The focus is primarily on the interpretation of toxicity tests and not on characterizing the basic pharmacokinetics of the substance. It is emphasized that toxicokinetics should be "... based on a flexible step by step approach......"
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and that no detailed procedures can be set out as each compound needs to be considered separately.

The objectives of toxicokinetics are said to be:

To describe the systemic exposure achieved in animals and its relationship to dose level and the time course of the toxicity study.

To relate 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 nonclinical toxicity studies.

To provide information which, in conjunction with the toxicity findings, contributes to the design of subsequent nonclinical toxicity studies.

The section on general principles includes a statement that:

Exposure is normally represented by plasma concentrations or AUC of parent compound and/or metabolite(s)

Possible species differences in pharmacodynamics should be considered.

The number of time points should not interfere with the study or cause undue physiological stress to the animals.

Complicating factors in exposure assessment include:

Species differences of all kinds...

Use of unbound concentrations where appropriate.

Possible antigenicity of biotech products.

Unusual routes of administration.

The setting of dose levels is of course primarily determined by the toxicology findings; however, the following suggestions are made:

At low dose levels:

Exposure should normally exceed that expected or known to be attained in humans at steady state following therapeutic dose levels.

The high dose level again is normally determined by the toxicology findings; however, if absorption is limited, the lowest dose giving the maximum exposure should be accepted as the high dose to be used; careful attention should be paid to non-linear kinetics.

Toxicokinetic measurements normally involve plasma concentrations of the administered substance. However, it may be appropriate to measure metabolite levels when:

The compound is a 'pro-drug'

Metabolites are 'active'.

This is the only practical means of assessing exposure.

The section on the statistics indicates that:

A high level of precision is not expected.

Calculation of mean or median values should be considered, individual animal data may in fact be more important than group data.

A section on analytical methods emphasizes:

Need for adequate sensitivity, accuracy and precision that the choice of analyte and matrix should be clearly stated and that additional justification needs to be made for the choice of the analyte for chiral compounds.

With respect to repeat dose toxicity studies it is stated that:

"Toxicokinetics may be limited to exposure profiling or monitoring at the start and towards the end of the treatment period of the first pivotal repeat dose study; thereafter, the procedure for later studies will depend on the results from the first study."

With respect to carcinogenicity studies:

"Toxicokinetic data may assist in the selection of dose levels."

A range of systemic exposure values should be achieved which exceed exposure in man by varying multiples.

[It is further noted that this may not always be possible with some classes of compound.]

Comparison with human exposure should contribute to the choice of the top dose level.

Exposure to be assessed 'early in the study, at one year, and towards the end of the treatment period'.

With respect to reproductive toxicology:

Information on pharmacokinetics should be obtained before conducting the studies; there are comments on possible differences in pharmacokinetics between pregnant and non-pregnant animals, placental transfer, and secretion in milk.

The document also contains a series of 'Notes' which provide definitions of some of the words used in the text, explanation of the symbols used, and further notes on items such as satellite groups, non-linear kinetics, control animals, diet and gavage dose comparisons etc.
There are of course many difficulties in drafting a 'Note for Guidance' of this kind. It is important to achieve consistency with existing guidelines and also with other position papers being prepared as part of the ICH process. It is difficult to judge at what level of detail the document should be written, and flexibility is clearly essential if toxicokinetic strategies are to be based on a compound by compound basis. It is clearly very difficult to accommodate the opinions of all those who have reviewed the draft documents; some commentators have held diametrically opposite opinions on some topics! Last but not least, it is noticeable that the principles of such a document tend to become diluted as efforts are made to accommodate all the differing views!

This 'Note for Guidance' will undoubtedly undergo much further redrafting, but it is hoped that we shall be able to present an improved draft at the ICH2 Conference in October of this year.

Before I conclude I would like to draw your attention to a further paper which is being prepared for ICH2. This concerns 'High Dose Selection for Carcinogenicity Studies'. At ICH1, in discussion about carcinogenicity studies, it was recommended that:

"....the criteria for dose selection should be neither the maximum tolerated dose (MTD), nor an arbitrary multiple of intended human dose. Dose selection should be accomplished considering exposure levels in the test species and in man".

The FDA have responded to this in a new position paper first drafted in March 1993 and discussed at the 'Six-Pack' meeting in Brussels that month. This states that it is:

".....reasonable to define a level of animal exposure that would be considered sufficiently great, compared to human exposure, to provide reassurance of an adequate test of carcinogenicity of a non-genotoxic therapeutic...."

The paper notes that "it is critical to compare exposure...rather then dose as the latter does not take into account inter-species differences in pharmacokinetics... AUC is the most comprehensive pharmacokinetic endpoint...." It is also noted that "....The unbound concentration should be determined for use in dose selection"

The paper proposes that "It is acceptable to use as an alternative to the MTD a high dose that represents a 30 [?] fold AUC unbound ratio of rodent to human exposure"

Clearly, there has to be further discussion of this paper and the 30-fold ratio is at present a proposal for debate. However, it appears that for comparatively nontoxic compounds we may in the future have an additional criterion to assist in the setting of the top dose level in these studies and one which obviates the need to administer higher and higher dose levels when no useful increased level of exposure is being achieved.

This FDA paper will also no doubt undergo further revision and it is assumed that a further draft will be presented at the Orlando meeting.

REFERENCES:

3. Current Situation of Toxicokinetics Studies in Japan

presented by Hideo Hakusui

The Pharmacokinetics and Metabolism Group, Nonclinical Evaluation Committee, Japan Pharmaceutical Manufacturers Association (JPMA) conducted a questionnaire investigation on the current situation of toxicokinetics studies in Japan in April 1992.

The questionnaire asked member companies whether they actually conducted any toxicokinetics in their toxicity studies. Out of 49 companies that answered, 18 (32%) were usually conducting some kinetic studies in their toxicity tests and 22 (39%) companies had experienced toxicokinetic studies from necessity. In total 81 toxicokinetic studies were conducted by 40 companies in Japan during 1985–1992 (April). The following was the chronological number of studies conducted:

- 1985 1 1989 7
- 1986 1 1990 10
- 1987 2 1991 46

These data clearly indicate that many pharmaceutical companies in Japan have just got under way in adopting pharmacokinetic studies in their toxicity studies. Most of these studies were performed in repeated dose toxicity studies (68%) and single dose studies (18%). However, there was no case of reproductive tests in which any toxicokinetic studies were involved.

Concerning GLP application to the toxicokinetic studies, 5 out of 49 companies (9%) conduct toxicokinetic studies under GLP, while most companies (74%) are conducting blood sample collection under GLP but analytical procedures outside of GLP facilities.

The questionnaire also asked member companies about their understanding of the relation between toxicokinetics and pharmacokinetics. The outstanding answers are as follows:

1) Information necessary for dose selection, determination of adequate route of administration and dosing formulation for toxicity studies is available from pharmacokinetic studies
2) Sex and species differences, dose-dependency and change in plasma levels by repeated dose administration can be predicted by pharmacokinetic studies
3) Some information necessary to interpret results of toxicity studies and/or related to the mechanism of toxicity such as protein binding, tissue distribution including embryos and fetuses, and secretion into milk can be studied in pharmacokinetic studies more precisely and efficiently than in toxicity studies.

As a conclusion, the pharmacokinetics traditionally conducted in Japan will provide a lot of useful information for toxicity studies, if the guidelines are partially but adequately revised.

