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NONCLINICAL KINETICS AND METABOLISM STUDIES IN SUPPORT OF THE SAFETY ASSESSMENT OF DRUGS

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Animal studies on absorption, distribution, metabolism and excretion (ADME) play an important role in the discovery and selection of drugs as well as in supporting pharmacological studies. The majority of ADME studies, however, are performed to support toxicity studies and, ultimately, safety assessment. In reaching this goal, nonclinical kinetics and metabolism studies form the pier for the bridge between toxicokinetics and toxicity studies; the combination of ADME data with human pharmacokinetics is essential for a drug-exposure based safety evaluation. Lists of ADME studies to be performed in the various stages of drug development are presented and discussed; they comprise standard in-vivo and in-vitro studies as well as some optional studies. ADME studies, however, should always be tailor-made, that is, designed on a case-by-case basis; in line with a global strategy; and anticipating toxicological, pharmacological, and pharmacokinetic findings by a flexible step-by-step approach. The continuous interaction and combined efforts of a pharmaceutical company's departments of toxicology and pharmacokinetics, including a toxicokinetics group, will result in a faster registration of new chemical entities.

Key Words: ADME; Animal kinetics; Toxicokinetics; Safety evaluation; Study design

"All things are poisons, for there is no thing without poisonous qualities, it is only the CONCENTRATION which makes a thing a poison."

after Paracelsus (16th century)

INTRODUCTION

FOR DRUGS DEVELOPED for human use, the study of the kinetics and metabolism in experimental animals has three main objectives:

1. To aid in the discovery and selection of drugs,
2. To support pharmacological studies, and
3. To support toxicity studies and, ultimately, safety assessment.

Recently, kinetics and metabolism studies of new chemical entities (NCEs) were integrated into the early phase of the drug discovery process, in order to optimize pharmacokinetics and metabolic pathways in parallel with the optimation of the phar-

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macological activity, and they are also used in the late discovery stage for the selection of the best overall compound from several pharmacologically similar candidates. Although some attention will be paid to this important new objective of pharmacokinetic studies, the present paper is mainly focused on the role of absorption, distribution, metabolism, and excretion studies in the safety assessment of drugs.

TOXICITY STUDIES AND TOXICOKINETICS

The ultimate goal of regulatory toxicity studies is the safety assessment of drugs by establishing the safety margin between the human therapeutic dose and its toxic levels (1). As a consequence, the four major objectives of toxicity studies are:

1. The determination of the highest no toxic effect level,
2. The characterization of the toxicity profile,
3. The identification of target organs or systems involved in toxicity, and
4. The investigation of the relationship between toxicity and dose level, that is, the determination of a dose response with respect to toxic effects.

Until recently, toxicologists have calculated safety margins as the ratio between the highest no observed toxic effect *dose* level in animals over the therapeutic *dose* in man. It is clear, however, that a safety margin based on dose levels alone may only have some value for well absorbed, nonmetabolized drugs which do not exhibit dose-dependent kinetics (2); in other cases, it does not account for the actual exposure of the toxicant in the toxicity studies nor for species differences in pharmacokinetics. Therefore, the importance of toxicokinetics was recognized gradually. Toxicokinetics is defined as the generation of pharmacokinetic data as an integral component in the conduct of nonclinical toxicity studies and the use of these data in the interpretation of toxicological findings and their relevance to clinical safety issues (3). The primary objectives of toxicokinetics are:

1. To measure exposure in nonclinical toxicity studies and compare it with exposure in man,
2. To search for possible changes in exposure during toxicity studies (eg, rate and duration of exposure),
3. To contribute to the design of toxicity studies (eg, dosing regimen), and
4. To contribute to the interpretation of toxicological findings.

Appropriate toxicokinetic data allow the determination of a safety margin as the ratio of the no observed toxic effect *exposure* level in animals over the human therapeutic exposure level. In order to measure the *exposure* in toxicity studies, the toxic substance, the toxicant, should be identified; it may be the parent drug and/or one or more metabolites. Further, it should be established whether exposure should be measured only in plasma or also in some target tissue(s). The exposure is best characterized by two parameters: the area under the concentration-time curve (AUC), since it reflects the total exposure within the dosing interval and is directly related to the absorbed dose and inversely related to the clearance of the drug, and C_{max}, the maximum concentration, because peak levels are most likely more closely related to toxic effects than trough concentrations. Obviously, toxicokinetics needs to interact with ADME studies, the processes of which it reflects, and with clinical pharmacokinetic studies. Due to its integration into toxicity testing and its bridging character between nonclinical and clinical studies, however, the focus of toxicokinetics is primarily on the interpretation of toxicity studies and not on the characterization of the basic pharmacokinetics (ADME) of the drug. The absorption, distribution, metabolism, and excretion have

to be investigated in a separate set of studies.

