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Optimal dose identification: predicting a safe dose in man from animal studies

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Abstract

Adverse drug reactions remain a major clinical problem and impairment to safe drug development. The CPMP provides guidelines for preclinical studies. Safety margins for target-organ toxicity must be calculated. It is necessary to be aware of species differences in pharmacodynamic effects and in routes and rates of metabolism. The relevance of the test system to the intended human use must be defined. It is important to learn lessons from past experience with drugs that have caused serious toxicity in man. Idiosyncratic toxicity is only detected late in drug development. The pathophysiology of such reactions is complex and poorly understood. In man, genetics and disease may be predisposing factors. Therefore, it is not surprising that conventional animal models fail to identify drugs which cause idiosyncratic toxicity. At present, we have to depend on the identification of structural alerts in drugs and metabolites to screen out the potential for toxicity. Recent advances in molecular toxicology allow the investigator to explore biochemical stress, in both in vitro and in vivo models, at doses of drugs and chemicals that do not cause overt toxicity. It is, thus, possible to address physiological, pharmacological and toxicological aspects of adverse reactions. Ultimately, it may be possible to relate cellular changes in these test systems to serious toxicities seen in man (anaphylaxis, hepatotoxicity, blood dyscrasias and severe skin reactions) and, thus, provide more physiologically relevant assays for the prediction of human drug toxicity.

Keywords: Drug toxicity; Preclinical evaluation; Molecular toxicology

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1. Introduction

Adverse drug reactions (ADR) remain a major clinical problem and impairment to safe drug development, despite the extensive preclinical evaluation that is undertaken for a new drug. ADR are common and are a significant cause of patient morbidity and mortality [1,2]. ADR may be classified from a clinical perspective as either type A or type B [3]. Type A—augmented reactions—are predictable from the known primary or secondary pharmacology of the drug and are dose-dependent. Type B—idiosyncratic reactions—are not predictable from knowledge of the basic pharmacology of the drug and show marked individual susceptibility and no simple dose dependency. Such reactions are often serious, and include anaphylaxis, blood dyscrasias, hepatotoxicity and skin reactions. From a chemical perspective, the above classification can be expanded to include type C reactions, which are predictable in terms of the chemistry of the drug, and type D reactions which are delayed effects such as carcinogenicity and teratogenicity, screened for in bioassays [4].

Most serious, but rare, ADR are usually only detected once the drug has been widely used long-term in patient populations after drug licensing. This is despite extensive preclinical evaluation in laboratory animals and extensive evaluation in clinical trials. There are two main reasons for this. First, the animal species may be inappropriate for the study of human drug toxicity. Second, there is marked interindividual variation in the human population to all aspects of drug response [5,6].

The type and extent of preclinical studies that are necessary depend on the biological properties of the drug and intended clinical use. Each drug must be assessed on its own pharmacological and toxicological merits, before its introduction into man (phase I studies) and patients.

2. The preclinical evaluation of a new drug

The CPMP guidelines for preclinical studies require investigations into:

- pharmacodynamics
- secondary (safety) pharmacology
- single-dose toxicology and repeat-dose toxicology
- reproductive toxicology
- mutagenicity and carcinogenicity
- local tolerance and special studies

Such studies are required for the safe introduction of the drug into man for phase I studies and for long-term use in patients. Safety margins for target-organ toxicity must be calculated and false positive and negative findings minimised. It is, therefore, necessary to be aware of species differences in pharmacodynamic effects and in routes and rates of metabolism for the new chemical entity under investigation. The relevance of the test system to the intended human use must be defined. For animal models of toxicity, in vitro bridging studies are of particular use.