4. Pharmacokinetics and toxicokinetics

presented by Kosei Noda

The main purpose of the toxicology program is to clarify toxicological profiles of test substances in animals and to predict their safety in humans. For this purpose, a concept of safety
margins which is the ratio of the highest non-toxic effective dose of the test substances in animals to their clinical dose in humans has traditionally been used. On the basis of this consideration, it may be assumed that the absorption rate of test substances is constant, regardless of doses used during toxicity studies and no species differences are observed in the absorption or metabolism of the test substances. However, it is well-known that there are sometimes non-linear relationships between the given doses and exposure of the body to the test substance and also marked differences among animals and humans in the rate process of the disposition and the metabolic pathways. In addition, it must be taken into account that dosing forms in toxicity studies differ from those in clinical studies. Aging may affect the results of toxicity studies, especially long-term toxicity studies. Since the toxicity is caused by exposure of the body to a toxic substance (the unchanged drug and/or metabolite(s)), the concept of safety margins calculated from dose levels alone is not really preferable for the prediction of drug safety for humans.

It is of vital importance for toxicokinetics to evaluate the relation between the given doses and toxic symptoms by measuring systemic exposure (mainly the plasma concentrations) in toxicity studies from these experiences. Therefore, toxicokinetics of the test substances is now spotlighted. On the other hand, pharmacokinetics and metabolism (absorption, distribution, metabolism and excretion (ADME)) studies of the test substance are generally performed in fasting animals after single dosing with small doses of a solution of the radio-labelled substance. These data may scarcely be related directly to toxicological findings in toxicity studies. Moreover, there are controversial problems as to whether ADME studies are conducted at an appropriate time in conjunction with the toxicity studies.

In Japan, "guidelines for nonclinical pharmacokinetic studies" and its manual were issued in 1991. Although there are no expressions on toxicokinetics in the guidelines, it is stated that ADME data on the test substance are not only useful for designing the toxicological and pharmacological studies in animals and evaluating the results of these studies but also essential for establishing the appropriate dose regimen in humans and obtaining the safety and efficacy of the test substance. Therefore, it is desirable that ADME studies are designed and conducted on the basis of the pharmacological effects and toxicity of test substances. Concretely, factors such as animal species, dosing routes, dose levels and dosing interval and duration are required to be appropriately used for ADME studies under the conditions of toxicity studies. In future, ADME studies may be further investigated in consideration of toxicity studies.

ADME data play a very important role in planning toxicity studies as well as in the interpretation of the results of toxicity studies. What kinds of ADME data are needed for toxicity studies depends on each stage of the toxicity studies. ADME studies needed to support toxicity studies at respective R & D stages are as follows.

1) Stage before toxicity studies
ADME data to support toxicity studies at the stage before toxicity studies are:

(1) Development of assay methods
   precision, accuracy

(2) Single dose kinetics
   i) Pharmacological doses
      Cmax, Tmax, t1/2, AUC
      bioavailability, first-pass effect
   ii) Toxicological doses
      especially dose-dependency
   iii) Formulations
      solution, suspension or powder
      investigation of solvents and solubility enhancers
   iv) Fasting and non-fasting

In this stage, it is first necessary to grasp the kinetic characteristics of test substances and to develop analytical methods for the application of toxicokinetics to the toxicity studies. The analytical methods by which the accuracy, precision and reproducibility are validated using biological samples of each animal species must be established. Subsequently, the physico-chemical properties of the test substances should be clarified, and then the basic pharmacokinetics after a single dosing should be investigated in the same animal species as those in toxicity studies. In this case, it is important to clarify substantial
kinetic characteristics of the test substances from
the pharmacokinetic parameters obtained from
the plasma concentrations after dosing with a
small amount, i.e. non-toxic doses. If possible,
a test substance should be given to animals in the
form of a solution. It is important to determine
the bioavailability of the parent substance which
is calculated by comparing the plasma concentra-
tions with those after intravenous dosing and to
study the extent of the first-pass effect. Because
test doses in toxicity studies are high, non-linear
kinetics occurs very often in ADME data.
Therefore, the information on the relationship
of blood concentrations to test doses should be
obtained before conducting toxicity studies.
When test substances are insoluble and poorly
absorbed from the gastrointestinal tract, an
adequate dosing form of the test substances
should be prepared in order to optimize its mode
of administration. Effects of feeding on drug
absorption should also be examined.

2) Pre-clinical stage
ADME data to support toxicity studies at the
pre-clinical stage are:
(1) Absorption of radioactivity
   blood concentration, urinary excre-
tion
(2) Whole-body autoradiography
   rodent (male, pregnant)
(3) Tissue distribution
   radioactivity, unchanged drug,
   metabolite(s)
(4) Protein binding and distribution in blood
   in vitro (rodent, non-rodent,
   human)
(5) In vitro metabolism
   S9, hepatic microsomes or hepato-
   cytes (rodent, non-rodent, human)
(6) In vivo metabolism (rodent, non-rodent)
(7) Enzyme induction/inhibition
(8) Balance study of radioactivity (rodent,
   non-rodent)
The pre-clinical stage in drug development
program corresponds to the time to perform
short-term (2-week to 3-month) toxicity studies.
It is also the time when a radiolabelled substance
may be available and basic ADME studies are
performed. Therefore, it is desirable that
ADME studies are performed in consideration
of the toxicity studies. When the radiolabelled sub-
stance is obtained, plasma concentrations and
mass balance studies are first studied in rats and
dogs. From the results of the studies on plasma
concentrations and urinary and fecal excretion
of radioactivity, the extent of absorption of test
substances can be elucidated for the first time.
When the bioavailability of a test substance is low
in studies with a nonlabelled substance, it is
usually difficult to know whether it may be due to
poor absorption or first-pass effect.

Tissue distribution studies are performed by
whole-body autoradiography in male and pre-
gnant rats to assess distribution, accumulation
and placental transfer of radioactivity. The tis-
sue distribution is also investigated by the method
of counting the radioactivity. In these studies, it
is desirable to elucidate the ratio of tissue to
plasma concentrations and the proportion of the
parent substance and its major metabolites in the
tissues with high concentrations of radioactivity.
These data are particularly important since such
information usually cannot be obtained in large
animals as well as in humans. Tissue distribu-
tion data in rats are useful for the prediction of
accumulation and/or retention of a test substance
in large animals and in humans.

In vitro studies on the protein binding,
distribution in the blood and metabolism study of
the test substance with S9 mixture and liver
microsomes or, if possible, hepatocytes should be
performed at this stage. Although in vivo phar-

Table 4-1. Serum protein binding of Compound C (in vitro).

<table>
<thead>
<tr>
<th>Species</th>
<th>50</th>
<th>500</th>
<th>5000</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rat</td>
<td>95.1±0.9</td>
<td>95.1±0.1</td>
<td>95.0±0.0</td>
</tr>
<tr>
<td>Dog</td>
<td>88.2±0.4</td>
<td>89.1±0.6</td>
<td>88.6±0.1</td>
</tr>
<tr>
<td>Man</td>
<td>99.1±0.2</td>
<td>98.8±0.1</td>
<td>98.9±0.1</td>
</tr>
</tbody>
</table>

% n=3, Means±S.D.
macokinetic and metabolism data in humans cannot be obtained in this stage, some interspecies comparison might be possible if tissue samples from humans are available for in vitro studies. Protein binding and distribution in blood compartments are factors affecting the tissue distribution of a substance. In particular, the protein binding is the most important factor. The unbound substance is considered to be the biologically active entity for pharmacological or toxicological effects.

