

Toxicokinetics and New Drug Development

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The enzyme is dependent on the presence of Ca^{2+} for its catalytic activity and it can interact with calmodulin.

In purified form, the enzyme has been shown to be a dimer composed of two different types of subunits. The dimer has a total mass of about 110 kDa, while the individual subunits are 55 kDa. In different tissues, the enzyme may contain individual isoforms of calmodulin. A separate species seems to vary in size, according to its source. This may allow the enzyme to dephosphorylate unique substrates in different tissues. Calcineurin has the ability to bind, and to be activated by Ca^{2+} in the absence of calmodulin, an effect mediated by the B subunit, which contains two distinct

binding domains with distinct amino acid sequences. The binding of these domains are in the form of a ring. The B subunit could be functional in the intact cell, however, since it is also found in the cytosol, which binds to the molecule by interaction with regions of the A subunit.

Ca^{2+} -calmodulin can increase the activity of the enzyme by up to 10-fold, which suggests that calcineurin has a dual mode of regulation by Ca^{2+} or, perhaps another way, that the enzyme activity is controlled by calmodulin.

It is suggested that the enzyme activity is controlled by calmodulin. The University of New York at Albany is currently conducting research in this area.

In some tissues (e.g. brain) calcineurin is found in association with the particulate fraction of the cell, suggesting that it may be attached to membranes. This is probably achieved via the B subunit which has been found to contain a myristate substituent near to the N-terminus, and this may serve to anchor the protein to the membrane.

The catalytic function of calcineurin is regulated by calmodulin. The A subunit since this can be proteolytically cleaved by trypsin to yield a fragment which shows full phosphatase activity but no Ca^{2+} sensitivity. In the cell, it is likely that the enzyme is regulated by the prevailing Ca^{2+} concentration.

Calcineurin is a calcium-dependent phosphatase. Calcineurin can be activated by calmodulin with a stoichiometric ratio of 1:1. Calcineurin is activated by increasing the K_{m} of the reaction without changing the V_{max} .

Calcineurin shows a rather narrow substrate specificity in that it is most active against sites phosphorylated by either PK-A or PK-C, but it will also dephosphorylate certain proteins, such as substrates for Ca-dependent Kinases. Since the protein itself is activated by Ca^{2+} , it seems likely that in the

intact cell. It will show preference for sites modified by kinases which are not directly Ca^{2+} -sensitive, and this idea fits well with its reported specificity. The precise regulatory role of calcineurin in any particular tissue has not been established, but its particularly high levels in brain are suggestive of a role in neuronal function. The calcineurin pathway is involved in a number of cellular processes, including the regulation of gene expression, cell cycle progression, and the regulation of the immune response.

This enzyme (MDPP) was first described in liver and heart where it was considered to be involved in the control of glycogen synthase activity. Subsequent studies have revealed that it is widely distributed among various tissues and that it has a very high specificity. It seems to be particularly effective against the Ca^{2+} -sensitive form of the rate-limiting enzyme of cholesterol biosynthesis, hydroxymethylglutaryl-CoA reductase, which may indicate a role in the acute regulation of this pathway in certain tissues.

Preface

Toxicokinetics combines the words *toxicology* and *kinetics* and is the application of pharmacokinetic principles to the investigation of toxicity and other adverse effects of drugs. Toxicokinetics encompasses the kinetics of absorption, distribution, metabolism, and elimination of large doses of drugs in the body and the safety evaluation and assessment of adverse reactions caused by excessive drug dosages. If applied rationally, toxicokinetics can be advantageous in drug development and therapy.

Generally, pharmacological activity and toxicity are associated with the blood and tissue concentrations of drugs, their metabolites, or both. It is well established that drug actions are better correlated with concentrations in blood and tissues than with administered dosages. Furthermore, the advances in analytical chemistry, separation methods, and instrumentation now easily allow the direct quantitation of drugs in the body and in the excreta. This quantitation has increasingly facilitated and encouraged the application of toxicokinetics in safety evaluation and in determining the exposure of animals to drugs. It accounts for the great interest shown by scientists in recent symposia and meetings held to address toxicokinetics and related issues.

This book provides reviews of the state of toxicokinetics and its role in drug development, with emphasis on the mechanisms of toxicity and the relationships between adverse effects and plasma concentrations in both animals and human beings. The chapters are arranged to provide continuity of subject matter. We are grateful to the distinguished scientists who contributed to the book. We are also thankful to the many pharmaceutical companies that provided generous contributions to make meetings of the American Association of Pharmaceutical Scientists and the symposia on this topic so very successful.