3. Species variation in drug response

There is interspecies variation in all aspects of drug action, which can be considered in three broad areas:

- Interspecies differences in absorption, distribution and rates of metabolism
- Interspecies differences in routes of metabolism
- Interspecies differences in pharmacodynamic effects

Interspecies variation in drug metabolism has been well documented over several decades. In the first instance, chemical analysis is required to define the chemical entities responsible for both beneficial and adverse effects of a drug. Further analysis provides the concentration–time course of all chemical entities to allow a full toxicokinetic analysis of relative exposure of tissues in both the test animal and man. Safety margins can then be established. Biochemical analysis will identify the major enzymes responsible for the metabolism of the drug. High throughput screens are available to determine the role of human and animal cytochrome *P*450 enzymes in the oxidation of a new drug. Human liver banks and commercially available human hepatocytes allow a more integrated evaluation of the role of phases I and II metabolism. Transgenic animals, which contain "humanised" cytochrome *P*450 enzymes, may provide more relevant models of drug toxicity.

More recently, attention has been focused on species differences in receptors, ion channels and enzymes [7]. Differences have been documented in terms of level of expression, affinity and responses to agonists and antagonists.

It is, therefore, essential to define the relevance of any test system used in preclinical safety evaluation to the human situation in which the drug is intended for use. This may involve not only species differences in the expression and structure of key proteins which ultimately determine the type, duration and intensity of drug response, but also the need for consideration of special groups such as children and the elderly. In addition, it is important to learn lessons from past experience with drugs that have caused serious toxicity in man, and also those that have caused serious toxicity in test animals. Such paradigms are used in both drug development and drug licensing, during the consideration of the safety of a new chemical entity.

4. Thalidomide and teratogenicity

It is a sobering thought that some 50 years after the thalidomide tragedy [8], the mechanism of thalidomide teratogenesis remains unknown, despite the publication of over 2000 papers on the topic. The toxicity of thalidomide serves not only as a paradigm for preclinical safety evaluation, but is also of direct clinical relevance. The best animal models are the rabbit and the primate, while rodents are relatively resistant to the effects of thalidomide and the day of dosing is critical for a positive effect in the rat [9,10]. There has been an increase in clinical indications for the drug, which was once marketed as a treatment for morning sickness. New clinical indications include leprosy, HIV and cancer. The drug has also been found to be a TNF antagonist.

There has been much speculation on the mechanisms of embryotoxicity. Ashby et al. [11], in a multi-centre study using all available test systems, have shown that thalidomide is not a (human germ cell) mutagen. D'Amato et al. [12] have found that thalidomide can inhibit angiogenesis. Neubert et al. [13] found that thalidomide can down-regulate certain integrins. Stephens et al. [14] have proposed a model whereby thalidomide (or a metabolite) specifically binds to the promoter sites on the genes for insulin-like growth factor 1 and fibroblast growth factor 2, decreasing transcription efficiency of the associated genes. Furthermore, the ultimate teratogen has not been defined, although both oxidative and hydrolytic metabolites have been implicated in the teratogenesis.

Thus, at present, the overall approach to reproductive toxicology must be one of caution in which an empirical approach, based on more than species, is used. A full toxicokinetic profile, that allows adequate exposure of the test foetus to both drug and all known human metabolites, is essential.

5. Fialuridine and hepatotoxicity

Fialuridine was an experimental treatment for hepatitis B. In a phase II clinical study, five out of 15 patients died from liver and kidney failure, and two required a liver transplant [15]. Histological analysis of the liver tissue revealed marked accumulation of microvascular and macrovascular fat, with minimal necrosis of hepatocytes or architectural changes, while electron microscopy showed abnormal mitochondria. Hepatotoxicity is thought to be due to delayed mitochondrial damage. Studies in HepG2 cells suggest that incorporation of fialuridine into mtDNA leads to marked mitochondrial dysfunction as evidenced by disturbances in cellular energy metabolism and detection of micro- and macro-steatosis [16] There was a welter of correspondence in the New England Journal of Medicine concerning the apparent failure of the preclinical safety evaluation of fialuridine during 1995. Although the issue was never fully resolved, one interesting suggestion to emanate from these discussions was that the appropriate animal model might have been the virally infected woodchuck [17].

