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# THE ROLE OF METABOLISM STUDIES IN DRUG SAFETY EVALUATION*

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There is a general recognition now of the importance of drug metabolism studies in the process of drug development and the drug regulatory authorities certainly expect to see the results of such studies. However, it is not always appreciated just how closely such studies must be integrated with many other aspects of drug development. In normal practice, metabolism studies are carried out by the metabolism scientist working closely with the pharmacologist , the pharmacist, the toxicologist and the clinician; these interactions can, and should play a key role in determining the strategy of such studies.

Let us first consider the time-scale which applies to metabolism studies in safety evaluation. In Fig. 1, the left hand column shows the major steps in the evaluation of the desirable biological effects of the drug. After identification of promising activity in animal models and completion of satisfactory initial toxicity studies, come the first studies in man, either in normal volunteers or in patients, followed by further studies (phase II) to establish the efficacy of the compound, phase III studies involving wide-scale comparative trials, and finally hopefully the launch of the drug. On the right hand side of the Figure, the drug may first enter a toxicity screen designed

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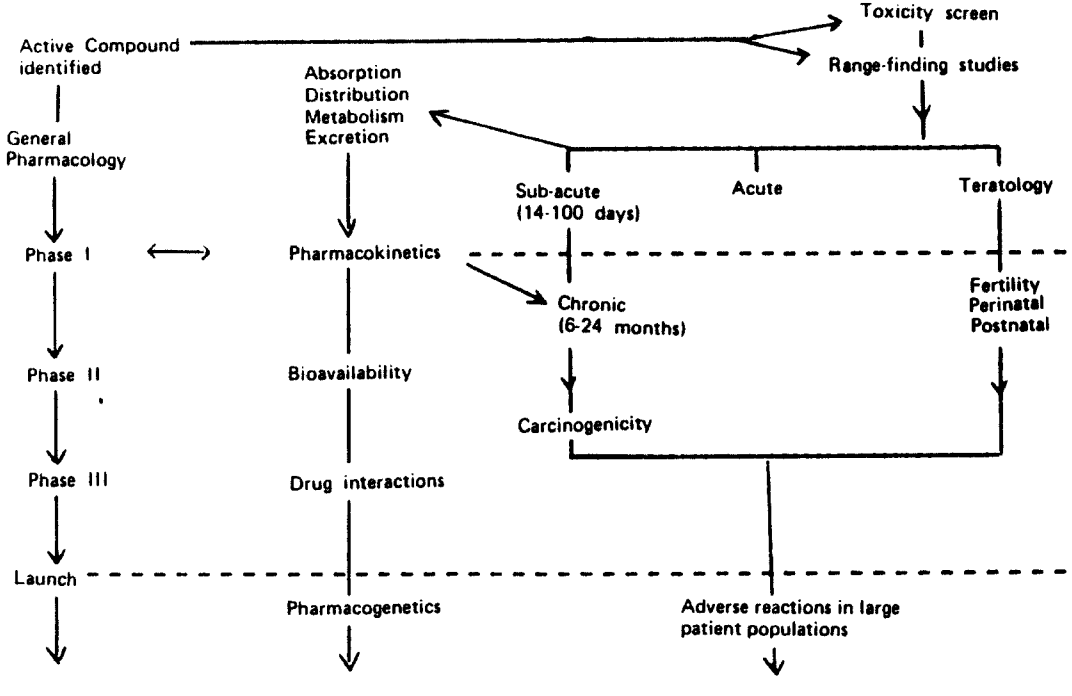


FIGURE I

Integration of Metabolism Studies with the Clinical and Toxicological Stages in a Drug's Development

to detect some specific toxic phenomenon expected from this class of drug or it may go directly into more general range-finding studies. After this the drug will probably be subjected simultaneously to short-term toxicity studies lasting for a period usually between two weeks and three months, and to teratology studies. In the event of a satisfactory result from phase I studies in man, chronic toxicity studies and further reproductive studies in animals will commence. In the centre of the Figure can be seen where drug metabolism studies come in. They may well be involved initially in a pharmacokinetic assessment of whether the primary activity of the compound in animal studies is likely to extend to man. But certainly thereafter they will be intimately involved with the toxicity studies - amongst other things with the absorption characteristics, species differences in metabolic fate, and routes of excretion of the drug. The time-scale of the overall operation is such that toxicologists are not going to wait for all this information before proceeding, but they will expect it to emerge during the course of their studies. Thus the first phase of metabolism studies is confined to a period of only a few months.

