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USE OF PHYSIOLOGIC PHARMACOKINETICS TO ASSESS INTERSPECIES DIFFERENCES IN TOXICOLOGY

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INTRODUCTION

It is clear from the evaluation of a large number of studies that there are often considerable quantitative as well as qualitative differences between species when evaluating toxicity including carcinogenicity. Therefore evaluating potential risk for man from experimental animal studies requires perspective both on quantitative as well as qualitative differences. Toxicity and carcinogenicity observed in animal testing frequently is not a linear function of administered dose. One quantitative tool being developed is the incorporation of physiologic parameters into pharmacokinetic models to facilitate extrapolation of the predicted effect as a function of the dose and route of exposure.

Mathematical models are extremely useful to help conceptually visualize and ultimately test hypotheses. In particular pharmacokinetic modeling has facilitated improvements in the interpretation of animal toxicity studies by providing a physiological basis for extrapolating from high to low doses, between species and routes of administration and characterizing nonlinear phenomena. In this technique, a computer model is constructed with compartments which correspond to actual tissues or tissue groups of the body. The size of these compartments, as well as the blood and air flows through these compartments, may be obtained from the medical literature. Once partition coefficients and metabolic constants have been determined, differential equations describing the dynamic mass balance in each compartment may be written. In the case of $MeCl_2$ two enzymatically mediated metabolic pathways, one involving mixed function oxidase (MFO) and the other involving glutathione transferase (GSH) must be considered. Figure 1 is the diagrammatic representation of the physiologically-based pharmacokinetic model for methylene chloride ($MeCl_2$). The objective of constructing this model was to be

able to predict concentrations of relevant metabolites in specific target tissues and thus facilitate extrapolation of potential effects from high to low doses and from experimental animals to man.

MATERIALS AND METHODS

Details of the physiologic pharmacokinetic model constructed for methylene chloride are given in Andersen[1].

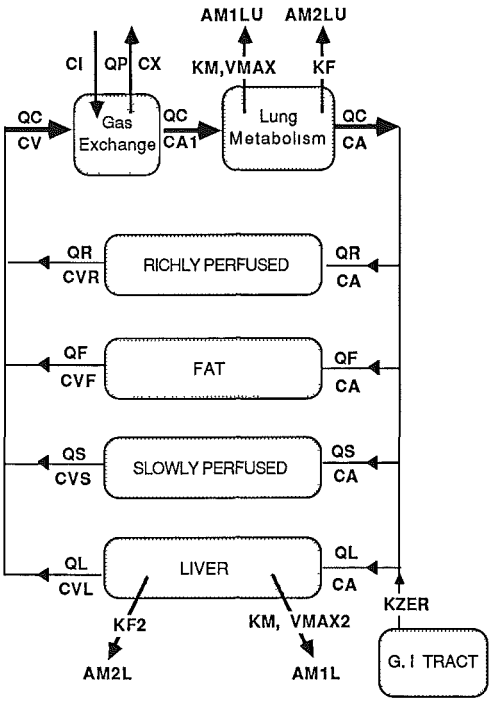

Figure 1:

Diagram of the physiologically-based pharmacokinetic (PB-PK) model utilized for methylene chloride. Tissues of the body are grouped into five compartments with similar flow and partition coefficients: Lung, Fat, Liver, Richly Perfused, and Slowly Perfused. Metabolism occurs in the Lung and Liver compartments. $MeCl_2$ enters the body through inhalation with absorption into pulmonary blood in the Gas Exchange Compartment, or by ingestion with absorption directly into the Liver compartment.

RESULTS AND DISCUSSION

Model Validation. To test the reliability of the model, the model predictions were compared with several sets of blood concentration/time course data. One of the strengths of physiological modeling is the ability to use the same model to predict the disposition of materials in a target species from pharmacokinetic data gathered from another species. The model was used to predict blood levels of $MeCl_2$ in F344 rats inhaling 200 or 1000 ppm $MeCl_2$ for 4 hr (Andersen[2]). Blood levels of $MeCl_2$ were well described by the model both during the 4 hr exposure, and for periods up to 120 minutes following cessation of exposure (Figure 2a).