ADME STUDIES: A GENERAL OVERVIEW

It is clear that ADME studies are the basis of toxicokinetics and play an important role in toxicity studies and in the safety assessment of drugs (Table 1). ADME studies try to elucidate the fate of the drug in the body, which responds to drug-related concentrations at which it is exposed, with pharmacodynamic and/or toxic effects as a result. The aspects of drug pharmacokinetics which are particularly important for the design and interpretation of toxicity studies are presented in Table 2. The studies should be performed in the same animal substrains as used in the toxicity studies, for example, specific pathogen-free (SPF) Wistar rats (not necessarily, however, in SPF condition).

Absorption and plasma kinetic studies in animal species begin with the determination of the bioavailability of the drug and metabolites and the duration of exposure within a dosing interval (τ), which is related to the half-lives of absorption and elimination. In contributing to exposure, rate as well as extent of absorption are important (1,4). Further, it is also important to see whether the absorption or metabolic processes are saturated at higher doses (5,6), resulting in dose-dependent absorption or elimination kinetics, respectively. Distribution studies in animals can help in the interpretation of toxicological findings by investigating the tissue distribution profile of the radioactivity, the parent drug, and, if possible, major metabolites. Plasma protein and tissue binding is a major factor affecting the distribution of drugs (4), and plasma concentrations of unbound drug are thought to be most relevant in considering tissue concentrations. Therefore, plasma protein binding, and, if possible, tissue binding, should be studied over a broad concentration range, since binding can also become saturated, which sometimes results in a dose-dependent accumulation or retention of the drug or metabolites in some tissues. Figure 1 presents the concentration-dependent binding of the active S-(-)-enantiomer of an NCE to erythrocytes in humans and rabbits. In these two species, drug concentrations of the active enantiomer in whole blood, in contrast with those of the inactive enantiomer, are 20–60 times higher than in plasma, indicating a high-affinity binding

TABLE 1
Objectives of ADME Studies in Support of Toxicity Studies

- To design and interpret toxicity studies
- To make comparisons between animals and humans
  - —selection of animal species in tox studies,
  - —extrapolation of toxicity data from animals to humans
- To investigate
  - —which compound should be monitored in toxicokinetics (parent drug and/or one or more metabolites)
  - —where (plasma and/or target tissues) to monitor
  - —and when to monitor

TABLE 2
Which ADME Studies in Support of Toxicity Studies?

1. Absorption and plasma kinetics:
   - bioavailability (F)—rate
     —extent
   - duration of exposure: $t_{1/2} \leftrightarrow \tau$
   - dose-dependent absorption?
   - dose-dependent kinetics?
2. Distribution:
   - distribution profile of radioactivity, unchanged drug, metabolite in organs and tissues
   - plasma protein and tissue binding
   - dose-dependent accumulation, retention?
   - specific binding characteristics
3. Elimination:
   - profiles and species differences in metabolism and excretion
   - saturation of metabolic pathways
   - auto-induction or -inhibition

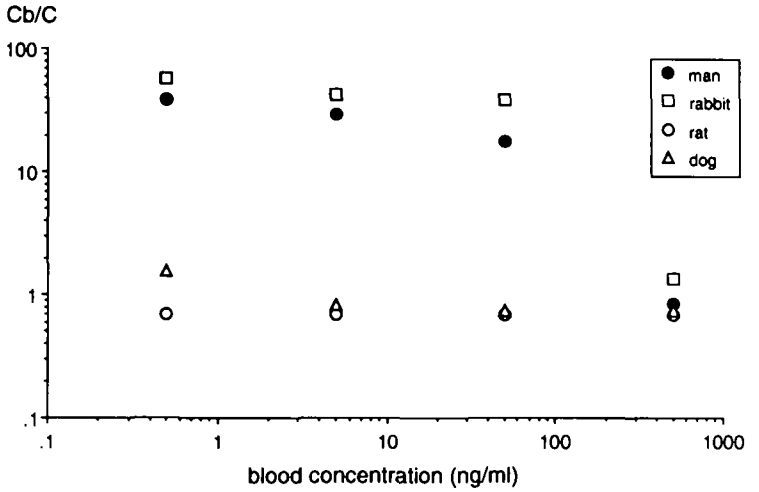

FIGURE 1. Species differences and concentration dependence in blood distribution. Blood to plasma concentration ratio (Cb/C) of the S-(-)-enantiomer of a new drug as a function of the blood concentration in rats, dogs, rabbits, and humans.