6. Tamoxifen and hepatocellular carcinogenicity

Breast cancer is a major cause of death from cancer in women, only recently overtaken by lung cancer in U.K. The death rate has fallen in both the UK and USA during the past 10 years, and both tamoxifen and increased surveillance programmes have contributed to this success. A major concern in the development of tamoxifen was the discovery of hepatic tumours in rats in the rodent carcinogenicity bioassay [18]. Tumours were not detected in the mouse. The metabolic pathway responsible for tumourigenesis is thought to involve sequential bioactivation via α-hydroxylation and *O*-sulphonation [19]. The resulting *O*-sulphonate is highly unstable, with a half-life of <1 min, and readily collapses to a carbocation that reacts with DNA [20,21]. The formation of DNA-adducts is thought to be the initial genotoxic step in tumourigenesis. We have investigated the formation and metabolism of α-hydroxytamoxifen in the rat. It is excreted as a stable *O*-glucuronide in

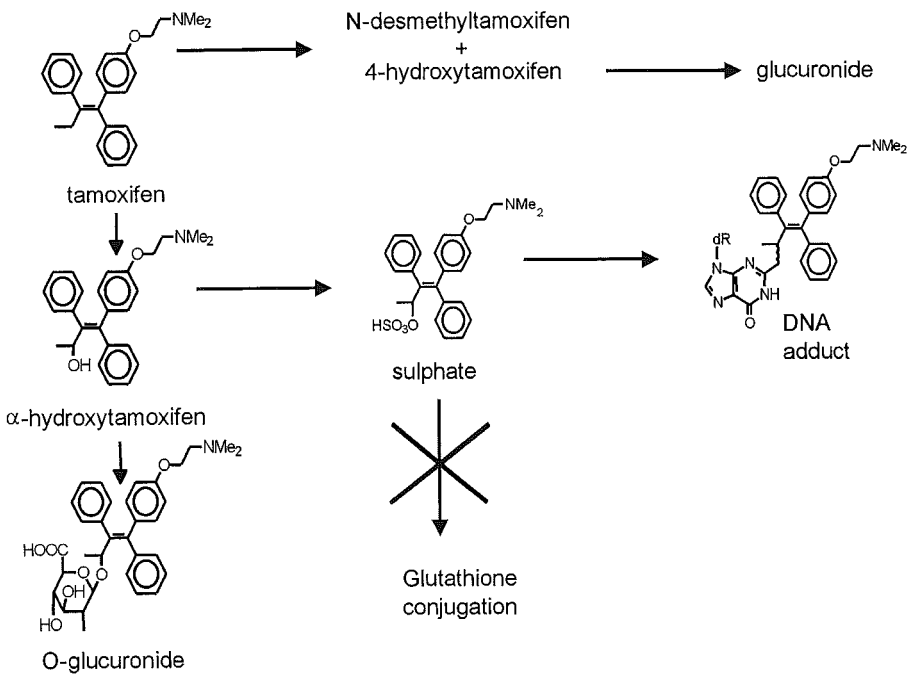

Fig. 1. Tamoxifen: metabolism, bioactivation and formation of DNA adducts in vivo.

bile. Estimates of rates of formation indicate that approximately 5% of the dose of tamoxifen undergoes α-hydroxylation and that the majority of this undergoes *O*-sulphonation. Inspection of the full metabolic profile for tamoxifen in the rat (Fig. 1), shows that the balance between α-hydroxylation, *O*-sulphonation and *O*-glucuronylation will determine the risk of DNA adduct formation. We, therefore, performed in vitro bridging studies with hepatic subcellular fractions as part of human risk assessment [22]. There were marked species differences in the metabolism of α-hydroxytamoxifen (Table 1). Rat hepatic cytosol was more active than human hepatic cytosol with regard to *O*-sulphonation (bioactivation). In contrast the rate of *O*-glucuronidation (bioinactivation) was at least 100-fold greater with human hepatic microsomes than for rat hepatic microsomes. These data indicate a "metabolic" safety margin of 1500. The dose of tamoxifen required to induce tumours in rats (40 mg/kg) is approximately 50-fold greater

Table 1
A metabolic risk assessment of hepatocarcinogenicity

		Safety factor
Hydroxylation of tamoxifen	rats greater than women	× 3
Sulphonation	rats greater than women	× 5
Glucuronidation	women greater than rats	× 100
Dose of tamoxifen	rats 40 mg/kg, women 0.3 mg/kg	× 100
Total		× 150,000