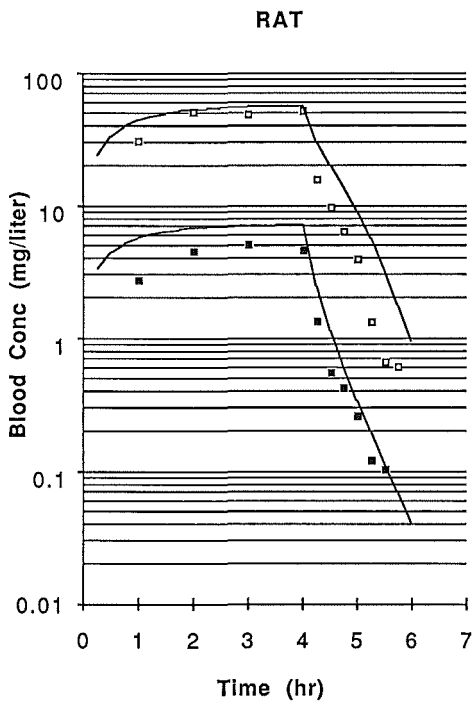

Figure 2a:

Validation of the PB-PK model with experimental data. Figure 2a presents data obtained in F344 rats during and following inhalation exposure to 1000 ppm (open symbols) or 200 ppm (closed symbols). The simulated data is presented as a solid line, while the experimental data is shown with closed or open symbols.

Experiments conducted with human volunteers (Schumann[3]) were also evaluated. In these studies, healthy volunteers were exposed to either 100 or 350 ppm of $MeCl_2$ for 6 hr and samples of venous blood were collected during the exposure, and for periods up to 24 hr after cessation of exposure. This simulation may be regarded as the ultimate test of animal extrapolation, and the predicted values for humans were in excellent agreement with the experimental data (Figure 2b).

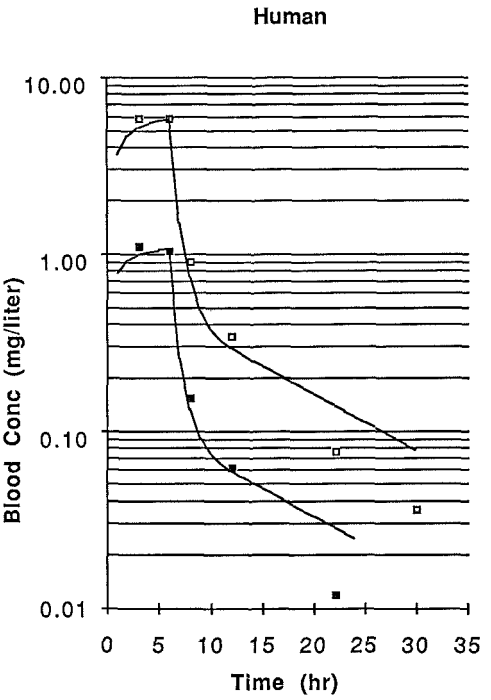

Figure 2b:

Validation of the PB-PK model with experimental data. Figure 2b presents data obtained in humans during and following inhalation exposure to 350 ppm (open symbols) or 100 ppm (closed symbols). The simulated data is presented as a solid line, while the experimental data is shown with closed or open symbols.

Hypothesis Testing. The model can now be used to compare the values of potentially relevant measures of target tissue dose during two chronic bioassays of $MeCl_2$ toxicity in the B6C3F1 mouse. An inhalation bioassay (NTP[4]) revealed significant increases in lung and liver tumors

while a drinking water study (Serota[5]) failed to show a dose-related increase in the incidence of either type of tumor in this same strain of mouse.

Concentration of metabolites related to the MFO pathway in liver and lung were nearly identical in the two studies (Table I). Values for

TABLE I

INHALATION vs DRINKING WATER TISSUE CONCENTRATIONS[1]

$B_6C_3F_1$ Mouse

Site	Inhalation 2000 ppm	Inhalation 4000 ppm	D-H_2O 250 mg/kg/day
MFO[2]/Liver	3575	3701	5197
MFO/Lung	1531	1583	1227

[1]mg metabolized per liter tissue per day
[2]MFO-mixed function oxidase pathway

metabolites in the liver expressed in units of mg/liter/24 hr were 3575 and 3701 after exposure to 2000 ppm and 4000 ppm respectively, while the value in mice consuming $MeCl_2$ in drinking water at the rate of 250 mg/kg/day was 5197. Similarly, the concentration of metabolites from the MFO pathway in the lung were 1531 and 1583 after 2000 ppm and 4000 ppm respectively, while in mice consuming $MeCl_2$ in drinking water was 1227. If the products of the MFO pathway were important in the toxic/carcinogenic reponse there should have been a similar, if not more pronounced response in mice consuming water containing $MeCl_2$ at a dose of 250 mg/kg/day. Since this was not observed it is concluded that the MFO pathway is not involved significantly in the carcinogenic response observed in mice.