In vitro studies are useful to get information about species differences in pharmacokinetics and metabolism. For instance, Table 4-1 represents a case of protein binding study. Protein binding was high (above 88%) in all of the rat, dog and human sera. The species differences in protein binding appear not to be remarkable. However, when the data are expressed by free fraction, the protein bindings (extent of free fraction) in rats and dogs are 5 or 10 times higher than in humans.

Even if chemical structures of major metabolites in animals and humans cannot be elucidated in in vitro metabolism studies, some information on similarity and disparity of metabolism between animals and humans can be obtained by studying the metabolic rates and patterns. It is desirable to characterize the key cytochrome-P450 isozyme(s) involved in the metabolism of test substances in animals and humans.

In addition to the in vitro metabolism, in vivo metabolism studies should be performed. Major metabolite(s) in animals should be identified and the concentrations measured in biological materials such as blood, plasma, urine and feces. Enzyme induction/inhibition studies in rats are also needed at this stage.

The studies mentioned above may be useful for a) comparing the absorption 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 substances. This knowledge, including toxicokinetics data obtained in this stage, is considered to be useful for validating short-term toxicity studies and designing subsequent toxicity studies.

3) Clinical stages

ADME data to support toxicity studies at the clinical investigation stages are:

(1) Human pharmacokinetics
(2) Human metabolite(s) in blood, plasma, urine, feces
(3) Milk excretion in rodents
(4) RI repeated dose ADME studies
(5) Comparison of bioavailability in alternative routes (dietary, gavage)
(6) Effects of aging
(7) Relevant ADME studies

Phase I studies provide pharmacokinetic data in humans. When an active metabolite common to animals and humans is found, the determination of plasma concentrations of this metabolite should be considered in further toxicity studies. In short-term toxicity studies, the monitoring of toxicokinetics is usually limited to the parent substance. In long-term toxicity studies, however, it should be determined whether blood concentrations of metabolites are assessed. When a major and active metabolite is found in humans but not in animal species for toxicity studies, it seems necessary to conduct additional toxicity studies with the metabolite. Milk excretion study should be performed before phase-II clinical studies.

The data of repeated dose ADME studies using the radiolabelled substance may provide useful information about the extent of accumulation, the time course of disappearance and changes in metabolic profiles, etc. For instance, Fig. 4-1 shows the tissue concentrations of radioactivity in rats 24 hours after single oral dosing with 10 mg/kg of 14C-labelled substance and repeated oral dosing for 56 days. The concentrations of the radioactivity in the plasma, liver, kidneys and lungs after the 56th dosing were about 7-10 times that after single dosing. Accumulation of the radioactivity in the blood and the spleen was marked, increasing respectively by 13 and 29 times that after the first dosing. Since this substance affected the erythrocyte system and spleen of rats in a repeated dose toxicity study, special attention should be given to the relationship of tissue accumulation and organ toxicity.
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When a dietary administration is used as the dosing route in chronic toxicities or carcinogenicity studies in rats, plasma concentrations by feeding should be compared with those by gavage. It is also generally suggested that effects of aging on the pharmacokinetics of test substances must be taken into account in long-term toxicity studies. When there is a poor relationship between toxicity findings and plasma concentrations of a parent substance, the possible cause of dissociation should be elucidated, e.g. the mechanism of the toxicity, implication of active metabolites, covalent binding and changes in endogenous substances, etc.

4) Conclusion

It can be stated that the purpose of toxicological and pharmacological studies in animals and clinical studies is to assess effects of test substances on the living body, but the purpose of toxicokinetic and ADME studies is to assess effects of the body on test substances. Therefore, when results of toxicity studies are discussed, overall evaluation should be made on the basis of not only the reactions to the body but also results of toxicokinetic and ADME studies.

Furthermore, the data for pharmacokinetics in humans and toxicokinetics in animals are of vital importance for establishing the relationship between clinical studies and toxicological 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.

Lastly, since the types of ADME studies to be performed in each R & D stage differ depending on R & D policies of individual companies and on the specificity of individual test substances, it is noted that the design and the timing of the ADME studies should be considered flexibly.

5. Significance of Toxicokinetics in the early stage of toxicity studies: Case Studies

presented by Fumio Sagami

During the past period of more than a decade, Eisai Research Laboratories has intensively adopted a pharmacokinetic approach in toxicity studies, especially in exploratory toxicity studies and dose-range finding studies to conduct toxicity studies on more rational bases. I will introduce some of our experiences of toxicity studies in which you may find the significance of so-called "toxicokinetics".
1) Dosing formulations and conditions

It is generally known that adequate formulation of test compounds in necessary for toxicity studies. Such was the case of a poor water-soluble Compound A. When the compound was prepared in suspension for dosing, the maximum available AUC was about 0.4 mg hr/ml at 0.2 mg/kg and above. However, we could get the maximum AUC of 10 times at 3mg/kg by adding some amount of polyethylene glycol 400 as a solvent. In cases of lipophytic compounds, the bile secretion is sometimes essential condition for the gastrointestinal absorption. We experienced some cases where the systemic exposure to test compounds was enhanced more than 10 times by oral administration after test animals were given food than under fasted conditions. Compound B was unstable in acid solution (stomach juice). Administration using enteric coated tablets was effective to get a level of reasonable systemic exposure in dog studies. However, we could not overcome the trouble in preparing enteric coated granules for the compound for rat studies. Dosing together with a sufficient amount of carbonhydrate was effective to enhance systemic exposure but not found practical in repeated dose toxicity studies. We conducted those studies by dosing to the empty stomach under a conditioned feeding.

2) Enzyme induction and inhibition.

Enzyme induction and inhibition are sometimes causes of non-linear kinetics. Compound C was a strong inducer of drug metabolizing enzyme (Fig. 5–1). The plasma levels definitely decreased within two weeks of repeated administration, especially at higher test doses. It was noteworthy that there was no remarkable reduction in plasma levels thereafter until the end of 12 weeks.

Compound D seemed to be a metabolism enzyme inhibitor. Test doses were 1, 3, and 10 mg/kg. No remarkable toxicity was observed at the first dose even at the highest dose. However, one of six animals in the highest dose died on the third day and all the rest of the animals died or were moribund within the first seven days. As shown in Fig. 5–2, the plasma level in 3 mg/kg at Day 34 was about 10 times that of the first dose and remained at enhanced levels thereafter. There was no remarkable change in plasma levels in the group given the lowest dose. These data suggested that there was a drastic increase in exposure by repeated administration of the highest dose for a few days.

3) Conclusion

I would like to conclude from our limited experiences that pharmacokinetic approaches in the early stage of toxicity studies (exploratory toxicity studies and dose-range finding studies) provide a lot of important information most needed to adequately conduct toxicity studies, i.e. extent of bioavailability, dose-dependency (linear or non-linear kinetics), and effects on drug-metabolizing enzymes. Change in systemic exp-

![Fig. 5-1](image-url) Plasma concentration (ng/ml) of Compound H during 13-week repeated dose toxicity study in dogs. Each point represents mean and S. E. of 3 males and 3 females.

- ○: 1 mg/kg ●: 3 mg/kg
Toxicokinetics: Its significance and practical problems

Fig. 5-2 Plasma concentration (μg/ml) of Compound C during 13-week repeated dose study in dogs. Each point represents mean of 3 animals.