in the erythrocytes. At high blood concentrations, these specific binding sites become saturated, and the blood to plasma concentration ratio (C_b/C) decreases to about one, the ratio which is also observed in rats and dogs, where such specific binding sites apparently are not present. Further, in some cases, specific binding characteristics should be studied, for example, binding to melanin-containing tissues (7).

The elimination processes, consisting of biotransformation and excretion, should be studied in the animal species in order to compare with man and to allow a valid extrapolation. Characterization of metabolic pathways and qualitative and/or quantitative measurements of major metabolites in relevant biological fluids and tissues are especially important in discovering interspecies differences. Moreover, identification and pharmacologic characterization of individual metabolites is key in comparing results of nonclinical studies with those of human studies (8). Saturation of metabolic pathways is observed more rapidly when high dose levels are administered by gavage, whereas the same doses in the diet may not give dose-dependent kinetics, because saturation of Phase 1 as well as Phase 2 pathways is concentration-related rather than dose-related. Processes of auto-induction or -inhibition can sometimes be seen, especially in rats. In this case, mechanistic studies should be set up to identify which enzymes are involved in the induction or inhibition process, and which substance, the parent drug or a metabolite, is responsible for the observed findings. In-vitro studies using hepatocytes in primary cell culture may often help in the elucidation of these mechanisms.

ADME STUDIES IN DRUG DEVELOPMENT

In drug development, four phases may be discerned (Figure 2). Tables 3, 4, and 5 present lists of ADME studies in support of toxicity studies to be performed in Prephase 1, Phase 1, and Phases 2–3, respectively. The studies presented refer to a drug for chronic oral administration. For drugs intended for use by administration routes and/or short duration of treatment, these lists of ADME studies should be adapted accordingly.

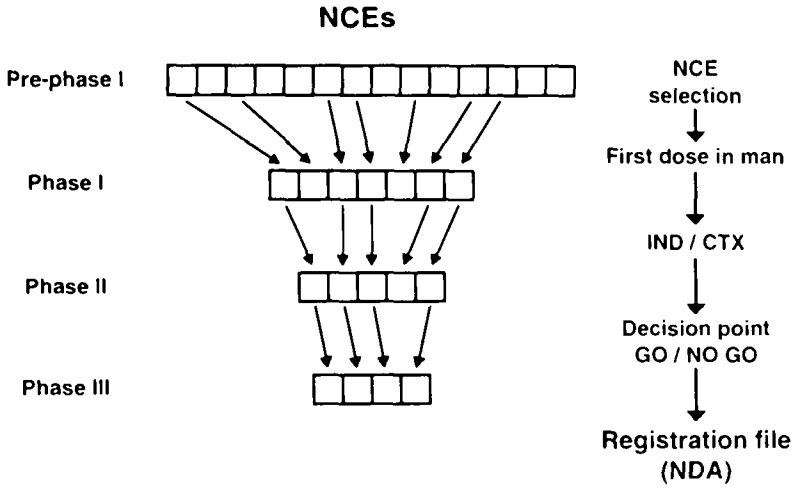

FIGURE 2. Phases and milestones in the development of drugs.

Prephase 1

Prephase 1 development starts from the moment one or more NCEs for the same indication are selected for development. As mentioned above, preclinical pharmacokinetics can already play a role in the early stage of the drug discovery process. In Prephase 1, only a few ADME studies should be performed: studies necessary to get Ethical Committee approval, and studies which can contribute to the drug selection process (Table 3). An extremely valuable study is a comparative in-vitro metabolism study using rat, dog, and human liver microsomes or, if possible, hepatocytes, in order to study the metabolite profile, major pathways, and the key cytochrome-P450 forms involved in the metabolism. From this study, (some) major metabolites can be identified by spectroscopic techniques (mass spectrometry, NMR), and the pharmacological activity of the purified metabolites can be tested in vitro, for example, in receptor binding experiments. If active metabolites are found, it must be decided whether they are valuable candidates for development and whether the NCE selection should be revised. The characterization of the key enzyme(s) involved in the metabolism of the drug in man can reveal a possible genetic polymorphism, for example, the debrisoquine-type oxidative polymorphism by cytochrome P450IID6, and then phenotyped subjects can be selected for Phase 1 clini-