The authors hope that *Toxicokinetics and New Drug Development* will be helpful to pharmaceutical scientists engaged in safety assessment and drug development and to graduate students interested in this discipline. The book

contains topics not reviewed adequately elsewhere and topics reviewed in literature not commonly followed by most toxicologists. The principles discussed in the book, although related to drugs, also apply to environmental and agricultural compounds. The book provides a forum for covering experimental and clinical toxicokinetics and their role in drug development.

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Chapter 1

An Overview of Toxicokinetics

Vijay K. Batra and Avraham Yacobi

A few years ago, little pharmacokinetic information was included in a safety evaluation study protocol. Today, as part of the validation of safety studies and proof of exposure of animals to a test compound, many pharmaceutical companies have established either full or partial toxicokinetic programs. The support provided by these programs, however, is variable and depends largely on the drug development philosophy of the company. Nonetheless, strong evidence indicates that toxicokinetics is essential in the safety evaluation of potential drug candidates (1-6).

Toxicokinetics is defined as the application of kinetics of drug absorption, distribution, and elimination to safety evaluation studies in which traditionally large but subtoxic dosages of a test compound are administered to test species (7,8). The need of such application is obviated by the objective of the safety evaluation studies of assuring safety of a potential drug candidate within a specific dose range. Safety evaluation of drugs is costly, is of utmost importance in drug development, and can determine the fate of a potentially valuable product.

Many methods used in drug safety evaluations, including autopsy, histology, and hematology, are empirical. They are based mostly on qualitative observations and sometimes lack a sound scientific rationale (1,9). For instance, the duration of conduct of a safety evaluation study to support the long-term clinical use of a drug is a matter of opinion. In the United Kingdom, a 6-month toxicity study done in two species is considered appropriate; in Canada, an 18-month study may be required; and in the United States, 12-month studies in a rodent and in a large animal are acceptable.

Some of the notable pitfalls of traditional toxicity testing methods (9) are as follows:

Methods are empirical and qualitative.

Data interpretation assumes complete drug absorption.

Assume animals handle high and low doses of the drug similarly.

Dosing conditions are different from those intended to be used clinically.

Species differences are usually ignored.

When an unusual toxicity is observed, the compound is abandoned and further development stopped without attempts to understand the fundamental reasons for the toxicity.

To interpret safety data when pharmacokinetic information is not available, one has to assume complete absorption of the drug. One must also assume that there is pharmacokinetic linearity between the doses being evaluated. These assumptions, unless tested, are groundless, because the kinetics of absorption, distribution, and elimination of large doses as given in safety evaluation studies can be completely different from the kinetics of smaller doses given for therapeutic purposes. For instance, what is the meaning of LD_{50} value of 5,000 mg/kg of a drug given orally when the LD_{50} of the same drug given intravenously is less than 100 mg/kg? Or, how can one claim that the highest tolerated dose of a drug is 5,000 mg/kg per day when the concentrations of the drug in blood for this high dose are essentially the same as those after the administration of the 250 mg/kg per day dose given orally?

One serious disadvantage of traditional safety evaluation testing is that when an unusual toxicity is observed, further development of the compound is generally stopped, with no attempts made to understand the mechanism of its toxicity. In many instances, the toxicity is simply due to selection of a very high dose or to the formation of a toxic metabolite that may be specific to the test species, with no relation to human beings at all. The addition of a sound toxicokinetic program to safety evaluation studies can help overcome some of these shortcomings. Since toxicokinetics deals with quantitative measures of drug dosing and body exposure, it will identify unanticipated toxicity if elicited by excessive and supratoxic dosages. Similarly, it will indicate if a lack of toxicity findings is a result of a lack of drug absorption. Overall, toxicokinetics will strengthen the safety evaluation studies and the interpretation of data, which in turn assures the administration of safe dosages to human beings.

Typical toxicokinetic support activities needed to substantiate safety evaluation data and to strengthen Investigational New Drug/New Drug Applications (IND/NDA) submission packages are as follows:

Mixed function oxidase induction potential
Selection of an optimal drug delivery system
Dose range selection
Short-term toxicity testing
Maximum tolerated dose
Species differences
Carcinogenicity, chronic toxicity, reproduction, fertility, and teratology studies
Age-dependent accumulation of drug/metabolites
Retrospective studies

In this overview, instances describing the usefulness of toxicokinetics are presented.

MIXED FUNCTION OXIDATION INDUCTION/INHIBITION

The potential of drugs to induce or inhibit the hepatic mixed function oxidase (MFO) system can be determined readily in rats using standard methodologies (10-12). Such determinations may be done early during the development of the compound, preferably during the predevelopment phase. The knowledge of potential liver enzyme induction is important for two reasons. First, it alerts the study director of the possible hepatic reaction or toxicity that may result from long-term use of the drug. Second, the information helps in the interpretation of the safety data because induction of a drug can lead to either lower plasma drug concentrations or higher metabolite concentrations that in turn determine the efficacy or toxicity of the drug or metabolite. Early availability of the MFO information can lead the pathologist to closer scrutiny of the liver tissues by electron microscopy. Unless there is a specific reason for including it, the electromicroscopic examination is usually not performed in the toxicology study protocol.