- - - - - : 10 mg/kg, male
- - - - - : 50 mg/kg, male
- - - - - : 250 mg/kg, male
- - - - - - - - - - : 10 mg/kg, female
- - - - - - - - - - : 50 mg/kg, female
- - - - - - - - - - : 250 mg/kg, female

Exposure by repeated dosing is one of the key items to be studied by toxicokinetics. As the cases of Figs. 5-1 and 5-2 show, those changes in exposure by repeated dose usually develop and reach a plateau during the first few weeks of repeated dosing. Most important toxicokinetic profiles of individual test compounds can be revealed by assessing systemic exposure in 4-week repeated dose studies.

6. Current Status and Perspective of Toxicokinetics

This paper deals with the following points concerning toxicokinetic (TK) studies: 1) to introduce how a pharmaceutical company conducts TK studies in the drug development process, showing cases of the past, the present status, and future perspectives, and 2) to give concrete examples of problems arising in the course of conducting TK studies in small animals (rats, mice).

1) Significance of TK study in the process of drug development

In terms of the significance of conducting a TK study, the proposition to be made clear is "what is essentially required in toxicokinetic study in the drug development research process?". Recently, TK studies began to play an important role, and accordingly in Japan, we encounter various situations to handle, such as a request for a TK study for foreign NDA and discussion in the International Conference for Harmonization (ICH). Toxicologists, especially those involved in the evaluation of drug safety, are now confronted with an important problem: how to analyze, utilize and evaluate TK data.

Generally, we can easily think of the necessity and significance of TK study based on the basic knowledge. For example: a TK study is necessary (1) for selection of dosage, formulation and dosing route, (2) for determination of TK profile in tox. study, (3) as a help for the evaluation of...
toxicities, (4) to serve for the elucidation of toxicological mechanisms, and (5) for comparative evaluation between experimental animals and humans. Those discussions nevertheless will often be interpreted as idealistic. Some may say that toxicokinetics is not scientific since it is difficult to establish the discipline of toxicokinetics in the field of science, or that toxicokinetic data are not imperatively required. However, it is still premature to draw such conclusions. We can expect new development and advancement in TK research when toxicology and pharmacokinetics get more integrated and when adequate TK data are accumulated; at that time, each item above will become fully meaningful.

Toxicokinetics is said to be interdisciplinary, but I emphasize that toxicokinetics should be rooted in the toxicology-related sciences. Fig. 6-1 shows the design of toxicological studies and pharmacokinetic studies in the process of drug development and the timing of incorporating TK studies. In relation to the toxicology scheme in drug development, the timing and the order of conducting TK studies are self-determining. The role of TK studies in the drug development process is shown in Fig. 6-2.

In recent years, a toxicological and/or pathological approach is required in the very early stage of exploratory research in the drug development process. In parallel with exploratory toxicological studies, a large number of TK studies is conducted to contribute to steering of drug development. TK data are valuable in selecting animal species and drug formulation, and in determining metabolic pathways and accumulation.

Later, in the course of conducting pivotal toxicity studies, TK data are taken into consideration to determine the method of administration, dose levels, etc. Toxicity evaluation also requires TK data at the late stage of toxicity study (IND/NDA); for example, to support the confirmation of any toxicity effect observed.

From the practical viewpoint, the necessity/significance of a TK study should be intensively considered: (1) To know the TK profile gives us a chance to investigate the dose (esp. minimum dose) and dosing procedures, and (2) to determine the TK profile gives us information to estimate the toxicity and efficacy in animals and humans.

A few sentences which describe the essence of significance of TK data may be pointed out as follows: "We are not always forced to interpret or evaluate toxicity with toxicokinetic analysis, but TK data are useful. That may sound "easy-going", but this attitude is important. We do not give a great significance to a TK study, but we know that TK data sometimes can be of great help, indicating a critical point."

In conducting a TK study, the factors to be determined are enormous: animal species, sex difference, drug formulation, solvent, administration route, feeding effect, method and time point of blood collection, monitoring point, etc. Those factors must be determined for each compound, and the problems that arise are different from compound to compound. A TK study should require a "case-by-case" and "step-by-step" approach.

![Fig. 6-1 Toxicokinetic (TK) study in drug development process (1)](image)

![Fig. 6-2 Toxicokinetic (TK) study in drug development process (2)](image)
2) Introduction of the present status of TK studies... in the case of Roche...

In the last 5 years, approx. 100 reports on TK studies were issued within the Roche group (Switzerland, England, USA and Japan), and more than 50 studies were issued as internal scientific memos. Approx. 90% of those TK studies were conducted in Switzerland and England for NDA in Europe. The animals used were rats (88%), mice (7%) and rabbits (5%) among rodents, and dogs (40%), marmosets (36%), cynomolgus monkeys (21%) and baboons (2%) among non-rodents. With respect to the dosing duration, approx. half of the TK studies were single dose studies or shortterm (up to 4 weeks) studies, and 13-week studies and 26-week studies amounted to ca. 40% of all TK studies. As a matter of course, feed admix is more frequently employed for studies with longer dosing duration.

Concerning the blood sampling schedule, Roche has determined an internal guideline. The guideline, however, is treated as a reference in making protocols. Table 6-1 shows the standard (example in rats) of a sampling schedule for internationally developed drugs. It is sometimes difficult to repeatedly obtain blood samples in accordance with a time schedule from individual animals in cases of small animals like rats and mice. Table 6-2 shows an example of our scheduled program for toxicokinetic profiling in rats. There are arguments for and against this scheme. This schedule may not be perfect; still, it can provide a lot of important information. Our attitude as toxicologists is this: “Get full information from TK studies but not rely solely on them”.

Table 6-1. Proposed blood sampling schedule during oral and intravenous toxicity studies in rats.

<table>
<thead>
<tr>
<th>Duration of study</th>
<th>Sampling day 2)</th>
</tr>
</thead>
<tbody>
<tr>
<td>4 weeks</td>
<td>administration via stomach tube: predose 3), day 1</td>
</tr>
<tr>
<td>13 weeks</td>
<td>stomach tube: predose 3), day 1</td>
</tr>
<tr>
<td>26 weeks</td>
<td>stomach tube: predose 3), day 1</td>
</tr>
<tr>
<td>52 weeks</td>
<td>stomach tube: predose 3), day 1</td>
</tr>
<tr>
<td>24 months (carcinogenicity study)</td>
<td>food admix: one day from around the 3rd, 6th, 12th, 18th and 24th months.</td>
</tr>
</tbody>
</table>

1) For other routes of administration, the sampling schedule has to be adapted appropriately.
   Blood should be taken at least at 2-4 time points per sampling day.
   Unless otherwise agreed, 2 male and 2 female rats are to be taken for each time point.
   At each sampling point, blood samples of 1-2 ml should be drawn from each animal. The study protocol should indicate whether blood samples are to be treated as individual samples or if they can be pooled. The procedure of pooling must be described in detail.
2) The first day of the experiment is the day of the first administration.
3) Predose does not mean that the blood has to be collected immediately before the first drug administration. Bleeding may also take place a few days before.
Table 6-2. An example of time-scheduled blood sampling for toxicokinetics in rat studies (blood sampling on Days 1 and 13 of a 4-week repeated dose study). Note: a total of 15 animals is allocated for blood sampling at each one of the scheduled time points (3 animals per time point).

<table>
<thead>
<tr>
<th>Time course (hrs)</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Animal No.</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>7</td>
<td>8</td>
<td>9</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td>11</td>
<td>12</td>
<td>13</td>
<td>14</td>
<td>15</td>
</tr>
</tbody>
</table>

3) Concrete examples in the course of conducting TK studies in small animals (rats, mice)

In chronic toxicity studies or carcinogenicity studies in rodents, feed-admix administration is frequently employed. For determining the proper blood sampling schedule, we monitored the feeding activity of rats and mice for 24 hours, and observed an hourly feed intake (Fig. 6-3).