It is highly desirable to get pharmacokinetic data from the early studies in man, since these can be critical to the design of further toxicity studies. As with other toxicity studies, teratology and reproductive studies can only really be interpreted fully along with pharmacokinetic and metabolism studies run concurrently. Also, the metabolism scientist will almost certainly be involved in studying bioavailability in order to optimise the formulation being used in man: this may happen at one of several points in the development process and, of course, can carry safety implications with it. Then as the drug proceeds in the clinics the metabolism scientists will be involved in studying drug interactions, and other factors affecting the metabolism of the drug in question. This later phase of metabolism studies may extend over several years.

Thus in drug safety evaluation, metabolism studies are inevitably carried out within certain time constraints. Although obviously there may be occasions when the other disciplines must wait for the results of some metabolism studies, in general practice the toxicologists and the clinicians will be proceeding stepwise, learning from the results of metabolism studies as they go.

There is one practical consideration to mention at this point regarding the timing of studies. Despite the advantages of using radioactively-labelled compounds for metabolism studies, it is not always necessary to prepare a labelled compound prior to commencement of toxicology studies. However, it is almost always valuable to have an assay for the unchanged drug in body fluids available as soon as possible. Indeed, the contribution of pharmocokinetic methods to the evaluation of the toxicity of a drug is determined to a large extent by the quality of the assay methods available at the time they are required. Firstly, the assay must be specific: too many assays do not distinguish between the drug and one or more of its metabolites. Secondly, the assay must be accurate to aid in distinguishing between the various pharmacokinetic alternatives (see later). Thirdly, the assay must be reproducible. Fourthly, the assay must have good sensitivity: this will allow the use of smaller samples, and so perhaps more samples, and allow also the study of the later, slow elimination phases of elimination of drug. Finally, the assay should be as simple and rapid as possible: complex assays give scope for error and can limit the number of samples which can be analysed within a reasonable time.

Let us now consider in more detail safety evaluation studies in animals. Study of the toxicity of a substance at several dose-levels should suggest a dose-respose relationship which may enable us to estimate a "no-effect" or "lowest effect" dose in that species. However, a no-effect dose-level in an animal species will not necessarily provide a direct indication of the

corresponding no-effect dose-level in man or any other species. In some circumstances the "no-effect" dose-level or the "lowest effect" dose-level can be used to determine a therapeutic ratio in that species, and such information can be a useful guide to the likely approximate safety of a compound in man. Another approach to the correlation of dose-levels and effect in different species is to use as a basis for comparison the blood-levels of the substance produced in each species at a given dose-level. There are some very impressive examples of successful corelations of this sort, but it is equally important to recognise that there are also many examples where no apparent correlation can be found by following this approach.

In Table I are listed some of the factors which contribute to species differences in the toxic response to a drug. There is, of course, a clear inter-relationship between some of the factors listed in different groups. Thus, a species possessing a high metabolic rate, or distinctive characteristics in its gastro-intestinal tract or excretory mechanism, will reflect these properties in the manner by which chemical substances administered to the species are handled and ultimately removed from the organism. Species differences in responses to toxic agents may be explicable simply in terms of the known physiology or bio-chemistry relating to the species, or alternatively on the basis of metabolism or pharmacokinetics. Frequently all these factors will need to be considered.

In the Table, metabolism is included under the heading of pharmacokinetics, since knowledge of the metabolic fate of a compound is insufficient without some understanding of its kinetics, i.e. the rate processes by which it is transformed into its various metabolites and by which these substances reach target organs and are excreted from the body.