Enzyme induction is usually accompanied by enlargement of the liver and proliferation of the smooth endoplasmic reticulum system. In some cases, enzyme induction is the direct cause of hepatotoxicity. Table 1-1 shows an example of an association between the MFO induction and toxicity of five drugs in rats. Coincidentally, all four compounds that were shown to induce liver enzymes also showed hepatotoxicity. The MFO findings alone should not be used to select or reject a compound, but these data do suggest that in such instances the possibility of liver toxicity should be explored.

The MFO induction potential is usually dose dependent. Significant in-

Table 1-1. Correlation Between Mixed Function Oxidase Induction and Hepatotoxicity

Compound	Class	Induction	Hepatotoxicity
A	Diuretic	Yes	Hepatocellular necrosis
B*	Metabolite	High	Yes
C	Diuretic	No	No
D	Antiasthma	Yes	Preneoplastic nodules, rat
E	Antiasthma	Yes	Proliferation of bile duct
F	Anticancer	No	No

*Metabolite of A.

duction of the liver enzymes may occur at high dosages but not at low dosages. A case in point is an example of compound D, which, when given orally to monkeys at 5 mg/kg twice daily showed no induction, as indicated by the comparison of drug levels in the body on days 30 and 1 (Fig. 1-1). Typically, a predicted drug accumulation was attained. In contrast, at 20 mg/kg, the plasma concentrations on day 30 were significantly lower than

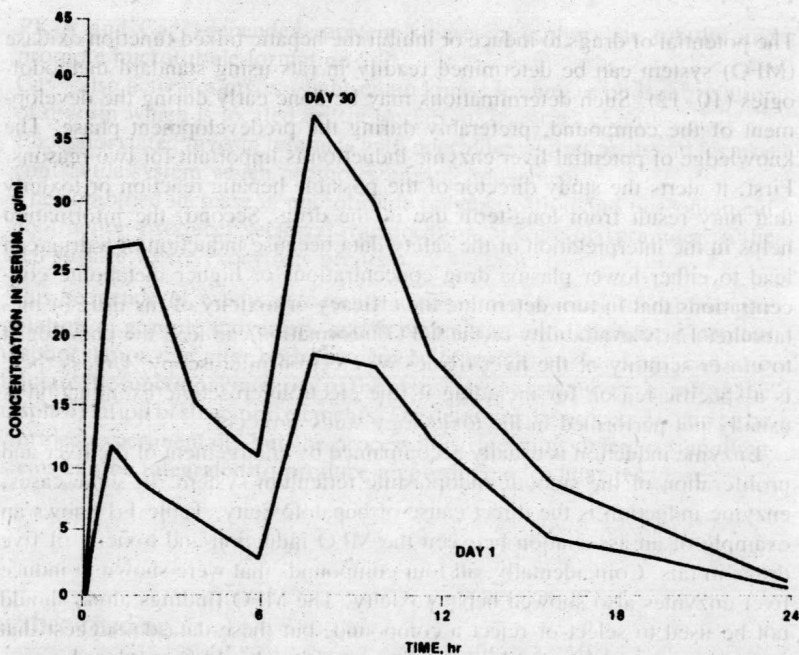


FIGURE 1-1. Serum concentrations of compound D following 5 mg/kg twice-daily administration to monkeys.