In the rats, approx. 70% of the daily feed intake was consumed during the dark hours (19:00 - 7:00 a.m.), and the mice ingested approx. 60% of daily intake in the dark hours. Analyzing the feeding patterns, we concluded that the most appropriate sampling time was 6:00 a.m. for rats and 22:00 for mice, since the animals showed a constant feeding activity.

In oral toxicity studies, gavage administration is generally used for short-term (2-4 weeks) repeated administration studies, and feed-admix administration is usually employed for long-term (13-26 weeks) studies. Fig. 6-4 shows the comparative toxicokinetic profile of a drug adminis-

![Fig. 6-3 Pattern of food consumption during 24 hours in male and female mice (left figure) and in rats (right figure).](image-url)
Toxicokinetics: Its significance and practical problems

Toxicokinetics: Its significance and practical problems

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The toxicity of drugs appears in various patterns; some drugs show AUC-dependent toxicity, and some show Cmax-dependent toxicity. When toxicity studies are conducted with both gavage dosing and feed-admix administration in the course of drug development, comparative TK studies should be conducted in parallel with the toxicity studies to evaluate the toxicity.

4) Integrated summary table in terms of TK data

Fig. 6-5 presents the example that the toxicity of a drug cannot be evaluated with TK data alone. Since the evaluation of toxicity requires total interpretation, we make, at each developmental phase, a table called an “integrated summary,” which contains the data of toxicology, toxicokinetics, pharmacokinetics, pharmacology and clinical study. It is not important whether the table itself is meaningful, but the point is that we must always be watchful of the related data. Toxicokinetic data are not always meaningful for toxicity evaluation of a drug, but are sometimes very helpful at a critical point. This is where the significance of a TK study resides.

Fig. 6-4 Plasma concentrations of Compound XXXX administered with gavage and feed-admix in male and female rats.

<table>
<thead>
<tr>
<th>Toxicokinetic parameters</th>
<th>Cmax (µg/ml)</th>
<th>Tmax (h)</th>
<th>AUC (µg · h/ml)</th>
<th>Dose (mg/kg)</th>
<th>AUC/Dose</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gavage male</td>
<td>11.56</td>
<td>2</td>
<td>162.7</td>
<td>100</td>
<td>1.63</td>
</tr>
<tr>
<td>female</td>
<td>10.30</td>
<td>3</td>
<td>202.3</td>
<td>100</td>
<td>2.62</td>
</tr>
<tr>
<td>Feed-admix male</td>
<td>5.43</td>
<td>16</td>
<td>151.3</td>
<td>82</td>
<td>1.82</td>
</tr>
<tr>
<td>female</td>
<td>6.03</td>
<td>16</td>
<td>184.4</td>
<td>82</td>
<td>2.35</td>
</tr>
</tbody>
</table>

Toxicokinetic data are not always meaningful for toxicity evaluation of a drug, but are sometimes very helpful at a critical point. This is where the significance of a TK study resides.
Fig. 6-5 An integrated Summary Table (Multiple Dose). What we wanted to show is given in boxes.
7. Practical Problems of Toxicokinetics; How to meet Scientific Needs

presented by Hiroshi Mayahara

1) Comparison of plasma levels of drugs in animals and humans

Fig. 7-1 shows the dose-concentration relation at toxic doses (the minimum toxic dose levels) in a total of 54 repeated-dose toxicity studies (21 rat, 30 dog and 3 monkey cases) which were conducted in several companies belonging to JPMA (Igarashi, 1993, The use and abuse of toxicokinetics: what does actual data tell us? Drug Information Journal, in press.). The abscissa represents relative dose (ratio to human therapeutic dose) and the ordinate relative Cmax (ratio to human therapeutic plasma level). The toxic doses (per body weight bases) ranged from 4 to 30,000-fold of the respective therapeutic doses depending on test compounds, while the toxic concentration ranged 1/2 to 10,000-fold of the respective therapeutic concentration. In three cases (one rat and two dog studies), the toxic Cmax in animals was lower than the therapeutic Cmax in humans. In most cases, plasma concentrations (Cmax) in animals were generally much less (sometimes 1/100) than those in humans. The reason why the plasma concentrations in animals were much less than that in humans can be partly explained by "interspecies scaling" (Mordenti and Chappell, The use of interspecies scaling in toxicokinetics. In "Toxicokinetics and New Drug Development", ed by A. Yacobi, J. P. Skelly and V. K. Batra, Pergamon press, pp 42–96, 1989). It is noteworthy, however, that the pharmacokinetic profiles such as bioavailability and plasma half-life extremely differ from compound to compound, and that there are not a few cases in which a reasonable level of systemic exposure to test compounds can not be achieved in available animal species.

2) How to enhance exposure levels in toxicity studies?

As mentioned above, exposure levels in animal models are lower than in humans in most cases. This is because the plasma half-lives of drugs are much shorter in animals than in humans. However, we sometimes experience cases in which the exposure levels in toxicity studies can be improved by some measures.

Table 7-1 shows an example of such cases.

![Fig. 7-1] Distribution of toxic doses and toxic plasma concentrations in rats, dogs and monkeys. The parameters are represented by ratios to human therapeutic dose and plasma concentration. Area above \( y = x \) indicates that plasma concentrations in animals are higher than the plasma concentration in humans, and area under \( y = 1 \) indicates that plasma concentrations at toxic doses in animals are lower than the human therapeutic plasma concentration.
Water-insoluble compounds are often prepared in suspension for oral administration. Compound X was one of such cases. The absorption from the gastrointestinal tract was much improved by use of a grinder to decrease the particle size in a test solution. The use of a smaller size (median size: 3.8 μm) could attain about 16 times larger exposure (AUC) than a larger size (median size: 10.8 μm).

In our laboratories, we investigate the effect of the following factors when the need for increasing exposure in toxicity studies has arisen:

1) dosing formulation (bulk in capsule, diet admix or suspension; kind of vehicles for suspension)
2) particle size and particle size distribution (use of motor grinder, grinding time)
3) timing of feeding (fasted or fed, duration between dosing and feeding)
4) dosing time and frequency of dosing within a day
5) crystal types
6) racemate or enantiomers
7) other dosing routes
8) other test species

3) How to decrease the number of animals for toxicokinetic studies?

One of the most serious problems in practicing toxicokinetic studies is that a large number of animals (additional animals in cases of rodents) will be needed for toxicokinetic determinations. In the Toxicokinetics Note for Guidance (ICH Draft III), there is the following description: "The number of animals to be used should be the minimum consistent with generating adequate toxicokinetic data (3.7)." This is a important message for industries, but there are no concrete words on how to decrease the number of animals for the studies.