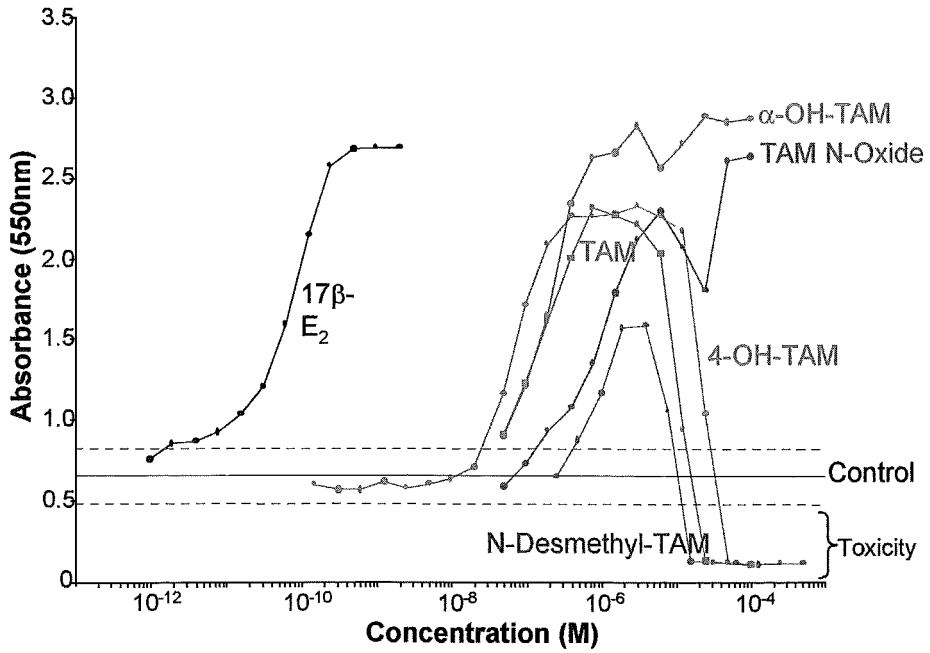

Fig. 2. Oestrogenicity of tamoxifen and its metabolites in relation to estradiol in the transfected yeast oestrogen receptor assay.

than the therapeutic dose (0.3 mg/kg). Overall, these data suggest a safety margin of 150,000 for the risk of tumours, which is consistent with the clinical experience of the drug to date. Similar metabolic considerations provide a chemical rationale for the lack of tumours observed in the mouse.

Of course, one cannot rely on chemical considerations alone in the safety assessment of a potent pharmacological agent such as tamoxifen. In a yeast reporter assay system, we found that α-hydroxytamoxifen behaved as a full agonist, whereas tamoxifen, 4-hydroxytamoxifen and desmethyltamoxifen acted as partial agonists (Fig. 2). Tamoxifen acts as a strong oestrogen antagonist in human breast but as an oestrogen agonist in the uterus [23]. The actions of tamoxifen are mediated through the oestrogen receptors ERα and ERβ, which bind to a variety of responsive elements to activate transcription. Transfection studies have shown that the activities of selective estrogen receptor modulators (SERM), such as tamoxifen, vary dramatically between cell types and promoter constructs [23]. These results suggest that the pharmacological effects of SERM, in vivo, cannot be predicted by their actions on simple elements, such as the estrogen response element (ERE), in isolation.