The headings in Table II provide a useful platform from which to examine the pharmacokinetics of a drug during toxicity studies. Under "absorption" we must consider not only the

TABLE I

Basis of Species Differences in Toxicity

Anatomical/physiological
- morphological
- endocrinological
- reproductive system
- gastrointestinal tract
- excretory mechanisms
- tissue responsiveness to stimuli

Biochemical
- metabolic rate
- enzyme levels and distribution
- nutrition basis
- intermediary metabolism

Pharmacokinetic
- metabolism
- rates of absorption
  distribution
  elimination

amount absorbed, but also the site at which a drug is absorbed and the rate at which it is absorbed. We must also consider how the absorption pattern during a 24 hour period depends on its method of administration – whether the drug is administered by gavage or mixed in the diet. "Distribution" is clearly important because most drugs exert their effects at some point outside the bloodstream. So does the concentration of drug in the blood reflect that in the tissues? Should we be concerned with drug or its metabolites? Under "metabolism" we must acquire basic data regarding species differences in metabolic handling of the drug, many of which can be anticipated to some extent now; then we want to know whether toxic effects are

TABLE II

Pharmacokinetics in Toxicology

Absorption
- amount
- rate
- dietary administration

Distribution
- blood vs tissues
- drug vs metabolites

Metabolism
- species differences
- toxic metabolites

Elimination
- route
- rate
- chronic administration



being mediated via the unchanged drug or via metabolites. "Elimination" is the word used to describe all means whereby the drug is removed from the body: this can be divided into metabolism (and the fate of the metabolites is an additional complication) and excretion of unchanged drug itself. We wish to know the route of elimination, the rate of elimination, and whether elimination rates are affected by chronic administration of drug.

Figure 2 illustrates the nature of the pharmacokinetic problem confronting the metabolism scientist trying to understand species differences in response in early toxicity studies. The data are derived from a mathematical model and show how a plasma concentration vs. time curve (A) can be affected by changing each of the parameters: absorption rate, amount absorbed, elimination rate, and tissue distribution. The magnitude of the changes have been chosen deliberately to demonstrate the

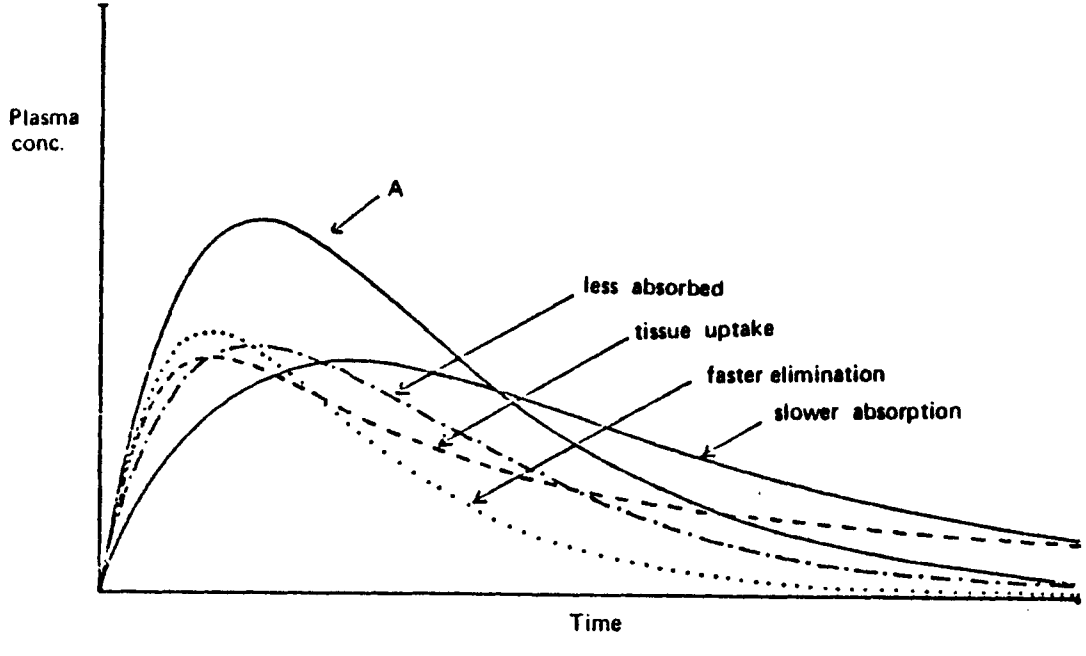


FIGURE II.