The following summarizes possible ways to reduce the number of animals needed for toxicokinetic studies without compromising scientific needs:

1) To define (reduce) the kind of toxicity studies in which toxicokinetic data are required. This should be encouraged by the regulatory authorities.
2) To decrease the number of time points per each determination and the number of animals in each time point. This can be achieved by well-designed studies and by using toxicokinetic data obtained in previous studies, as the Note for Guidance clearly mentions that duplication of similar kinds of studies should be avoided.
3) Pharmacokinetic data obtained outside of toxicity studies should be accepted for validation of toxicity studies and in interpreting toxic findings. This has been intensively recommended by Japanese EWG members. However, the guidelines for pharmacokinetics and metabolism studies in animals should be revised adequately from this viewpoint. I would like to recommend the following additions to the Note for Guidance:

1) Toxicokinetics data should not necessarily be required when overt toxic changes are demonstrated in a toxicity study, because the purpose of the toxicokinetic study is validation of systemic exposure, and one of the expressions of systemic exposure is occurrence of toxic changes.
2) Especially for reproductive toxicity studies, toxicokinetic data should not be required when toxic changes are demonstrated in dams, because the value of toxicokinetic
data is lower than those of general toxicity studies for the following reasons:

a. The toxicokinetics data from general toxicity studies can be applicable.
b. Expression of overt toxic changes in dams is not required in reproductive toxicity studies.
c. Exposure to fetuses or embryos is not prerequisite. For example, they need not be exposed to the compound, which will not be transferred across the placenta.
d. The quantitative data of exposure in fetuses or embryos cannot be compared with those of human fetuses or embryos.
e. Pharmacokinetic data using isotope-labelled test compounds can offer data on embryonal exposure at higher sensitivity.
f. The causal relationship between the embryonal toxicity and the test compound is obscure in most cases.
g. The extrapolation of animal data to humans is difficult due to the anatomical difference in the structure of the placenta.

3. The toxicokinetics data in the micronucleus test may be required when in vitro mutagenicity tests showed a positive result but an in vivo micronucleus test gave a negative result to validate the local exposure of target tissue (bone marrow) to the test compound. However, two questions were raised at the EWG meeting; one was a practical problem (difficulty) to measure local distribution of test compounds in tiny mouse bone marrow, and another was a doubt of the necessity to determine the local exposure of tissue where no specific barrier system has been known. Fig. 7–2 shows an example of tissue distribution of a test compound into bone marrow comparatively determined in rats and mice. The data suggest that a) there is no tissue distribution specific to bone marrow (compared with plasma, hematocytes and spleen), indicating that there is no need of direct determination in the target tissue if it is validated by surrogate data, and b) there is no remarkable species difference between the mouse and rat. The bone marrow is usually (or at least often) included as one of the test organs for the tissue distribution studies in basic pharmacokinetics using rats.

In Fig. 7–3, an algorithm of toxicokinetic studies during the course of the development is presented. In this Figure, the arrows indicate the use of information. The toxicokinetic studies in a series of toxicity tests for each test animal species are divided into three categories, a, b and c. Category a is a stage of preliminary toxicokinetic to find optimum conditions for subsequent studies. Category b is the profiling study in which an adequate number, say 5–8, of blood samples during a dosing interval are taken in order to make an estimate of both Cmax and AUC. I would like to call these studies pivotal toxicokinetic studies. One month repeated dose study and dose range finding studies for carci-

Fig. 7–2 Distribution of Compound Y (oral administration) into blood, spleen and bone marrow in mice and rats (T. Igarashi, 1993, unpublished data).

- - - : bone marrow --O-- : plasma
- - - : hematocytes ---X-- : spleen
**Fig. 7-3** A possible algorithm of strategy of toxicokinetic studies.

Note: a) preliminary toxicokinetic studies
   a') not necessary if any reliable data are available from ascending studies
   b) profiling (determination of AUC, Tmax and Cmax)
   c) monitoring (determination of Ctime with small number of animals)

?) see text
nogenicity tests may be carried out for the typical cases. Category c is the monitoring study in which a small number (1-3) of blood samples during a dose interval are taken in order to provide reassurance that the exposure levels obtained are consistent with those predicted by pivotal studies. These are applicable to long-term repeated dose studies and carcinogenicity studies. This algorithm may offer a pragmatic approach to toxicokinetic studies using a minimum number of animals.

8. Toxicokinetics; The United States Pharmaceutical Industry View

presented by Mitchell N. Cayen and Thomas B. Marriott

1) Toxicokinetics principles as viewed in North America

Because toxicokinetics is a developing scientific discipline, there is still no uniform definition of what the term toxicokinetics means. Although toxicokinetics generally refers to the development of pharmacokinetic parameters at doses used in toxicology studies, some people include in their definition all absorption, distribution, metabolism, and excretion studies when they use the term “toxicokinetics”. We therefore need to harmonize our definition of toxicokinetics.

Toxicokinetics is a combination of toxicology and pharmacokinetics. It is not the purpose of a toxicokinetic study to characterize the pharmacokinetics of a drug in a laboratory species. Rather, it is to develop a tool which can be most helpful in the interpretation of toxicity data.

It is the dose of the drug that is being studied that is the principle difference between toxicokinetic and pharmacokinetic data. Toxicokinetic studies use the doses used in toxicology studies and are therefore significantly higher than doses used in pharmacokinetic studies.

Toxicokinetics is an extension of preclinical safety and pharmacokinetics programs. Toxicokinetic data is important both for interpreting preclinical safety data and for providing guidance in the planning of future safety studies.

2) Integration of toxicokinetics into safety assessment programs

Kinetics can play a useful role in the support of drug discovery studies as well as in support of drug preclinical safety assessment. Using a semi-validated assay, the development of kinetic data can be a powerful tool to assist in interpreting pharmacology studies, and when combined with specific ADME studies, it can be most useful in interpreting initial toxicology studies. Toxicokinetic data, developed using a validated assay, has significant benefit when interpreting findings in the subacute toxicology studies. Further, when preclinical kinetic data are combined with preclinical metabolism studies like tissue distribution and balance studies, they provide strong support for initial AME studies in man.

If one plans to use kinetic studies to support the drug discovery process, kinetic studies must be designed to answer very specific questions. But to answer any question, one has to first determine what should be assayed, drug or metabolite, and then develop and validate the required assay(s) to achieve that goal. In contrast to the discovery process, the goal of kinetic studies in toxicology is to demonstrate dose-related exposure to drugs and to facilitate interpretation of the findings in the toxicology studies.

The goal of toxicokinetic studies is to help explain (a) unexpected toxicity, (b) lack of toxicity, (c) to relate plasma drug levels to toxic response, (d) to help explain species differences in response, and (e) to help extrapolate data from animals to man.

Using “exposure” as indicated by AUC and/ or Cmax for parent drug or major metabolite in laboratory animals and man, one can calculate a
human safety margin. This human safety margin is the ratio of the AUC of the "no effect dose" in animals in the toxicology studies to the AUC to the therapeutic dose in man.

Toxicokinetic data developed in a toxicology program may demonstrate (a) that there is a major metabolite in the laboratory species that is specific to that species and appears to account for the toxicity. (b) Toxicokinetic data may suggest the doses used in a toxicology study were too high since plasma concentrations beyond a given dose no longer increased with dose. (c) Toxicokinetic data may suggest that the compound appears to be an enzyme inducer and (d) in developing all the information needed to interpret toxicokinetics, one may find clear species differences in plasma protein binding.

3) Toxicokinetic program: strategies and implementation

Most toxicology programs conducted in North America today have a toxicokinetics component. A step-wise approach is used by using compound-specific decision points. These decisions include:

(a) what to measure (drug or metabolite),
(b) appropriate exposure data (total or unbound drug)
(c) appropriate exposure parameters (AUC and/or Cmax)
(d) what specific, goal-oriented ADME studies to conduct.

The need for incorporating toxicokinetics into reproduction studies is still being debated. There is additional data required to meaningfully interpret toxicokinetic data.