7. Preclinical evaluation of idiosyncratic drug toxicity

Idiosyncratic toxicity is only detected late in drug development and usually at the post-marketing stage. The pathophysiology of such reactions is complex and poorly

understood, but may involve drug bioactivation and an immune response [4]. In man, these reactions show a high level of host selectivity, indicating a genetic predisposition. Thus, species differences in drug metabolism, immune responsiveness and in the physiological response to chemical stress may all explain the lack of suitable animal models for type B reactions [4]. Furthermore, disease (primary or incidental) may also be a predisposing factor. Therefore, it is not surprising that conventional animal models fail to identify drugs that will cause idiosyncratic toxicity. At present, we have to depend on the identification of structural alerts in drugs (metabolites) to screen out the potential toxicity. Such sub-structures are well recognised by the drug metabolist during early drug discovery and such information is rapidly relayed to the medicinal chemist. Chemical structures regarded as hazards, for either the drug per se or its metabolites, include acyl halide, aromatic amine, carbocation, epoxide, furan, quinone, quinoneimine and hydroxylamine. Chemical techniques used in both in vitro and in vivo studies include LC-MS detection of glutathione conjugates, radiometric analysis of protein adduct formation and Western blot analysis. More recently radiometric 2-D proteomic analysis has been used to define specific protein targets for a particular drug.

8. Use of molecular biology in hazard identification and risk assessment

During the past 50 years, there has been a continuing evolution of the techniques used in safety analysis, and in particular in the field of drug metabolism and drug toxicity. There is a need to investigate novel means of investigating drug-induced stress at an early stage in drug development, in order to reduce the attrition. One approach is to measure sub-clinical markers of drug toxicity that may predict various forms of human drug toxicity. The ultimate aim of such studies would be to apply formal structure–activity relationship (SAR) analysis to molecular markers of chemical stress, cellular damage and clinical toxicity.

Recent advances in molecular toxicology (transcriptomics, proteomics and metabolomics) allow the investigator to explore chemical stress, in both in vitro and in vivo models, at doses of drugs and chemicals that do not cause overt toxicity. Cellular stress, at levels that do not result in overt toxicity, is now recognised to up-regulate a battery of cellular defense systems aimed at removal of toxic species and cellular repair. Studies in mice from our laboratory with a variety of hepatotoxins, including paracetamol, indicate that very early changes in intracellular signalling proteins (c-jun, c-fos, AP1) can be detected in the liver, even after relatively low, sub-toxic doses [24]. Moreover, these changes are reflected in the altered function of certain key antioxidant enzymes.

It is envisaged that such approaches will provide a better understanding of the physiological response of various organs to the chemical stress induced by drugs and their metabolites. Ultimately, it may be possible to relate cellular changes in these test systems to major toxicities seen in man (anaphylaxis, hepatotoxicity, blood dyscrasias and severe skin reactions) and, thus, provide more physiologically based biomarkers of human drug toxicity in preclinical test systems.

9. Conclusions and future perspectives

The aim of our research is to determine the fundamental mechanisms of serious ADR in order to predict both the chemical and individual basis of drug toxicity. The general approach we have adopted is that of 'molecule to man', whereby the disposition of a drug (and its metabolites), is related through biochemical and molecular analysis, to cellular events and ultimately to toxicity in patients.

We have been able to use animal models successfully for type A and type C ADR. For example, a murine model has been use to explore the early, critical chemical, biochemical and molecular events which define both the physiological and toxicological response to model hepatotoxins such as paracetamol [24]. In addition, we have, in collaboration with Wolf (Dundee) used transgenic animals to explore downstream events in the pathophysiology of drug-induced hepatotoxicity [25].

Other groups and we have so far been unable to develop an animal model of immune-mediated drug toxicity, despite the frequency and severity of such reactions in man. Our approach to this problem has been to use a combination of genomics and ex vivo cell assays to investigate the individual and chemical basis of drug hypersensitivity [4]. On the basis of such knowledge, we can select candidate genes as susceptibility factors for both drug-and chemical-induced auto-immune disease in man. The appropriate use of transgenic animals, in which the levels of key drug-metabolising enzymes, cytokines, growth factors and transcription factors can be regulated experimentally, can be used to test hypotheses generated from clinical studies.

By a careful use of clinical and (transgenic) animal studies, linked by molecular analysis to the chemistry of the drug, it should prove possible to dissect the mechanisms of these presently poorly understood ADR. Most drug companies are investing heavily in a combination of genomics, transcriptomics, proteomics and metabolomics in order to exploit the massive potential of the post-genomic era. At present, such techniques can only be used for hypothesis generation and should, therefore, only be regarded as part of drug discovery. Further work is required to define which molecular changes can be used to predict toxicity in the test system. It will then be necessary to determine whether such molecular-based test systems can be used to detect the hazard of toxicity in man, and then for risk assessment.