TABLE 3
Prephase 1 ADME Studies in Support of Toxicity Studies

(Assay methods: HPLC and eg, MS).
1. *In-vitro* metabolism—rat, dog, man
   —metabolite profile
   —major pathways
   —key enzymes
2. Single-dose absorption—rat, dog, solution
3. Toxicokinetics of one-month tox studies, solution (eg, HP-β-CD):
   —rats: at autopsy
   —dogs: C-t profile last dose

Evaluation
   —Species differences in absorption and metabolism?
   —Major (active?) metabolite present in plasma?
   —Dose-linear kinetics?

→*Dose selection for first dose in volunteers*
→*Ethical Committee approval*

MS: mass spectrometry; HP-β-CD: hydroxypropyl-β-cyclodextrin

cal studies. It can also provide indications of possible drug-drug interactions.

Further studies in Prephase 1 are single-dose absorption studies in rats and dogs. In these studies, the drug should always be given in a solution in order to have a maximum absorption. Therefore, drug salts and solvents (eg, polyethylene glycol, whether or not mixed with water) can be used. Solubility enhancers such as cyclodextrins might be used for even the most lipophilic substances; 2-hydroxypropyl-β-cyclodextrin appears to be the most suitable entrapping agent available at this moment, due to its high water solubility, its very low oral bioavailability, and its safety (9). The one-month pilot toxicity studies should also be performed with the drug in solution, by gavage, in order to avoid absorption problems. The presence in plasma of active metabolites revealed in in-vitro metabolism studies should be established in the Prephase 1 studies.

The evaluation of the Prephase 1 studies (Table 3) fosters the selection of a starting dose of the NCE which can be safely administered to human volunteers.

TABLE 4
Phase 1 ADME Studies In Support of Toxicity Studies

(Assay method for UD and major active metabolite(s))
(Synthesis of radiolabeled drug ($^{14}C/^{3}H$) and of metabolites)
1. Pharmacokinetics (IV/PO)—rat, dog
2. F_{rel} capsule vs solution—dog
3. WBA—rat (male and pregnant)
4. Pilot ADME—rat (male or female)
5. AME—pregnant rabbit
6. Pilot PPB and blood distribution—rat, mouse, dog, rabbit, man (*in vitro*)
7. Toxicokinetics of three-month tox studies
   —rat (solution): + induction/inhibition at autopsy
   —dog (caps.): + distribution at autopsy
8. Toxicokinetics of 14-day IV tox studies—rat, dog
9. Toxicokinetics of segment 2 studies—rat, rabbit

Evaluation
- Plasma levels at pharmacological and toxic doses?
- Identification of target tissue(s)?
- Dose linearity/dependence?
- Induction/inhibition?

→ *Dose selection for further tox studies based on exposure*

Unchanged drug (UD), intravenous (IV), oral (PO), relative bioavailability (F_{rel}), whole-body autoradiography (WBA), absorption (A), distribution (D), metabolism (M), excretion (E), plasma protein binding (PPB).

Phase 1

Based on findings in the Prephase 1 studies, an assay method—usually high performance liquid chromatography (HPLC)—to measure unchanged drug and, if necessary, a major active metabolite in plasma and tissues, is further developed and validated, and the synthesis of radiolabeled drug and of a number of metabolites is performed. In the meantime, studies on the basic pharmacokinetics after intravenous administration in rats and dogs and on the absolute bioavailability can be initiated (Table 4). In the dog, the bioavailability of a capsule formulation, intended to be given in the three-month toxicity study, is compared with that of a solution. If the relative bioavailability of the capsule formulation is much lower, a better formulation should be developed.

As soon as the radiolabeled substance is available, and after its radiochemical and metabolic stability has been proved, the tissue distribution is studied by whole-body autoradiography (WBA) in male rats as well as in female pregnant rats, only at a few time points, but allowing a judgment to be made as to whether the drug shows any unusual distribution properties or whether there is an important retention of radioactivity in any particular organ or in the fetuses. Other Phase 1 studies with the radiolabeled drug are a limited ADME study in male rats (an extensive study in male and female rats should be performed later, in case of positive clinical Phase 2 studies) and an ADME study in female pregnant rabbits. Further, a pilot in-vitro

study on the plasma protein binding and distribution in blood is performed in a small number of samples from rats, mice, rabbits, dogs, and man at low and high concentrations, covering the pharmacological and toxicological range. The latter study will allow an interspecies comparison of plasma concentrations of unbound drug, which is the most relevant in considering tissue concentrations.

