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Drug Elimination Interactions: Analysis Using a Mathematical Model[1]

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A mathematical model was developed to analyze the elimination kinetics of drug interactions in the rat. The model is based on physiological blood flow rates and organ weights and includes Michaelis–Menten equations for enzymatic processes which are involved in the elimination of the drug; competitive inhibition interactions are computed for shared pathways. Using data from the single drugs, the model can simulate the results of experiments of the acute warfarin–BSP interactions in rats.

KEY WORDS: mathematical models; drug interactions; warfarin; bromosulfophthalein; bile.

INTRODUCTION

In modern therapy, multiple combinations of drugs are often used for different therapeutic purposes. Numerous drug interactions have been reported in recent years. In the case of the anticoagulants, interactions with other drugs can occur at various sites in the elimination pathways which affect the kinetics of elimination. Studies have shown that the excretion of warfarin in the bile of the rat is a sensitive indicator of some of these different types of interactions (1). Bromosulfophthalein (BSP) dramatically decreases the elimination of warfarin in the bile. This interaction is useful for kinetic or mathematical analysis because the onset of action, the peak effect, and the duration of action can be studied over a short period of time. Furthermore, both substances can be readily measured in both the bile and the plasma. This combination proved to be a useful experimental study to use to test a mathematical model and a computer program which was developed to facilitate the analysis.

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GLOSSARY

B	substrate concentration (BSP)nmol/ml
C	drug concentration, nmol/ml
E	enzyme concentration
ES and EB	enzyme complexes
$g(t)$	drug administration function (impulse function for i.v. injection)
k	rate constants
K_m	apparent dissociation constant, nmol/ml
P	drug metabolite
n	exponent
Q	blood flow rate, ml/min
r	drug elimination rate, nmol/min
R	tissue-to-plasma binding ratio
S	substrate concentration (warfarin), nmol/ml
t	time, min
V	organ volume, ml
V_{max}	rate constant, nmol/min/ml of liver
τ	residence time of bile duct, min
Subscripts	
B	BSP
K	kidney
L	liver
M	muscle
P	plasma
S	warfarin

In a previous article (2) we showed that a model based on physiological flows and volumes of organs in rats could satisfactorily represent the interactions that occurred during the simultaneous elimination of BSP and warfarin. Most of the physiological parameters in that model, such as organ weights or volumes and blood flow rates, were taken from the literature. Values for other needed parameters such as tissue-to-plasma binding ratios were in part derived from values reported in the literature but were subject to limited modification on the basis of obtaining a better model fit of the experimental data. Elimination kinetics were represented by Michaelis–Menten equations, but the data were of insufficient accuracy and range to warrant a full statistical fit on all of the elimination rate constants. Therefore,

a simplified linear approximation to these equations was used. An interaction parameter was also introduced which represented the fractional rate of decrease in rate of elimination of warfarin as proportional to the instantaneous concentration of BSP. The introduction of this one new parameter gave an excellent (but empirical) representation of elimination kinetics involving an interaction.

The present work represents a more ambitious goal: Can the magnitude of drug elimination interactions be represented from data and parameters of single drugs? Prediction of dynamic drug distribution within an animal is one of the attractions of using physiologically based flow models. But prediction of interaction effects based on single drug data would represent an important step in the application of mathematical modelling.

In this study we again worked with the BSP–warfarin system using the same experimental data that was described in our earlier article (2) to test the interaction model. We propose to incorporate the Michaelis–Menten equations for the elimination rate using classic inhibition kinetics. If the drugs under consideration share a common elimination pathway, the competitive elimination rates can be written in terms of the rate constants V_{max} and K_m for the individual drugs. However, the BSP–warfarin system does involve one complication. In their work on the elimination of BSP and bilirubin, Clarenburg and Kao (3) reported that BSP was eliminated in the rat by not one but two important pathways. Only one of these, the so-called primary pathway, was shared with bilirubin. The secondary pathway was not. In the present study we assumed that the primary route is also the shared pathway between warfarin and BSP.

THE MATHEMATICAL MODEL

The mathematical model was programmed to be compatible with Fortran G, H, and WATFIV compilers. All computations were done on an IBM 370 computer system.

The mathematical description of the behavior of drug elimination and interaction is based on a model similar to that developed by Bischoff *et al.* (4). Compartments representing physiologically meaningful body regions are connected by blood flows (Fig. 1). The concentration of the drug in the blood and in the tissue in various regions of the body are governed by mass balances, tissue-to-plasma binding ratios, and the chemical kinetics of the rate-limiting elimination steps. A set of ordinary differential equations may be derived from the "lumped" properties of the body regions.