In contrast, metabolic products from the GSH pathway in liver and lung (Table II) increase following 2000 ppm and 4000 ppm inhalation exposure which is consistent with the dose related oncogenic response in these target tissues. Moreover, the concentration of metabolites from the GSH pathway following drinking water exposure are much lower than following inhalation exposure. This reduced concentration of metabolites following consumption of water containing $MeCl_2$ is consistent with the lack of toxicity and oncogenicity observed in the drinking water bioassay.

TABLE II

INHALATION vs DRINKING WATER TISSUE CONCENTRATIONS[1]

$B_6C_3F_1$ Mouse

Site	Inhalation 2000 ppm	Inhalation 4000 ppm	D-H_2O 250 mg/kg/day
GSH[2]/Liver	851	1801	15.1
GSH/Lung	123	256	1.0

[1]mg metabolized per liter tissue per day
[2]glutathione pathway

Dose and Interspecies Extrapolation. The PB-PK model can now be used to calculate the values of the toxicologically relevant chemical species in various tissues under a variety of exposure conditions. Since the metabolites from the GSH pathway are likely relevant to the toxicity and oncogenicity observed at high doses in the liver and lungs of mice, it becomes important to be able to predict the concentration of these metabolites in the target tissue of humans. The value of the liver metabolite concentration related to the GSH pathway in B6C3F1 mice and human following inhalation of various concentrations of $MeCl_2$ for 6 hr/day is plotted in Figure 3a.

The calculated metabolite concentrations are displayed on a log/log plot for exposure concentrations from 4000 ppm to 1 ppm. Metabolite concentrations for B6C3F1 mice are represented by the heavy solid line, while those for humans are represented by the heavy dashed line. Metabolite concentrations which would be obtained by linear extrapolation of data from the 4000 ppm mouse exposure are depicted as a lighter solid line in this and other figures. The concentration of metabolites calculated from the PB-PK model are close to those estimated by linear extrapolation with the mouse above 1000 ppm, but deviate from linearity in the region below 1000 ppm. The non-linearity in the curve is apparent in the region where the MFO pathway saturates. Saturation of the MFO pathway allows a larger percentage of the $MeCl_2$ available for metabolism by the GSH pathway, resulting in a disproportionate increase in the metabolites of the GSH pathway at exposure concentrations above 100 ppm. Similarly in humans (exposed 6 hr/day) the curve also displays a non-linearity in the region between 100 and 1000 ppm although this is less pronounced than in mice. Importantly the values of the human metabolite concentrations are lower than the mouse throughout the entire exposure range.

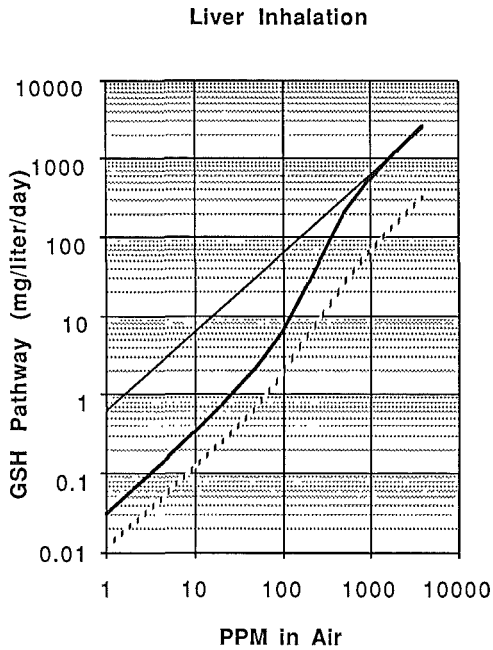

Figure 3a:

Relationship of the metabolite concentrations associated with the GSH pathway to external dose. Figure 3a shows the values in B6C3F1 mice and humans during inhalation exposure (6 hr/day) in the liver. Data for mice are shown as a heavy solid line, while data for humans are shown as a heavy dashed line. In each case, a reference line depicting a linear extrapolation from the highest administered dose of $MeCl_2$ (4000 ppm) is shown as a light, solid line for comparison. Units for the Y-axis are mg metabolized per liter tissue per 24 hr.