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Appendix A. Discussion 2

A. Breckenridge: In an example, you showed how predictable were the endometrial and the GI cancers from the pre-clinical work?

K. Park: That is really why I emphasise the need for pharmacology and the chemical genotoxicity. It is really a slightly controversial topic at the present time. One group has

claimed that there are actually endometrial cancer and that has been published, but other groups I know have failed to reproduce that with slightly better techniques. Going back to the pre-clinical evaluation, if you give the drug to young animals and feed them from a pre-pubertal age, I think you can then actually induce the endometrial tumours as some sort of hyperplasia. However, in the conventional rodent bioessay, it would have been missed. With respect to actually caring for patients, the hepatotoxicity has not been seen yet, but maybe it takes 20 years. There is what is thought to be this pharmacologically mediated toxicity, so at the present time, mechanistically, one would think, there is a pharmacological basis for that. Just a rodent bioessay obviously has great limitations, but, at the same time I think one has to refer to whoever is doing this. If we look at the evolution of the absolutely huge knowledge of the pharmacology of tamoxifen, it is not just even on the oestrogen receptors, then it becomes difficult to predict everything in the sense of the complexity of the receptors. I think that the animal studies, if you looked at them carefully, do show hyperplasia, and I think the mechanism is probably pharmacological rather than chemical.

X. Carné: You are dealing with a very interesting topic but I think that in your conclusions, you should also include two other "-omics": politiconomic and logisticonomic aspects in drug safety are very important. We studied, in Spain, all the drugs withdrawal in the 1990s due to safety reasons. We found 22 drugs and in the great majority they were related to hepatic toxicity (8 out of 22) or cardiac toxicity (QT lengthening problems or arrhythmia; 6 out of 22). In both cases, they were, in my opinion, clearly type B-reactions. It means no clear dose–response relationship. But we found a lot of political and logistical issues when we deeply studied the origin of these drug withdrawals. When the CPMP discussed these problems in London, there were many other non-scientific issues on the table. Tolcapone for example, has been withdrawn from the market with only two—if I am not wrong—letters saying that it was hepatotoxic, without knowing the mechanisms and so on. And when they were discussing, a telephone rang and said "I think we have a third one", like an epidemic of hepatic toxicity due to tolcapone. In conclusion, I accept that we must improve our techniques to predict drug safety in man, but always, unfortunately, there will be other not scientific things that will come on, just political or logistical. If we have many drugs in the same drug class, if there is one small slight possibility that that particular drug is toxic, so the registration authorities will be much eager to withdraw the drugs from the market that otherwise.

K. Park: With all due respect, in all my experience, every decision that I have seen made, both outside and within regulatory authorities, have been based on science and the available facts.

L. Sheiner: I found the topic quite interesting. I think that the suggestion that we are getting closer to being able to predict from biochemical events is heartening. You talked about tamoxifen and the complicated mechanism by which the drug might cause tumours, and suggested that this would lead to great difficulty in modelling. You also talked about paracetamol, which led you finally to a discussion of various new sources of information, and you said that these will this allow pattern recognition to be used to make predictions. In the first case, it is not the modelling that is the problem, but a lack of understanding.. A model is an expression of our understanding. So, one may have some qualitative understanding, but not yet be able to put it together in a quantitative way. At that point,

one often turns to pattern recognition, as you suggest for paracetamol, but when the desired degree of extrapolation is as great as it is there, from cells in animals to whole human beings, pattern recognition will not suffice, and the only way to proceed, I believe, will be through mechanistic understanding. I know that is your goal, but I did want to clarify my objections to the suggestion that there was either in the first case something the matter with modelling per se, or in the second case, that pattern recognition should be our goal. I think pattern recognition is a very early intermediate goal which may give us a few practical clues, but in the end we must strive for a clear understanding of the mechanism which can be expressed as a mathematical model.