A complete set of the seven equations must be written for each drug under consideration. The blood flow rates and the organ volumes are the same for all drugs; the binding ratios and the elimination rates are drug

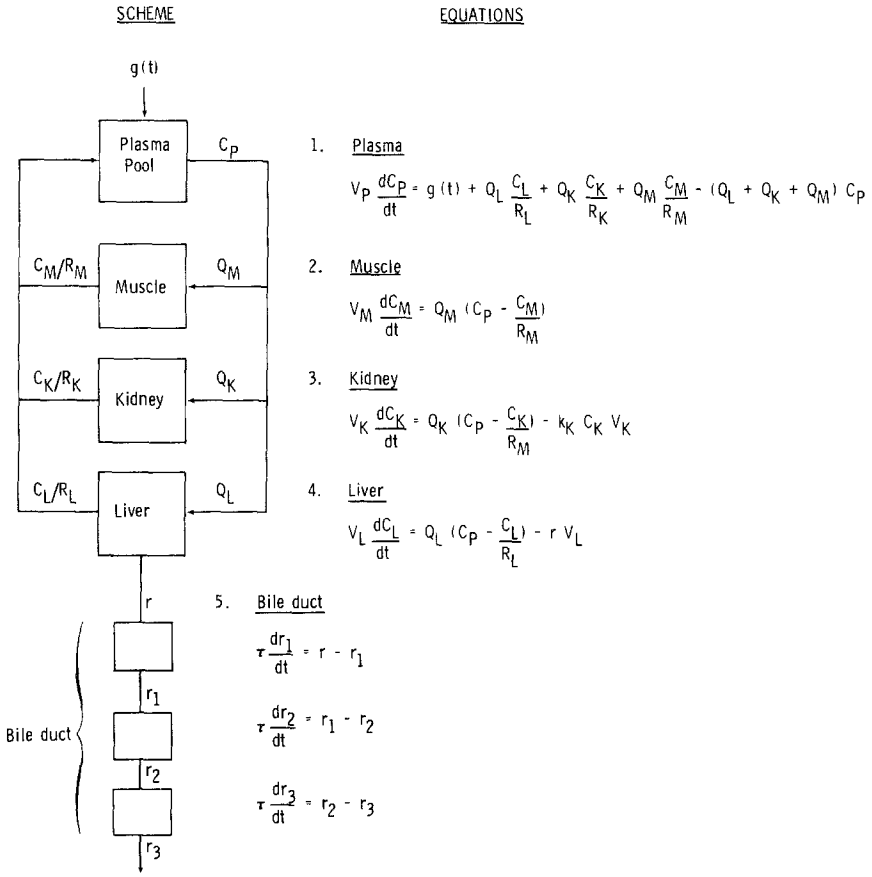

Fig. 1. Block diagram of model showing seven compartments and blood flows and concentrations.

specific. Additional equations can be added to this set to describe reabsorption of the drug in the gut and gut lumen if this should occur. However, all of the drugs used in this study have negligible reabsorption and hence those equations are not needed.

The equations that describe flow through the bile duct are an approximation to a pure delay time. This three-compartment approximation probably reflects actual conditions more accurately than would a pure delay time (which also could be programmed). The compartment model simulates the delay time plus some axial diffusion that occurs along with the flow.

For drugs which are removed slowly, such as warfarin, the plasma is essentially in equilibrium with the tissue so that

$$C_p \approx C_M/R_M \approx C_K R_K \approx C_L/R_L \tag{1}$$

As a result,

$$dC_L/dt \approx R_L\, dC_p/dt \approx -r \tag{2}$$

The relationships in equations 1 and 2 are useful for direct evaluation of rate data when tissue concentrations have not been measured.

The form of describing the equations is identical to that proposed by Bischoff *et al.* (4) except for the elimination rate terms for the kidney and liver. Here we have adopted the suggestion of Montandon *et al.* (5) and have written the rate in terms of a unit weight or volume of the organ. The implied assumption is that the amount of enzyme involved in the metabolism of the drug is proportional to the weight of the organ. While not rigorously correct, this assumption nonetheless does provide a reasonable approximation for the effect of weight differences between animals. The normal liver weight can be based on correlations with body weight (4). This formulation also provides a first-order correction for elimination rates involved with such factors as sex differences or diseases whose effects result primarily from differences in liver weight.