During the three-month toxicity studies, the enzyme induction and/or inhibition is studied in rats by incubation of a number of enzyme substrates with microsomes prepared from liver parts taken at autopsy, whereas in dogs, the tissue distribution is studied at autopsy. If induction or inhibition of liver enzymes is observed in rats, this will also be investigated in dogs. Toxicokinetic data obtained in 14-day intravenous toxicity studies in rats and dogs pave the way for a single-dose pharmacokinetic study after intravenous administration in volunteers. Toxicokinetic data obtained at the end of the segment 2 reproduction studies in rats and rabbits are supported by single-dose studies in pregnant rats and rabbits. By combination of the results of the toxicity studies with those of the Phase 1 ADME and toxicokinetic studies, doses can be proposed for further toxicity studies, based on exposure-related toxicological findings. In the meantime, the maximum tolerated dose and the basic pharmacokinetics in man will have been determined in the Phase 1 studies in volunteers.

Phases 2–3

After granting investigational new drug/clinical trial exemption (IND/CTX) approval, worldwide Phase 2 clinical trials can be initiated. At that time (see Table 5), the relative bioavailability and the plasma level time profile are determined under steady-state conditions in rats after administration of the drug in the food in comparison with those after administration by gavage. This allows an appropriate selection of the dose levels in the chronic toxicity and carcinogenicity studies, based on exposure. Data obtained in the Phase 1 ADME studies, for example, on plasma protein binding, can also offer valuable information. In early Phase 2, an ME study with the radiolabeled drug is performed in dogs, including an investigation of the metabolite pattern in plasma, and, shortly thereafter, a similar study is performed in a small number of volunteers. Thereafter, an extensive ADME study is performed in male and female rats after single dosing. This study includes the dissection of a large number of organs and tissues at 6–8 time points. A selection of more than 30 tissues is governed by WBA and other distribution data obtained in Phase 1 and the radioactivity, and, in a selection of tissues, the unchanged drug and active metabolites are quantified. This time-consuming study results in a lot of information useful for the interpretation of toxicological findings in rats and especially for the identification of target organs in which the drug or its metabolites show retention. It also investigates possible sex differences.

Further, a more extensive plasma protein binding study is carried out in samples from the various animal species used in toxicological studies, as well as from humans; the possible pH-dependence, binding proteins, and in human samples a number of drug-drug interactions are investigated as well.

Apart from the ADME studies mentioned above, there are a number of studies, especially distribution studies and mechanistic studies, which might not be considered standard in phases 2 or 3 of drug development (Table 5). These studies include a quantitative study of the placental transfer with dissection and determination of unchanged drug, a milk excretion study, regional distribution studies, which might be very useful for the elucidation of pharmacological or toxicological mechanisms of action, and, as already mentioned, a study of melanin binding in pig-

TABLE 5
Phase 2–3 ADME Studies in Support of Toxicity Studies

1. F_{rel} food vs gavage—rat at steady-state
2. AME—dog, solution
3. AME—man, solution
4. Extensive ADME—rat, single (male and female)
5. Extensive PPB and blood distribution—mouse, rat, rabbit, dog, man
6. Specific distribution studies—rat or dog:
   —placental transfer
   —milk excretion
   —regional distribution
   —pigmented animals (melanin binding)
   —retention, covalent binding (repeated)
7. mechanistic studies, eg, bile excretion, enterohepatic circulation, age-related kinetics in rats
8. AME mouse

(6–8: not standard)

9. Toxicokinetics in three-month dose finding—mouse
10. Toxicokinetics in six-month (rat) and 12-month (dog) tox studies
11. Toxicokinetics in carcinogenicity studies—rat, mouse
12. Toxicokinetics in segment 3 study—rat

Evaluation

- Selection of animal species in toxicity studies
- Comparison animals vs humans
- Interpretation of pharmacological data
- Interpretation of toxicokinetics and toxicological findings:
  —safety margin based on exposure
  —identification of target tissue(s)
  —changes in kinetics during tox studies
  —contribution of mechanism of toxicity

Relative bioavailability (F_{rel}), absorption (A), distribution (D), metabolism (M), excretion (E), plasma protein binding (PPB).

mented rats. For a first study of the binding to melanin-containing tissues, WBA is the method of choice, and the results can be useful not only in relation to toxicological findings, but also to estimate organ radiation doses anticipated in tissues, especially in the eyes, of human volunteers (7). In the United Kingdom, this information is needed to support a proposal to the Administration of Radioactive Substances Advisory Committee (ARSAC) (10). Whether the melanin binding is reversible can be studied in a subsequent in-vitro study (11).