Figure 3b presents the values of the lung dose of metabolites related to the GSH pathway in mice and humans exposed to various concentrations of $MeCl_2$ in air. In this case the non-linearity in the mouse curve is of smaller magnitude than for mouse liver, indicating a smaller effect of the MFO pathway upon the material available for the GSH pathway. As in the liver, the concentration of metabolites in human lung is lower than mouse at all concentrations probably because of the lower activity of GSH transferase in human lung relative to mouse lung.

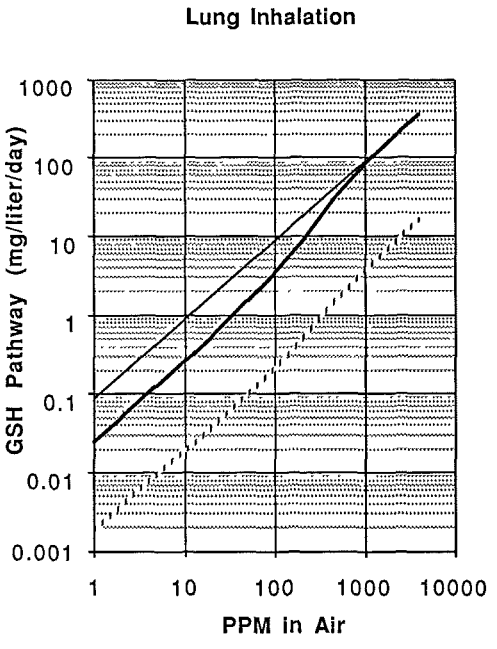

Figure 3b:

Relationship of the metabolite concentrations associated with the GSH pathway to external dose. Figure 3b shows the values in B6C3F1 mice and humans during inhalation exposure (6 hr/day) in the lung. Data for mice are shown as a heavy solid line, while data for humans are shown as a heavy dashed line. In each case, a reference line depicting a linear extrapolation from the highest administered dose of $MeCl_2$ (4000 ppm) is shown as a light, solid line for comparison. Units for the Y-axis are mg metabolized per liter tissue per 24 hr.

As demonstrated, physiologic pharmacokinetics can be a powerful tool to facilitate extrapolation between doses and species. It can be used also to generate and test hypotheses concerning mechanisms of action as well as facilitate extrapolation. Most importantly, if sufficient knowledge is available it allows direct interspecies comparisons for predicting potential effects which precludes the use of arbitrary factors which assume greater or lesser sensitivity of man compared to experimental animals.

REFERENCES

1. Andersen, M. E., Clewell III, H. J., Ganges, M. L., Smith, F. A. and Reitz, R. H. (1986) Toxicol. Appl. Pharmacol. (accpeted for publication).

2. Andersen, M. E., Archer, R. L., Clewell, H. J. and MacNaughton, M. G. (1984) The Toxicologist 4: 443

3. Schumann, A. M. (1984) In: Food solvents workshop I: Methylene chloride. Proceedings of the workshop, Nutrition Foundation Inc.; 888 Seventeenth St. N.W., Washington, D.C. pp 162-188

4. NTP, National Toxicology Program, USA (1985). NTP Technical Report on the Toxicology and Carcinogenesis Studies of Dichloromethane in Fischer 344 Rats and B6C3F1 Mice. NTP-TR-306.

5. Serota, D., Ulland, B. and Carlborg, F. (1984). In: Food and Solvents Workshop I: Methylene Chloride. Proceedings of the workshop, Nutrition Foundation Inc.,; 888 Seventeenth St. N.W., Washington, D.C. pp 43-56.

DISCUSSION - Pharmacokinetics and mechanism of action in evaluating animal carcinogenicity.

G.J. Mulder

I think that to link pharmacokinetics to effects, including carcinogenic effects, is very important and it is quite worthwhile to try to expand the model by including different organs, but there are two questions of interest. One of them concerns the validation of your model, in terms of organ distribution of metabolites, and the other refers to the assumptions made in the extrapolation to man.