K. Park: I agree with that entirely. What I was really trying to say was that usually in toxicological issues, the mechanisms are more complex than the pharmacology of efficacy. And one starts off with a relative simple model, and I think in PK/PD modelling, that works through, because that works through to efficacy, as we have seen in many models. I was just really trying to draw attention to the fact that really more from a mechanistic point of view, is that when we begin to look at something like tamoxifen, I have focussed on the chemistry and I would lead you all down that pathway and show you 150,000 diminutions of risk factor. But then I was just being aware of the fact that there is the pharmacology as well. So, there are two separate pathways, but both of them are necessary: you need the oestrogenicity as well as the genotoxic insult to actually produce those tumours in the rat. One has to take account, simultaneously, of species difference in pharmacokinetics, routes of metabolism, very minor routes of metabolism, and species difference in the response as well as the affinity of those receptors as well. So, I was just trying to broaden out the pattern and show that really toxicology, unfortunately, is a lot more complicated than pharmacology when it comes to actually modelling.

L. Sheiner: I am sure that that is the case. Unfortunately toxicology has not historically been an area where mechanistic thinking, the kind of work that you are doing, has been much applied. Yet, that is the way we will have to go; that is ultimately what will allow us to make predictions.

A. Bye: From an industrial perspective we usually have a whole range of chemicals in class, and you can see toxicity in one meanwhile another might be clean. Do you ever see the day that we will be able to look at the whole series of compounds in a class, to start working out whether you can prevent people progressing compounds that allow you to make reactive and toxic metabolites.

K. Park: I think the first answer is yes, because what I was really trying to stress was that one could go towards physiologically based toxicology (rather than pharmacokinetics) so one can look at chemical stress. For example, with drugs like clozapine you get 1% agranulocytosis. Now we can show chemical stress in animals, but not overt toxicity. In academia if we go backwards and we look at human toxicity that was not predicted and understand the physiological reasons for that, that gives us the basis for then looking at a series of compounds, without absolute certainty, and I think that will enable you to go forward. But the final point, I would say is that also it is not just a matter of eliminating compounds. A lot of compounds are eliminated in industry through fear and actually you may have lost some very important compounds as well, so there are two sides to that point.

A. Bye: I was fascinated to see that this effort is going on in almost one academic group, your own. In Glaxo-Wellcome, it would be a foreign language to many people and

it worries me intensely that we are not carrying this kind of thinking through where it is needed. Over 30% of our compounds in Glaxo Wellcome are failing for, I think you said, "bizarre toxicology reasons". Now, they are only "bizarre" because we have no idea what we are looking for, and we tend to just run up the routine pre-clinical toxicology. When we have four or five leads to choose from, we just choose the one with the cleanest toxicology profile. In this situation now, we may be choosing the wrong one. It might be better to say how the animal toxicology differs from the human toxicology. But in the human toxicology the only toxicology we see are the bizarre examples, because hopefully we stop them in their tracks. It is an incomplete science unless things have gone horribly wrong, then you do a retrospective examination of the problem. The question is, how can we use this in a more prospective way? What elements do we need to start putting together, to be able to use this in a much more predictive way?

K. Park: First of all, that you could say that toxicology is pharmacology we do not understand, and we have got to understand the problem in man. I tried to show in my presentation that if we can go back and start with clinical toxicology in man, go back to the animal studies and to see whether there are biological events occurring there which would help explain those events in man, then we may be able to go forward and have a more physiologically based approach to chemical stress. It is really just taking on what Spindon said many years ago: "extensive studies on a small number of animals, looking at realistic doses, are far more relevant than using irrelevant doses in a huge number of animals". The tools are beginning to appear, the problem is how do you apply them, what questions do you ask? These tools will generate huge amounts of information, and people will say, "Oh well, we will use computer-driven bio-informatics to sort it all out at the end of the day", but I really think there is got to be some guidance through, with those, and so the analogy that some people have used is that we were using a gene-cluster transcription factor enzyme approach is like a rifle shot to look through, rather than the blunderbuss approach of using a gene chip net: rather a crude way of putting things about. I think it is just a matter of directing things in a sensible way. But the potential from these studies are immense. The difficulty in a sense is applying them to man; we are struggling with a concept of utilising some of these techniques in man, and how can you use that during a serious adverse reaction. We use clozapine as a model for agranulocytosis because there is a reasonable frequency of that: 1%. That may be a useful tool to explore about the tip of the iceberg, goes from a clinical event to sub-clinical events, related to chemistry and bio-chemistry, and ultimately back to the tip. I think we have to take paradigms in man that we can use at the present time, with drugs that are giving problems in industry and study them in an academic environment; that will enable us to, perhaps, then, eventually develop methods that can be used prospectively.