When the drug is retained in a particular tissue after a single dose, this can be further evaluated after repeated administration of the radiolabeled drug. An investigation might show, for example, if retention of the radioactivity is due to covalent binding of reactive metabolites. This kind of study also meets the special Japanese requirement for repeated radiolabeled dose pharmacokinetic and metabolic studies (12). Other mechanistic studies can be very useful too, but should not be considered standard, for example, a study of the bile excretion and enterohepatic circulation in rats, which should be performed in the case of a pronounced fecal excretion, and a study on age-related kinetics in rats, which should be performed in the case of substantial differences in plasma kinetics between young adult rats and rats at the end of chronic toxicity and carcinogenicity studies.

The various ADME and toxicokinetic studies provide input for the final evaluation, which is written down in the Nonclinical Expert Report (the so-called Pharmaco-Toxicological Expert Report) required by European Community (EC) countries. This should comprise the validation of the selection of animal species used in toxicological studies, and an interpretation of pharmacological data and of toxicokinetics and toxicological findings. Target tissues should have been identified and changes in kinetics during toxicological studies should have been explained. Most important of all, the combination of ADME data and toxicokinetics with toxicological findings and human pharmacokinetics must result in the calculation of a safety margin based on drug exposure.

The question of which ADME studies are necessary and which are optional or perhaps redundant cannot be answered by a simple list of studies. In general, those studies which substantially contribute to:

1. An understanding of the fate and disposition of a drug in animals and man,
2. The relation of plasma and/or tissue concentrations with pharmacological findings,
3. The interpretation of a safety margin, based on the absorbed dose (13) and exposure levels, and
4. The elucidation of mechanisms of toxicity

should be performed.

It is essential that ADME studies should never be done simply in order to fill in a box on a checklist from the regulatory authorities. On the contrary, they should be tailor-made, which means well-designed, in line with a global strategy, and matched to pharmacological and toxicological findings. ADME studies should be performed based on a flexible step-by-step approach and case-by-case decisions, directed to the main objectives (see Table 1). Therefore, the timing of ADME studies should allow a continuous anticipation of major steps in nonclinical and clinical development. Based on these principles, there are a number of studies which can be looked at as optional, and have to be selected for each specific drug candidate being investigated (4), for example, the biliary excretion study and the extensive tissue distribution study after repeated dosing in rats, as already mentioned. A quantitative placental transfer study in rats or rabbits is clearly not needed if the drug is not embryotoxic or teratogenic and if there is a substantial maternal exposure, and the same holds true, mutatis mutandis, for a milk excretion study. Moreover, these studies could even be looked at as being redundant, since, even if there is a whole battery of negative reproduction studies and if sufficient exposure of fetuses and sucklings has been substantiated, there will always be a warning in the package insert for pregnant and breastfeeding women.

An optimum design in gathering ADME data, with a step-by-step approach and including in-vitro studies, will substantially reduce the number of animals used. In this context, it is clear that toxicokinetic studies provide the best means of obtaining multiple-dose pharmacokinetic data in the toxicity species, avoiding duplication in the use of animals (3). This is also one of many arguments in favor of a toxicokinetics group as a subdivision of the department of (nonclinical) pharmacokinetics.

CONCLUSIONS

Apart from their important role in the discovery and selection of drugs, nonclinical kinetics and metabolism ADME studies may be considered one of the pillars of drug development and registration. They form the pier for the bridge between toxicokinetics and toxicity studies. ADME data, in combination with human pharmacokinetics, are also essential for the link between toxicity studies in animals and

clinical studies, resulting in a safety assessment based on drug exposure. As a consequence, ADME studies should be tailor-made, that is, designed on a case-by-case basis, in line with a global strategy, and matched to pharmacological and toxicological findings. The continuous interaction and combined efforts of a pharmaceutical company's departments of toxicology and pharmacokinetics, including a toxicokinetics group, will considerably speed up the preparation and acceptance of an international registration file (14) or a new drug application.

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