Representative Plasma Concentration vs. Time Plots

potential difficulty in distinguishing between these factors. It can be seen that if blood samples are taken at only one or two arbitrarily chosen times after administration of the dose, comparison of the concentrations will not provide an answer to the question of why a drug gives rise to a certain plasma concentration vs. time profile in one species, and a quite different one in another. For example "low" plasma concentrations may not represent poor absorption (which may possibly be improved by reformulation) but instead may reflect an unalterable intrinsic property such as a high elimination rate, or high tissue distribution. An additional handicap to interpretation is that, at an early stage of toxicity evaluation, the assay for the drug in plasma may not be sufficiently well developed to provide the necessary accuracy and sensitivity.

Returning to Table II, let us discuss "absorption". How does one measure absorption of an orally administered drug? The height of the plasma concentration-time curve depends not only upon the rate and amount of absorption, but also upon the distribution and elimination rate of the substance in question. Thus plasma concentrations of a drug are of limited value as a means of determining the amount of drug absorbed. The major and important exception to this is if one can administer the substance in question by intravenous or other parenteral route: then the comparison of the plasma-time profile after oral and parenteral administration can, under certain circumstances give a measure of the amount of drug-absorbed.

Measurements of the substance or its metabolites excreted in the urine are obviously sound evidence for the minimum amount of drug absorbed. Conversely though, failure to excrete drug or metabolite in the urine does not mean that the substance has not been absorbed. This is because elimination via the saliva or bile can lead to excretion of drug and metabolites with the faeces. Again the pattern of excretion of drug and metabolites in urine and faeces after a parenteral dose of the drug can

provide a valuable base-line against which to compare data after oral administration of drug.

One piece of information which should always be available before commencing toxicity studies is the solubility of the drug in simulated gastro-intestinal fluids. There are two points to be made here relating to compounds of low solubility: the first is that some compounds which have exceptionally low solubilities are in fact absorbed to quite a surprising extent. This is because it is not solubility per se which determines absorption, but the rate of dissolution under what are called "sink conditions", that is when the material dissolving is being continuously removed, for example by absorption through the gut wall. The second point about compounds of low solubility is that there can come a stage when, as the dose level is increased, even the rate of dissolution is inadequate, and only a limited amount of compound can dissolve in the gut fluid per unit time. The result is that increasing the dose results in no further increases in absorption. Such a finding can be most illuminating in explaining why dose-response curves are not as expected.

Dietary administration of drugs to rodents produces, as one might expect, a rather different absorption profile from that following gavage administration. Rodents tend to eat most of their food by night and to extend this activity over a considerable period of time. In order to determine what this means in terms of drug absorption we at Pfizer have carried out a number of studies with compounds administered to rodents via the diet; plasma concentrations were determined at intervals throughout the day and night. Not surprisingly we found a broad peak of drug concentration during the hours of darkness, the peak level being perhaps no more than a quarter of that achieved when the whole daily dose was administered as a single gavage dose. The through level during daylight hours depended upon the half-life of the drug, those drugs with half-lives of more than about

three hours maintaining significant concentrations in the plasma throughout the day.

The affinity of drugs and metabolites for tissues is governed to a major extent by their physiochemical properties. As metabolites are usually considerably more polar than the parent drug, the proportions of drug and metabolites which are circulating in the plasma may well not be representative of the proportions which are stored in tissue sites, including target organs for toxic effects.