There are many reasons why elimination may *not* scale strictly in proportion to empirically computed liver weight. It would not be unusual to find 10–20% differences in liver mass even among normal individuals of a single species. Actual enzyme content of the liver could be expected to vary even more. Thus extrapolation of model values between species such as was suggested by Montandon *et al.* (5) should be approached with great caution. In fact, totally different elimination pathways may dominate in different species with totally different rate limiting steps.

For the removal rate kinetics, Bischoff *et al.* (4) suggested an equation of the Michaelis–Menten form:

$$r = V_{\max}/(1 + K_m/C_L) \tag{3}$$

Of course, the enzyme kinetics can be represented by more fundamental and complex expressions if the experimental information is available. The effects of enzyme inducers and inhibitors may also be incorporated into these expressions. Since in many cases drug toxicities dictate that much of the data be collected at quite low concentrations, equation 3 is frequently approximated by its linear form:

$$r = (V_{\max}/K_m)C_L \tag{4}$$

In the present work, however, we retain the more rigorous representation of equation 3 for the elimination kinetics.

Elimination Rate Equations

The form of the Michaelis–Menten equations for a single drug (S) follows from the assumption of a reaction mechanism as follows:

$$S + E \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} ES \overset{k_2}{\rightarrow} P$$

The assumption that the concentration of the intermediate ES remains constant leads to equation 3. Even when the kinetics are far more complex, the overall results may often be well represented by the form of equation 3.

Interactions would occur when a second drug (B) is present which is eliminated using the same enzymatic pathway:

$$B + E \underset{k'_{-1}}{\overset{k'_1}{\rightleftharpoons}} EB \overset{k'_2}{\rightarrow} P'$$

Assuming that the concentrations of the intermediates EB and ES remain constant leads to a pair of symmetrical rate expressions often referred to as the "competitive inhibition" equations:

$$r_S = V_S \Big/ \left(1 + \frac{K_S}{S} + \frac{K_S}{K_B}\frac{B}{S}\right) \tag{5}$$

$$r_B = V_B \Big/ \left(1 + \frac{K_B}{B} + \frac{K_B}{K_S}\frac{S}{B}\right) \tag{6}$$

These expressions represent the only linkage—and hence all of the interaction—between the drug systems S and B in the describing equations.

For some drugs the elimination kinetics may differ significantly from equation 3. For example, in an analysis of the interaction between BSP and bilirubin, Clarenburg and Kao (3) found that BSP was eliminated in rats through two important pathways. One pathway, designated the primary pathway, followed the kinetics of equation 3 and interacted with bilirubin to yield expressions of the form of equations 5 and 6. However, a significant fraction of the drug was eliminated via a secondary pathway:

$$r_{B,\text{sec}} = V_{B,\text{sec}} \Big/ \left[1 + \left(\frac{K_{B,\text{sec}}}{B}\right)^n\right] \tag{7}$$

where the exponent n was approximately 2.

The secondary pathway was not shared by bilirubin. When bilirubin conce,trations were high, thus interfering with BSP elimination by the primary route, a large fraction of the BSP was eliminated through the secondary route. The total rate of elimination of BSP is given by

$$r_{B,\text{total}} = r_{B,\text{pri}} + r_{B,\text{sec}} \tag{8}$$

where $r_{B,\text{pri}}$ refers to the value computed by equation 6 and $r_{B,\text{sec}}$ refers to the secondary pathway of equation 7.

RESULTS

Interactions Between Warfarin and BSP

In a series of experiments described in previous articles (1, 2), 1.0 mg/kg of warfarin was injected intravenously into 300-g male Sprague-Dawley rats. One hour later, 50 mg/kg of BSP were injected intravenously. The injections of BSP reduced the concentration of warfarin in the bile (Fig. 2), which indicates competition for a shared pathway.

The physiological parameters of the blood flow rates and the organ volumes of the rat are known fairly well. Independent data are also available for the tissue–plasma binding ratios and the individual clearance rates for warfarin and BSP. Tissue-to-plasma binding ratios for warfarin were estimated from film densities recording the relative concentration of radioisotopes in the blood and tissue (2). Binding ratios for BSP were taken from several literature sources (6,7) which indicate that the binding ratio for liver-to-plasma is quite variable with changes in concentration. These data were correlated with a Michaelis–Menten form expression:

$$R_L = 2456/(C_p + 271.7) \tag{9}$$

with C_P in nmol/ml. This equation closely reproduced the binding ratios observed by Clarenburg and Kao (3) and Montandon *et al.* (5) and gave an average value close to that used in our previous study of this system (2) in which R_L was taken as a constant.