P.G. Watanabe

To validate the model we performed studies with ^{14}C labelled compounds in the rat and mouse. We used carbon monoxide as an indicator of the mixed function oxidase pathway and carbon dioxide as a marker of the gluthatione pathway. There is some debate because some CO_2 is generated by the mixed function oxidate system, but we tried to account for that. Although I only showed here blood kinetics, we measured tissue concentrations out of those metabolic pathways, and they correlated fairly well with the predicted values. With regard to extrapolation to humans, we relied on data about metabolic constants of these or similar agents in man. Actually, we are now going back to this problem and checking, from data now generated from in vitro human liver samples, how close our initial assumptions were.

G.J. Mulder

The constants you used, were they based on the exposure levels or on in vitro activity?

P.G. Watanabe

They were not based on methylene chloride at all. They were based on other materials metabolized by those pathways.

G.J. Mulder

Which to my mind makes it quite dangerous to extrapolate from animal data to man, particularly with regard to regulatory decisions.

P.G. Watanabe

I agree. That is why we are currently verifying our data for methylene chloride.

G. Zbinden

Did you detect any covalent binding to liver and lung DNA in mice exposed to methylene chloride?

P.G. Watanabe

No. Some time ago we evaluated DNA binding in these target tissues in the rat. At that time the sensitivity limit was about 1×10^{-6} molar and we did not detect any. I believe that in studies conducted elsewhere no covalent binding has been detected in the mouse.

T. Wieland

I wonder whether one should necessarily look at nucleic acid binding. It would be easier to evaluate macromolecular binding and see whether adduct formation in the target organs is in line with the assumption and calculations.

P.G. Watanabe

I agree.

P. du Souich

Taking into account the importance of the metabolites, it would be worthwhile to study how your model works in animals treated with enzyme inducers.

P.G. Watanabe

Of course. It would be fine to induce certain pathways and look at the ultimate response. We could do that, but one of the problems is that it is a two year bioassay.

R. Lauwerys

Your assumption that the oncogenic effect of methylene chloride in the mouse is related to the concentration of the active metabolites of the glutathione pathway in the target organs may be right, but since there is no proof of binding to some macromo-

lecule, one should be very careful in trying to extrapolate to humans. Other factors, such as interference of the metabolites with the immune system might play a role.

P.G. Watanabe

This is actually a very critical point. One of the greatest deficiencies that we have in toxicology is that we do not know what receptors we are dealing with. Even if we consider DNA a general target, we cannot forget that there is much DNA that is just redundant.

E.E. Ohnhaus

The inclusion of tissue partition coefficients in your model interested me most. We have data on monooxigenase levels in lung tissue from patients treated with rifampicin and subjected to surgical interventions. Monooxigenase activity was much higher in inflammed tissue than in fibrotic or normal tissue, and we found that this correlated quite well with the increased partition coefficients of rifampicin in these tissues.

G. Zbinden

Your observation that the mixed function oxidative pathway may be less important than the glutathione pathway would invalidate most of the in vitro mutagenicity tests as valuable pieces of information, since the glutathione pathway is not contemplated in these studies. Have you data on the in vitro mutagenicity of methylene chloride?

P.G. Watanabe

This has been studied at ICI and the results seem to implicate the glutathione pathway. The situation is complicated by the fact that methylene chloride itself, without any metabolic activation, gives positive results in bacterial mutagenicity studies. It seems that bacteria activate this material in a unique way, not shared by mammalian systems.

G.L. Plaa

It should perhaps be stressed that your data do not necessarily indicate that a glutathione metabolite is involved in the

oncogenicity of methylene chloride in mice. Actually, what you show that once the P 450 pathway is saturated the glutathione pathway plays a more important role, so that an inflection appears in the curves. I think that the saturation of the system is what is most important for any extrapolation. In this regard, I would like to know if there is any work that shows that there is a way of monitoring the two systems in humans so that we could get an indication of the shift in favor of the glutathione pathway.

P.G. Watanabe

In the case of methylene chloride the difficulty is that the glutathione is rapidly regenerated. Anyhow, this is a point that deserves some effort, but it is unlikely that we can find an indicator as simple as the measurement of thioethers in the urine.

R. Lauwerys

Since formic acid is a metabolite of the glutathione pathway, perhaps it could be used as an indicator.

P.G. Watanabe

There are two problems here. On the one hand the concentrations would be rather low, and on the other the background would be high, but it can be considered.