(1) one must know, in the species being studied, something about biotransformation and major metabolites, to determine whether to measure parent drug or metabolite in the kinetic studies.
(2) one needs a validated assay for the species being studied.
(3) one must have information on protein binding to know whether to express data as total or unbound drug.

Concomitant toxicokinetic studies are conducted in combination with the toxicology study using animals from the study or satellite groups in the study. There studies directly relate dose and exposure with time.

Ancillary (prospective) toxicokinetic studies refer to studies conducted using a range of doses, and are conducted for the purpose of assisting in dose selection for chronic studies, such as carcinogenicity studies.

Ancillary (retrospective) toxicokinetic studies determine the kinetics associated with doses used in completed toxicology studies. The purpose is to provide information on dose-related exposure that occurred in those studies to further validate the completed study.

One must recognize that there are limitations on the interpretive value of toxicokinetic data. Plasma concentrations of drug and end organ toxicity may not always correlate. Further, evidence of clear differences in the kinetics between laboratory species and man may still have no effect on selection of a laboratory species for future toxicology studies on a drug. This is due to the fact that there are so few readily available and well characterized laboratory species to use in toxicology studies. For carcinogenicity studies, if exposure in the rodent species is not considered to be adequate when compared to man at the therapeutic dose, based on toxicokinetic data, then one must use the concept of the Maximum Tolerated Dose to select the highest dose to be studied in the carcinogenicity study. Finally, the toxicokinetic data in the laboratory species being studied may be so different from that in man, that only the results from the toxicology studies must be used to make the decision on whether or not to proceed to man.

4) Toxicokinetics: views and opinions

There are some specific recommendations to industry coming from the US FDA on toxicokinetics: (a) you must have a specific, sensitive assay, (b) you must develop comparative metabolic profiles for animals and man early in your program, (c) you need to evaluate protein binding, (d) you need to identify metabolites, (e) you must have information available on tissue accumulation of the drug, (f) you must attempt to correlate plasma concentrations to toxicity, and (g) you need to evaluate the utility of extrapolating exposure ratios in animals to exposure levels in man.

The FDA in the USA has challenged the
The pharmaceutical industry to integrate into their drug development programs two additional parameters besides pharmacokinetics and toxicokinetics. These are pharmacodynamics, defined as readily detectable pharmacological endpoints, and toxicodynamics, defined as readily detectable toxicological endpoints.

Further, the FDA has stated that sponsors must justify, on a case-by-case basis, why correlations cannot be obtained for pharmacokinetics/pharmacodynamics and toxicokinetics/toxicodynamics on a given drug.

The purpose of the RK/RD correlations is to aid in developing human dosage regimens, indicate the likely steepness of the dose-response curve, assess the suitability of animal models, and to aid in adjusting individual patients to dosing regimen. To implement these goals one must relate (a) dose administered to plasma concentration, (b) dose administered to pharmacological effect, (c) dose administered to toxicity, (d) plasma concentration to pharmacologic effect, and (e) plasma concentration to toxicity.

Dr. Peck from the FDA recently stated that understanding the pharmacokinetics and pharmacodynamics of a drug should lead to identification of dosing regimens for individual patients that optimize a therapeutic outcome.

The role of kinetics in drug safety evaluation was the topic of a meeting held in May of 1993 in Rockville, Maryland. A number of topics were discussed at this workshop:

1. what parameters to use
2. use of toxicokinetics in selection of route of drug administration
3. the ideal toxicokinetic study design, and several topics on interpretation of data.

These latter topics included how to deal with non-linear kinetic data, how to respond to changes in exposure with repeated dosing, how to deal with exposures at "no effect doses" that are below the human dose, and the need for toxicokinetics in reproduction studies.

From the workshop a number of issues and opinions appeared to be emerging. It was generally agreed the most important toxicokinetic parameters in order of significance were AUC, Cmax, Cmin, and T1/2. It was clear from the discussion that there is no ideal design for a toxicokinetic program. The toxicokinetic program must be flexible and dependent upon the results being generated. Further, some laboratories in the USA are using population toxicokinetics in early toxicity studies to reduce costs, resources, and use of animals. These laboratories only do a detailed toxicokinetic program when they are sure the drug will be moved through development. Lastly, the selection of the high dose to be used in a planned toxicity study is based upon results obtained in earlier toxicity studies in that species and is not based on toxicokinetic data.

Two important points on this slide are the first point at the top of the slide and the last point. The toxicologists attending this meeting clearly indicated they would not change dose during a toxicity study even though the toxicokinetic data suggested plasma concentrations in the animals were changing with continued dosing. The idea was totally impractical. The point on the bottom of the slide that I would like to emphasize is as follows. If the exposure to the laboratory species is low at the "no effect dose", there clearly is a need to be conservative in conducting the clinical dose range finding studies in man because blood level data does not always correlate with toxicity.

Based upon requests for additional information that we receive, it would appear, from our perspective, that the primary focus is on preclinical ADME studies (especially tissue distribution) while pharmacokinetics is a secondary focus. This, however, may only be a perception. In contrast, in North America and Europe the primary focus is on preclinical pharmacokinetic studies, toxicokinetic studies, and the "M" in ADME and only secondarily on ADE.

In our opinion, no one in any geographic area has adequately addressed the issue of appropriate toxicokinetics for biotechnology-derived drugs.

5) Conclusion

In concluding this presentation, I would like to re-emphasize some of the points made earlier.

a) Toxicokinetics are now a part of virtually all toxicology programs and studies.
b) To make this possible requires that early in the study of a new drug it must be determined whether only parent compound
or parent compound and a major metabolite are to be assayed. 
c) This must be followed by development and validation of the appropriate plasma assay procedures. 
d) Most toxicokinetic studies use plasma concentration data supported by relevant ADME data. 
e) It is clear that toxicokinetic data can frequently be useful in interpreting toxicity findings and in planning future studies, including clinical programs. 

f) However, we need to recognize that there are limitations on the significance of toxicokinetic data because plasma levels do not always correlate with toxicity 
g) Finally, the toxicokinetic program for a drug must be developed on a case-by-case basis and in a step-wise manner. The unique differences between individual compounds in pharmacologic activity, metabolism, and protein binding indicate that this must be the approach.

9. Panel Discussion on “Significance and Controversial of Toxicokinetics”

chaired by Ryuichi Kato and Toshiji Igarashi

This panel discussion provided a good opportunity to directly listen to opinions of regulatory authorities of CE and the USA, because EWG members for ICH Topics of Toxicokinetics who attended the Tokyo Meeting (July 26 and 27, 1993) were invited to this Symposium. This paper will take up only comments and discussion to help an understanding of the ICH Guidance under discussion.

1) The main focus of toxicokinetics

Dr. Shoji Awazu (Tokyo College of Pharmacy) made a comment on the ICH draft for toxicokinetics. The ICH draft under discussion clearly defines toxicokinetics as “assessing systemic exposure”, as indicated by its subtitle. He was all for this idea because: 1) toxicokinetics should primarily focus on the interpretation of toxicity tests and not on characterizing the basic pharmacokinetic parameters of test compounds, and 2) a part of the purpose of basic pharmacokinetics is to support toxicity studies by providing sophisticated data of Absorption, Distribution, Metabolism and Excretion for evaluation and interpretation of data obtained in toxicity studies. From the view-point of efficacy and limitations of available technology, there is usually no need to study all of the ADME in toxicokinetics.