A striking example of this occurred recently in the Pfizer laboratories during the study of the hepato- and cardiotoxic effects of a very lipophilic drug. No drug was excreted unchanged in the urine; this was predictable for such a lipophilic substance which one would expect to be metabolised extensively at the liver before excretion. Similarly examination of the drug-related substances in the plasma showed that there was only traces of drug present, but a multitude of polar metabolites. At this point the problem was almost abandoned because of the daunting prospect of identifying the metabolites, and then synthesising them and evaluating their toxicity. However, on analysis of the drug-related substances present in the critical organs, namely the heart and liver, more than 90% of the drug-related substances was found to be unchanged drug; it was therefore with some confidence that the toxic effects of this drug could be ascribed to the drug itself and not to a metabolite.

A commonly used method for studying the distribution of a drug and its metabolites is that of whole-body autoradiography after administration of radioactivity labelled drug. The technique is well described in several tests[1,2a]. Obtaining the results can be time consuming (from several days to several months). By varying the plane of section and time after drug administration one can learn much about the kinetics of distribution and of the clearance of the drug and its metabolites from the tissues. However, there are some drawbacks to the technique.

The first is the cost: drug with fairly high specific activity ($^{3}H$, $^{14}C$ or $^{35}S$) is required, and the drug must of course be labelled in an appropriate metabolically-stable site in the molecule. A second drawback is that it is only radioactivity which one detects; additional time-consuming studies may be required to determine whether the radioactivity emanates from the drug or one or more of its metabolites.

Another aspect of the distribution of drugs which has come under scrutiny in the last few years is that in the pregnant animal. Although teratogenic effects can be mediated by several mechanisms and not all of these require the drug to cross the placenta and reach the foetus, it is nevertheless greatly reassuring, when one finds a negative result in a teratology study, to know that the drug has actually reached the foetus. The distribution within the foetus itself can, furthermore, give us an insight into the mechanism of any teratogenic or embryotoxic effects which are observed.

The subject of species differences in metabolic pathways is too vast to discuss here, and the reader is referred to standard texts[2b,3]. Suffice it to say that identification of the metabolites and the extent of their formation in the species under study can be vital for a comparison of the toxic effects of a substance in different species. For instance, if a substance is converted to one major metabolite in one species and to a different major metabolite in a second species, when we try to compare the toxicity of the parent substance in these two species, we may in effect be comparing the toxicity of the respective metabolites and not that of the parent substance. Another way of stating the problem is to ask, when one measures the plasma concentrations of a compound under study, whether one is measuring the right substance. One must also remember when comparing the toxicity of a drug administered by several routes that some metabolic reactions occur only while the drug is being absorbed, whether it be from the gastro-intestinal tract, or from the lung,

or perhaps through the skin. Thus there may be a first-pass metabolism effect unique to a particular route of drug administration.

The discussion of excretion processes will be confined to the kinetic aspects of the elimination as they bear on toxicity studies. Firstly, what is the effect of repeated dosing (as occurs during a toxicity study) upon drug accumulation? Drug accumulation is essentially a function of the dosage regimen, and this relationship can be expressed mathematically[4] (Eq. 1.) It can be seen that the plasma level* in a dosage interval at the steady state will increase if the dose is increased, or if the rate of elimination is reduced, or if the dosage interval is decreased. Conversely, the plasma level can be reduced if the dosage interval is made longer.

$$\bar{C} = \frac{FD}{V\tau K} \text{ or } \frac{FD}{V\tau} \cdot \frac{t_{\frac{1}{2}}}{0.69} \quad \ldots\ldots \text{Eq. 1.}$$

$$\text{"Steady state plasma concentration"} = \frac{\text{fraction absorbed x dose x half-life}}{\text{vol.of distribution x dosing interval x 0.69}}$$

These are trends one would expect from common-sense considerations. It is important to realise that this equation tells us that any drug has the potential to accumulate.