For the elimination rate kinetics, the form of the kinetic equations 6 and 7 as described by Clarenburg and Kao (3) was adopted. Equation 5 was assumed for warfarin elimination, thereby implying that the shared pathway for warfarin and BSP is in the primary route—the same as for bilirubin and BSP.

Values for the rate constants $V_{\max}$ and K_m for BSP for both the primary and secondary routes for BSP were derived from the data of Clarenburg and Kao (3) and are listed in Table I. The values shown differ from those reported by those authors but seem to fit their data better. Note that an exponent of 2.0 was chosen for the secondary path rather than a fractional power.

Values for the rate constants for the elimination of warfarin are somewhat more difficult to evaluate. There are several reasons for this. Warfarin is tightly bound by serum albumin, and, in the analyses of drug levels in organs, inaccuracies can be caused by the presence of variable amounts of blood remaining in the tissue (up to 0.25 ml/g).

Table I. Parameters Used in Model

Rat weight	300 g	
Organ volumes V	ml	
Plasma	13.36	
Muscle	150.0	
Kidney	2.66	
Liver	11.93	
Blood flow rates Q	ml/min	
Muscle	4.29	
Kidney	7.15	
Liver	9.28	
Tissue to plasma binding factors R	Warfarin	BSP
Muscle	0.060	0.10
Kidney	0.48	1.20
Liver	1.08	2456
		$C_p + 271.7$
Reaction rate constants (literature values)		
Primary: $V_{mas}(10^{-9}$ mol/min/ml of liver)	0.051	23.6
$K_m(10^{-9}$ mol/ml)	65.0	174.0
Secondary: $V_{max}(10^{-9}$ mol/min/ml of liver)	—	15.0
$K_m(10^{-9}$ mol/ml)	—	64.1
n	—	2.0

In many types of experiments, such as the one described here, tissue concentrations which require sacrifice of the animal often are not even available. In such a case the model fit is governed by plasma concentration that in effect operates through the approximations of equation 1 so that the value of the apparent K_m cannot be resolved independently from the value of the binding ratio R_L. In the work reported here, a value of 1.08 was used for the binding ratio of warfarin in the liver.

The warfarin elimination rate data reported by Wosilait (8) were obtained over a very wide range. At the lower concentration levels, corresponding to those considered in the work here, the apparent K_m was about 65 nmol/ml. This value was adopted in the simulation. It should be noted, however, that the apparent K_m was not constant over the entire range reported in that work. This variation could in part be the result of effects of association equilibrium between warfarin and serum albumin.

Some support for this value of K_m is offered by Pohl *et al.* (10), who measured the rate of hepatic hydroxylation of various isomers of warfarin. This reaction is a possible limiting step in warfarin elimination. Values for the apparent K_m of 30–200 nmol/ml were reported. These results bracket Wosilait's value, although the concentration levels were much higher; a concentration dependence for the apparent K_m was not indicated.