A. Breckenridge: I am left with an overwhelming feeling that the drug regulatory pre-clinical toxicology that we insist on, apart from geno-toxicity and reproductive toxicity, has become—I would not say irrelevant—but it is ignoring modern science. Am I unfair or are you still happy with the predictive value of it?

K. Park: At the present time, these are tests we have to do because we are frightened of removing them. If you take the carcinogenicity assay, one could question that, now we have gone from two species to one species with the rider that they should be relevant to human use, which is rarely proven. You could actually look at the Haines test and using a

rat S9, why do not we use human P450? But we have got to remember that all the time in those tests we are testing the chemical in any biological system to see if the chemical can cause biological damage. And then you ask the second question, whether it is relevant to man. I think a lot of these procedures are out there and they are growing all the time, which is the problem with the new molecular techniques; there is a huge expansion of work and it is just that when one can actually say that we can take those away. Because if you think about it, all the data that is presented for rodent bio-assays, for mutagenicity studies, for a lot of studies in animals, refers to historical controls. It is going to take a long time and quite a brave man who removes all of that. And the problem with these toxicities is we really understand very little about, especially for teratogenicity. The carcinogenicity, which can come from many different directions, again one has to use historical controls, and people feel comfortable with, sort of case histories rather than taking on the new things. There are limitations and one hopes one refines them. We have taken them out at the present time, and I think that is how we move forward, but cautiously.

D. Back: You raised a couple of issues which in a sense left things 'up in the air' in terms of the use of models. For example, with fialuridine you suggested that maybe we should have used a virally infected animal model, and that would have possibly given us another answer. And also with tamoxifen, the ability to express receptors in different cell types, and the potential complications arising from that. Can you just expand on the relevance of going along that road and actually trying to make sure we do have the right models for predictive toxicology?

K. Park: First of all, thinking about predictive models which are relevant to the clinical setting, one huge area which we are concerned about on the Committee of Safety and Medicine at the present time is paediatrics. Going back to the viral infection, there was a huge amount of correspondence in the New England Journal of Medicine during the period of the fialuridine debate as it became, after the disaster. People were suggesting to use woodchucks which could be infected with the sort of parallel form of the human disease. Perhaps to use the virally infected animal would be more relevant, to look at the symmetry between the infection and the drug to cause paratoxicity. The drug was very efficacious, it cured the viral infection and then caused toxicity because it was delayed later on. But I think the concept of actually thinking sensibly about an animal model for specific groups, if one takes examples, for paediatrics, for the elderly, for HIV infection where there is ten or a hundred-fold increase in ADR, then one could sensibly think about reasons why there is an increase risk. And then if you did have a drug with a history, or from a class of a history, then you could use it. For the nucleotides, clearly fialuridine, people then looked at DNA polymerase gamma, and do a sort of chemical test which could be used quite sensibly for targeting the mitochondria. I think it is really a matter of exploring in depth, scientifically, the relevance of the animal models. You can go through this starting with P450, then the receptors, and so on. Now, what I was talking about with tamoxifen was really giving you a very long view, because that took 15 or 20 years of research. You could not do that in drug development. But if we learned those lessons, then when new drugs come along, we can use all of these paradigms. And it is maximising the use of information which will come out of traditional tests like histology, by using the molecular biology to walk from molecule to man, through the animal studies.

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