One can use this equation to calculate steady state plasma levels from single dose data, but so many assumptions are necessary for this extrapolation to be valid in practice that it is generally advisable to measure the actual plasma concentrations achieved during the repeated dosing of a toxicity trial. In the Pfizer laboratory this is normally done on the first day of the study, again about 10 - 14 days later, and finally again near the end of the study. The reason for making measurements

* The "steady state plasma level" in this context is that plasma concentration which when multiplied by the dosage interval gives the area measured under the plasma concentration/time graph in one dosage interval at the steady state.

at several points during the study is that repeated administration of a drug can modify a) the drug's absorption by an effect upon gastric motility, intestinal secretion processes or intestinal microflora, and b) the drug's rate of elimination – either by inhibiting or stimulating its own metabolism. This last phenomenon of hepatic microsomal enzyme induction is of widespread occurrence amongst lipophilic drugs; the subject has been well reviewed[2c,5].

Another aspect of the kinetics of drug elimination which has implications for the interpretation of toxicity is the possible dependency of the kinetics upon the dose administered. Various instances of this phenomenon have been reported. The data in Table III demonstrates how the half-life of three drugs varied when administered at two dose-levels to dogs[6]. Although there was a little overlap in some cases, it can be seen that raising the dose by a factor of between 3- to 7-fold resulted in the half-lives of elimination being more than doubled at the higher dose. Reference has already been made to the case where plasma levels of a drug do not increase with dose because absorption is limited by the intrinsic solubility of the compound in the fluids of the gut. Table III shows the opposite situation, where an increase in the half-life of elimination as the dose is raised

TABLE III

Dose Dependent Elimination Kinetics in Dogs

| Drug | I.V.dose (mg/kg) | N | Half-life (hr) Range | Mean |
|---|---|---|---|---|
| Phenylbutazone | 10 | 10 | 2.0–3.3 | 2.8 |
| | 50 | 11 | 3.5–13 | 8.1 |
| Biscoumacetate | 10 | 8 | 9–24 | 13.7 |
| | 75 | 9 | 20–41 | 31.5 |
| Diphenylhydantoin | 20 | 3 | 1.5–2.8 | 2.3 |
| | 50 | 3 | 5.5–7.8 | 6.4 |

leads to higher concentrations of drug in the plasma than would have been predicted. This may happen for a variety of reasons including saturation of first-pass metabolism effect, or saturation of an active transport process involved in excretion or in transport of drug to its site of elimination, etc.

One further aspect of the importance of pharmacokinetic studies for safety evaluation is the effect of pregnancy upon drug kinetics. Clearly pregnancy subjects the maternal metabolism to considerable disturbance which could well be reflected in differences in drug absorption, distribution and kinetics of elimination. The first question to be considered is how to express the dose-level. Should it be referred to total body-weight of the dam, or to the corresponding non-pregnant weight? Maternal liver weight increases during pregnancy, but drug metabolising enzymes concentration drops, so that in general there is not an increase in drug-metabolising capacity. In fact, the activity of a number of the hepatic drug metabolising enzymes is depressed by pregnancy. In the rat these include biphenyl hydroxylase, aniline hydroxylase, nitroanisole reductase, aminopyrine-N-demethylase and UDPGA-glucuronyl transferase[7,8]. In the rabbit, some depression of hepatic enzyme activity has been recorded, although there can apparently be stimulation of the metabolising enzymes of the lung[9]. As yet relatively little work has been done in this area, but undoubtedly it is one which is going to attract increasing attention in the future.

It may be useful to conclude by returning to the opening theme: drug metabolism studies should be geared to answering the most urgent questions arising from the emerging results of toxicity trials. This paper has presented a partial list of the many such questions which may be asked: viz. those relating to anomalous dose-response curves or to species-differences in toxic response which are a consequence of differences in absorption, biotransformation, tissue distribution, or excretion; or of changes in drug kinetics following multiple dosing or in pregnancy. The

limitations of laboratory resources means that it is not usually practicable to tackle all these aspects simultaneously, and the emphasis to be given to each should be determined through discussion with the toxicologist and clinician. Metabolism, and preferably pharmacokinetic, studies must be closely integrated with toxicity studies if one is to obtain the best interpretation of the toxic effects observed and particularly if one wishes to extrapolate from laboratory data to man.

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