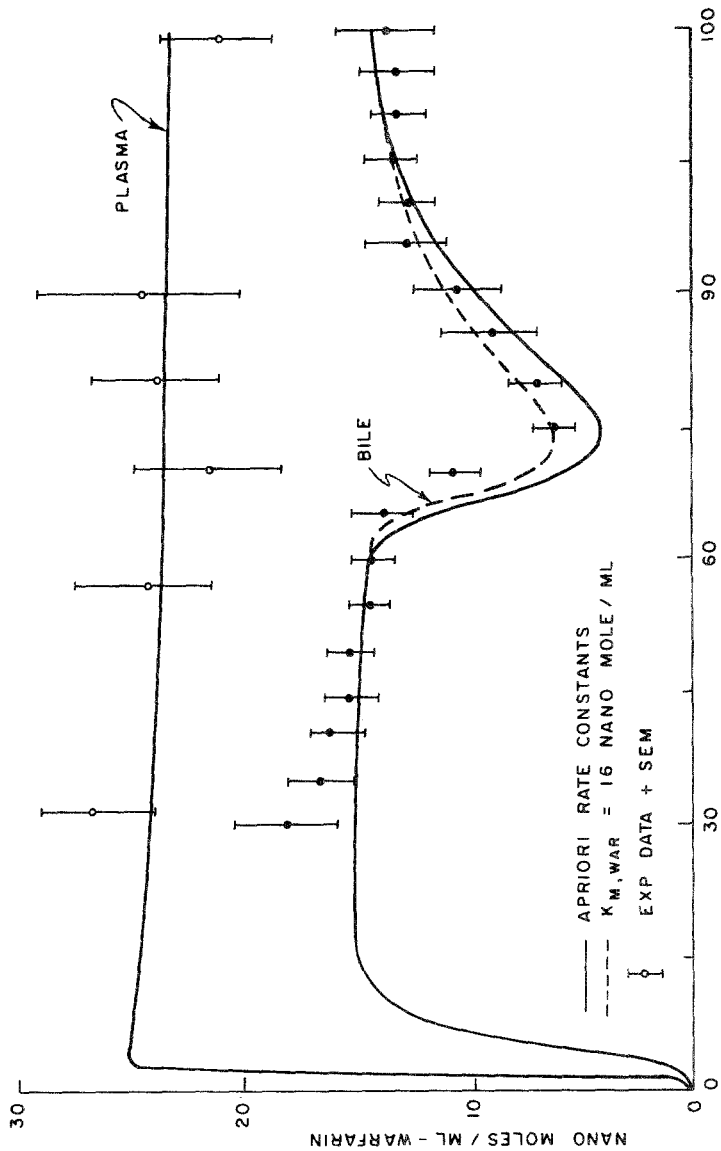

Fig. 2. Clearance of warfarin. [^{14}C]Warfarin (1.0 mg/kg, 2 μCi/kg) was injected intravenously at zero time and BSP (50 mg/kg) at 60 min. The experimental points for three rats are presented with SEM. The curves were computed using the simulation model. The dashed curve shows the weighted least-squares fit obtained by adjustment of K_m for warfarin and V_{max} for BSP (both the primary and secondary routes). There is negligible change in the plasma concentration of warfarin with the parameter changes.

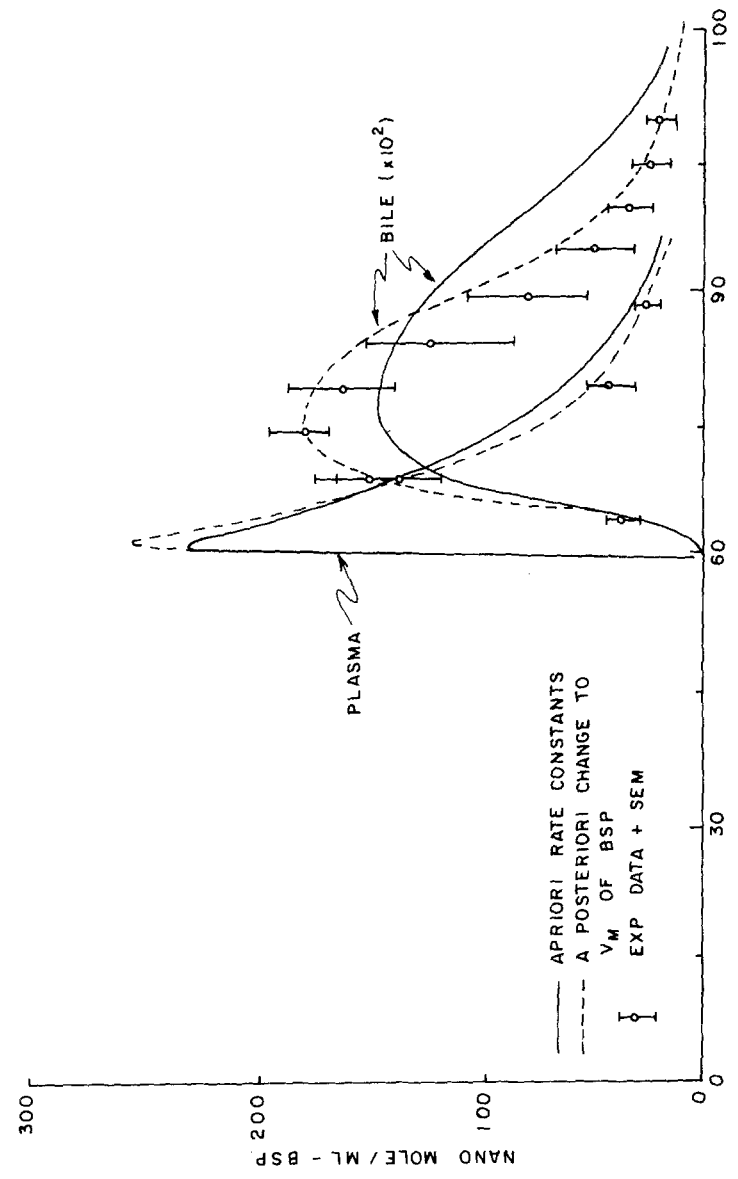

Fig. 3. Clearance of BSP in the interaction experiment. BSP (50 mg/kg) was injected intravenously 60 min after warfarin. Plasma and bile samples were collected and assayed at the indicated points ±SEM. The simulation using Clarenburg and Kao's parameters for BSP is shown by the solid line; the dashed line shows the weighted least-squares fit for K_m of warfarin and V_{max} (primary and secondary) for BSP.