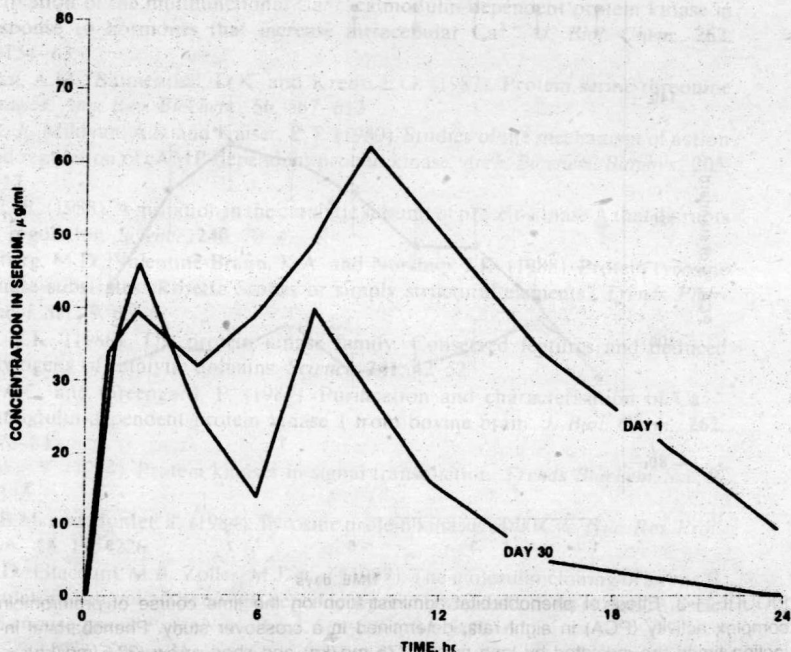


FIGURE 1-2. Serum concentrations of compound D following 20 mg/kg twice-daily administration to monkeys.

those on day 1 (Fig. 1-2). A compound with such behavior may cause more toxicity at lower doses and thus confuse the interpretation of the safety data. Occasionally, a compound might inhibit its own metabolism, thereby resulting in very high concentrations of unchanged drug in the blood, which in turn may lead to toxicity.

Repeated drug administration can influence the toxicity of the drug by changing its metabolism (MFO induction) and also by stimulating synthesis of certain proteins that can have an effect on drug activity. For example, Yacobi and colleagues (13) have shown that phenobarbital can produce a procoagulant effect on its own. The researchers showed that a 75 mg/kg intraperitoneal administration of phenobarbital for four days to Sprague-Dawley rats produces a significant increase of prothrombin complex activity (PCA) in plasma (Fig. 1-3). The rate constant for the decline of PCA after administration of a PCA synthesis-blocking dose of warfarin was not affected by treatment with phenobarbital, but the rate of synthesis of PCA was increased appreciably. It was concluded that phenobarbital increased synthesis of one or more clotting factors.

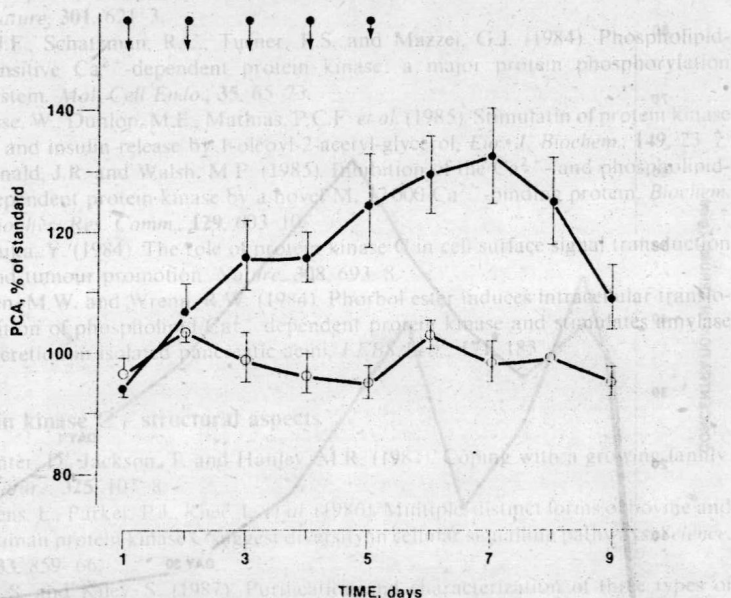


FIGURE 1-3. Effect of phenobarbital administration on the time course of prothrombin complex activity (PCA) in eight rats, determined in a crossover study. Phenobarbital injection times are indicated by long arrows (75 mg/kg) and short arrow (37.5 mg/kg). ○ = data obtained during the control phase (saline injection); ● = data obtained during and after treatment with phenobarbital. Vertical bars show standard error (13).

SELECTION OF AN OPTIMAL DRUG DELIVERY SYSTEM

In the safety evaluation studies, whenever possible the test compound is administered orally in a solution or a starch suspension to large animals, and as a drug-diet admixture to rodents. For soluble drugs, oral administration is acceptable, as exposure in most cases can be documented, but for many insoluble drugs, oral administration in suspension or drug-diet admixtures can lead to very poor drug absorption, thus leading to a lack of body exposure and consequently jeopardizing the entire safety evaluation study. A classical example is the experience with a hypolipidemic compound, CL 277,082, which is presently being tested. It is insoluble in water (<1 µg/ml) but quite soluble in various lipid solvents. In starch suspension it exhibited poor absorption (6–25%) (Table 1-2). Its absorption from various practical drug-delivery vehicles was evaluated, and the results are shown in Table 1-2. The absorption based on total radioactivity concentrations in serum was greater than 50 percent from triolein, oleic acid, and triolein