2) Toxicodynamics supporting evidence of exposure

It is generally understood that pharmacodynamic or toxicodynamic effects might also give supporting evidence of exposure or even replace pharmacokinetic parameters in some circumstances. Dr. Alan Taylor (FDA) commented on toxicokinetics in reproductive toxicity studies. The FDA believes that the primary indicator of exposure in reproductive toxicity is usually evidence of maternal toxicity. The presence of minimum toxicity in the dams indicates that the study was performed at a maximum tolerated dose. Therefore, while the collection of pharmacokinetic data in reproductive toxicity studies may be valuable in some instances, especially for compounds with low toxicity, or when calculations of potential “safety margins” is desirable, such data is not generally required for all studies.

3) Toxicokinetics for intravenous drugs

Dr. Mitsuji Tateishi (Nihon Unipjohn) raised a question as to whether any toxicokinetic studies should be considered for a test compound intended use by intravenous administration. Dr. David Case said that, to support the clinical i.v. route, there would have to be some intravenous toxicology and therefore presumably some i.v.
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toxicokinetics. On the other hand, Dr. Thomas Marriott plainly denied the necessity of toxicokinetics for such kinds of drugs by intravenous use, because we understand toxicokinetics as assessing systemic exposure, provided that the pharmacokinetic profile of the test compound after intravenous injection is well studied in basic pharmacokinetic studies. As a practical issue, Dr. Kuo Horii introduced their strategy in Roche. Not only for drugs administered by intravenous use but those by oral or other route use, assessing plasma levels after intravenous injection is a basic process of toxicokinetic studies for all kind of drugs. This is because 1) those data are very useful, for example, to estimate the relative bioavailability by oral administration, and 2) the in-house reason that toxicokinetic studies sometimes precede pharmacokinetic studies.

4) Case-by-case application of toxicokinetics

Dr. Hiroshi Mayahara presented his personal understanding concerning in which circumstances toxicokinetic studies may not be needed or should be intensively conducted. Concerning this “case by case principle”, Dr. Alan Taylor also commented as follows: Appropriate application of toxicokinetics should enhance the value of the animal toxicity data by allowing better design and interpretation of studies, resulting in more meaningful extrapolations to humans. At the same time, it may not be necessary for toxicokinetic data to be collected in all toxicity studies or for all pharmacokinetic parameters to be determined in all toxicokinetic studies. Scientific judgment parameters are the most important factors to assess.

5) Application of GLP

Toxicity studies are conducted world-wide under GLP. A statement of the Commission of EC (III/3824/92) recommends that not only toxicity studies but also pharmacological and pharmacokinetic studies closely related to safety studies should be conducted under GLP. FDA is now considering GLP application to all processes for toxicokinetic studies, e.g. including chemical analysis of a test compound in blood and tissue. This issue has great impact on Japanese pharmaceutical companies as shown in the presentation of Dr. Hideo Hakusui. A question concerning this issue was raised from the floor to European and USA regulatory authorities.

The FDA comment was made by Dr. Joseph DeGeorge as follows:

As has been the traditional practice at the FDA, any data derived from a GLP toxicity study should also conform to GLP regulations. As such, concomitant toxicokinetics conducted as part of GLP toxicity studies should be GLP. Pharmacokinetic data from non-GLP studies may also be acceptable for review.

The EC comment was given by Dr. Richard Lee (Medical Control Agency, England) as follows:

The objective of GLP is to reassure the regulatory authorities that the most important safety studies using animal models have been conducted to the high standards required. In order that these most important or “pivotal” studies can be as meaningful as possible, they must contain information which enables an interpretation of the findings to predict the significance of those findings for man.

Part of the information is a knowledge obtained concomitantly (i.e. from the same animals as studied toxicologically or satellite groups treated identically) or incidentally (i.e. in animals that are treated in a way that sufficiently mimics the conditions of the pivotal study to allow valid extrapolation of results) of the systemic exposure profile which pertained during the testing, now called toxicokinetics.

Since all other aspects of the safety testing—clinical chemistry, histology, pathology etc.—are all subject to GLP monitoring, it makes sense to him (and to his EC colleagues) that the kinetic data which is an essential part of the toxicity study—even if generated separately—should also benefit from GLP status.

This leaves the regulatory authorities with the possible sanction of downgrading the value of any study not done to GLP. If pivotal data lacks GLP status, then the whole application may be put in jeopardy, depending upon what assurance as to the quality of the data can be offered instead.

One difficulty for the drug developer seems to be not knowing in advance which particular toxicity studies with animals will be the pivotal ones and from this, which toxicokinetic data will...
benefit most from GLP status. This problem may be alleviated if ancillary studies can be done to cover the whole range of doses (exposures) used in the toxicity testing, including the studies of carcinogenic potential.

It is not for him, as a regulator, to propose strategy, but the acceptance by regulatory authorities of ancillary study data for each species used should ease the burden of resources needed to generate GLP-standard toxicokinetics.

What Dr. Lee stressed is that all parts of the toxicokinetics study need to be under GLP conditions, i.e. collection of blood samples, processing and storage of blood (plasma) samples and assay of the samples for the parent compound, metabolites or markers such as radio-label for concomitant studies. Ancillary studies should be entirely done to GLP standards.

10. Closing Remarks

presented by Toshiji Igarashi

I would like to emphasis the following three points:

(1) Toxicokinetics to rationally conduct toxicity studies: As suggested by Dr. Kosei Noda, Dr. Fumio Sagami, Dr. Ikue Horii and Dr. Mitchell Cayen, I find the most significance of toxicokinetics in the preliminary stage to GLP toxicity studies, namely to get a lot of scientific data needed to conduct good toxicity studies. Those data are needed for the toxicologists themselves but not much for regulatory authorities. I am anxious to have the understanding of the authorities about this. Suppose a case in which it has been elucidated by a toxicokinetic approach at the end of a long-term study that the exposure to a test drug was probably not sufficient because of the inadequate administration of the drug. What can we do other than restarting the study? The loss of great amount of resources is not only for the project team.

(2) Case-by-case principle specific to toxicokinetics: The words “case by case” repeatedly appear in the draft guidance of toxicokinetics as they do in every guideline. Most cases of “case by case” which we encounter in toxicity studies are due to compound-specific differences in bioactivity and physico-chemical properties. As mentioned by Dr. Kosei Noda, toxicokinetics consists of duplicated reactions, namely the reaction of a living body to chemicals (toxicity) and reactions of chemicals to living body (pharmacokinetics). Therefore, it may be rather a matter of course that the compound-specific difference is especially striking in toxicokinetic studies. For example, if the bioavailability is 0.5% in a test compound (this occurs not seldom in animal studies) but 50% in another compound, there is a 100 times difference. As an other example, if the protein binding is 60% in a test compound, while 99.6% in another compound, the difference in free fraction amounts to 100 times. These great variations occur very often in toxicokinetic studies, depending on the pharmacokinetic characters of test compounds (from compound to compound). Compared with these great variations, a possible difference of 2 or 3 fold between male and female animals or between strains of wistar and Fisher seems negligible, whether they are statistically rats significant or not. The case-by-case principle is very specific in toxicokinetic studies.

(3) Harmonization in understanding of guidelines: As Dr. Yasumoto Kikuchi (Takeda Chemical Industry) comments during the panel discussion, there is a distinct disparity in understanding of guidelines among Japan, Europe and the USA.

As presented by Dr. David Case, we will have a common guidance on toxicokinetics in the near future, as a fruit of ICH activities. However, are we Japanese ready to understand the guidance and put it into practice in a common way as our colleagues in Europe and in the USA have done? Another effort to achieve harmonization in understanding of guidelines is being asked of both regulators and industries.