The V_{max} for warfarin was computed from the rate of elimination during the first 60 min of these experiments (Fig. 2). Since BSP had not yet been injected, these data represent only a single drug system.

Thus all of the rate parameters needed to integrate the describing differential equations (Fig. 1) can be evaluated. Only data obtained from single drug systems have been used; no parameters specific for interaction effects have been added to the simulation model.

Computed values for the dynamic behavior of the interacting system using these *a priori* parameters are shown in Figs. 2 and 3 for warfarin and BSP, respectively. The concentration curves predicted by these calculations are very similar to those obtained from the measured values. The agreement is impressive when the diverse sources of the experimental data are considered.

The data fit may be improved, of course, by adjustment to the values of the parameters. In the derivation of the Michaelis–Menten equations it can be noted that the rate constants V_{max} are direct functions of the amount of enzyme present for the elimination reaction. A moderate discrepancy in the values of this rate constant would not be unexpected for different experimental animals even though similar rat strains, same sex, and similar weight ranges are used. A weighted least-squares analysis of the data indicated that an increase of about 25% for $V_{max,pri}$ and 50% for $V_{max,sec}$ over the values of Clarenburg and Kao (3) would significantly improve data fit. No change was needed in the value for V_{max} for warfarin since it was based on data from these experiments.

Some adjustment of the apparent K_m's is also needed to improve the fit of elimination rate depression during the interaction. If we assume that the values for K_m of BSP are not to be changed, a reduction of K_m for warfarin to about 16 nmol/ml would be indicated—a reduction to about one-fourth of the *a priori* value. While the apparent K_m involves several intrinsic chemical reaction parameters which would not be expected to change, this constant is also influenced by the degree of association with serum protein. Variation in serum protein levels for different animals could alter elimination rates and interactions.

With these *a posteriori* changes to the rate constants, considerable improvements in model fit were attained. The dashed curves in Figs. 2 and 3 show that the agreement between calculation and measurement is within the standard error of the mean for most data points.

Analysis of Elimination Kinetics

This type of computation can be a relatively sensitive measure of certain kinetic elimination parameters. The magnitude of the decrease in elimina-

tion rate during the peak interactive period (Fig. 2) is heavily dependent on the ratio of $K_{m,BSP}/K_{m,war}$ (see equations 5 and 6). Since the actual concentration in liver tissue was not monitored in these experiments the ratio of K_m's can be determined only to within a factor of the binding ratios. However, if the values of K_m for BSP and if the values used for binding ratios of warfarin *and* BSP are accepted as correct, then these experiments suggest that the apparent K_m for warfarin is lower than average values previously reported.

Because of the large intrasubject variability in these experiments, the formal statistical significance level of this difference is low. Moreover the effect could be due to a large K_m for BSP rather than smaller for warfarin, although the fit obtained for the rate of elimination of BSP over its relatively wide range of concentrations would tend to support the apparent K_m values used for that drug. The lower apparent K_m computed for warfarin may represent a real difference in the intrinsic rate parameters from previous data, or the discrepancy could be attributed to the effects of association equilibrium. Current work is directed toward the modeling of elimination reactions on the basis of free, unbound drug concentrations. There are indications that association equilibrium plays an important role in elimination kinetics.

CONCLUSIONS

It is apparent that physiologically based mathematical models can be very useful tools in the analysis of pharmacokinetic drug elimination data. The concepts, the mathematics, and the programs described here are sufficiently flexible that this approach could be readily applied to other paired and multiple combinations of drugs. By placing limitations on values of some fundamental chemical parameters, some elimination pathways can be confirmed or excluded. Further, it is possible to predict interactive effects from kinetic rate constants derived from the analysis of the behavior of the single drugs if competitive inhibition occurs in a shared pathway. A single interaction experiment such as is described in this article is sufficient to confirm the existence of the shared pathway.

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