Membrane Transport in Hepatic Clearance of Drugs I: Extended Hepatic Clearance Models Incorporating Concentration-Dependent Transport and Elimination Processes

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Purpose. The objective of the present study was to develop hepatic clearance models which incorporate a unidirectional carrier-mediated uptake and bidirectional diffusional transport processes for drug transport in the sinusoidal membrane of hepatocytes as well as nonlinear intrinsic elimination.

Methods. Two models were derived which view the liver as two separate compartments, i.e., sinusoid and hepatocyte. Model I assumes the instantaneous complete mixing of drugs within each compartment (similar to that of the "well-stirred" model), while model II assumes that the drug concentrations in both compartments decrease progressively in the direction of the hepatic blood flow path (similar to that of the "parallel-tube" model). Computer simulations were performed using a range of steady-state infusion rates for a substrate, while varying the V_{max} (capacity) and K_m (Michaelis-Menten constant) for the carrier-mediated uptake process, the diffusional clearance, the V_{max} and K_m for the intrinsic elimination process, blood flow and protein binding.

Results. Simulations in which V_{max} and K_m for the sinusoidal membrane transporter and the diffusional clearance were varied, demonstrated that these membrane transport processes could affect the clearance of drugs to a significant extent in both models. The estimates for clearance of substrates with the same pharmacokinetic parameters are always lower in model I than in model II, although the quantitative differences in parameter estimates between models varied, depending on the steady state infusion rates.

Conclusions. These more general hepatic clearance models will be most useful for describing the hepatic clearance of hydrophilic compounds, such as organic anions or cations, which exhibit facilitated uptake and limited membrane diffusion in hepatocytes.

KEY WORDS: hepatic clearance; pharmacokinetic models; facilitated transport; diffusion; nonlinear intrinsic clearance; computer simulation.

INTRODUCTION

Isolated hepatocyte, liver slice, liver perfusion and in vivo animal studies have demonstrated the nonlinear hepatic transport of several organic and inorganic substrates (1–4). Recently, studies with rat liver membrane vesicle preparations have elucidated the presence of several distinct carrier-mediated transport systems that are capable of transporting a wide range of endogenous/exogenous compounds, and a number of carrier proteins have been characterized (reviewed in refs. 5 and 6). However, most investigations that have examined the hepatic clearance of xenobiotics have utilized models in which the sinusoidal membrane transport processes for uptake and efflux are completely ignored (7) or regarded as linear (8).

Four types of models have been developed to describe the hepatic transport and clearance of substrates: the "compartmental model", the "parallel-tube model", the "distributed model" and the "dispersion model" (reviewed in ref. 9). The conventional compartmental model views the whole liver as a single equivalent hepatocyte, while the rest of the body is assumed to be a single well stirred plasma compartment. The membrane transport processes between these two compartments are considered to be linear. Several "extended compartment models", including the "well-stirred model", which have a sinusoidal blood and/or peripheral tissue compartments, have been developed to describe the hepatic transport of various compounds under linear kinetic conditions (8,10). The "undistributed sinusoidal perfusion or parallel tube model", assumes that the liver is composed of a series of identical and parallel tubes, along which the substrate concentration decreases progressively in the direction of the hepatic blood flow. This model assumes that uptake is a first order process and that the concentration governing the uptake rate is the logarithmic mean sinusoidal concentration (7). deLannoy *et al.* (11) have incorporated a changing sinusoidal drug concentration along the flow path in this model, to study the effects of a diffusional membrane barrier on hepatic clearance under linear conditions. The effects of a diffusional barrier were also studied for 4-methylumbelliferone and its sulfate and glucuronide conjugates (12,13). The "distributed model" views the liver as an array of parallel tubes, each receiving a fraction of blood flow or intrinsic clearance according to a specified distribution pattern. The membrane transport processes in this model have generally been considered to be linear (14). The "dispersion model" describes the hepatic clearance process in terms of bulk (convective) flow, axial dispersion (mixing of blood), and disappearance of solutes by elimination, assuming a linear (diffusional) membrane transport (15).

Most of the early transport studies using the described models were based on linear conditions, although different uptake and efflux rate constants could imply the possible presence of carrier-mediated transport systems in the sinusoidal membrane. Several approaches have been suggested to describe the nonlinear transport processes with various assumptions.

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GLOSSARY: C_b, C_s, C_t, drug concentration in the central, sinusoid and hepatocyte compartments, respectively; $C_{s,x}$, $C_{t,x}$, sinusoidal and hepatocyte drug concentration at any point x along the sinusoidal blood flow path (L), respectively; CL : hepatic clearance; CL_1, CL_2, CL_3, CL_d, uptake, efflux, intrinsic and diffusional clearances, respectively; $CL_{1,x}$, $CL_{2,x}$, $CL_{3,x}$, $CL_{d,x}$, uptake, efflux intrinsic and diffusional clearance at x, respectively; E, hepatic extraction ratio; f_b, f_t, fraction of drug unbound to blood and tissue components, respectively; $K_{m,1}$, $K_{m,3}$, Michaelis-Menten constant of the transporter and metabolizing enzyme, respectively; k_o, steady state infusion rate; Q, blood flow rate; V_b, V_s, V_t, volume of the central, sinusoidal and hepatocyte compartments, respectively; $V_{max,1}$, $V_{max,3}$, maximum capacity of the transporter and metabolizing enzyme, respectively; $V_{max,1,x}$ $V_{max,3x}$, capacity of the transporter and metabolizing enzyme at x, respectively.

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Deroubaix *et al.* (16) used a compartmental model to describe the saturation of the carrier-mediated hepatic transport of taurocholate (TC). In this study, they estimated V_{max} and K_m of a unidirectional facilitated transport system for TC, by simultaneously fitting the Michaelis-Menten equation to the sinusoidal concentrations and the uptake rates estimated from the compartment model at different steady state infusion rates of TC. Forker *et al.* (14) estimated the average rate constants for nonlinear transport processes by simultaneously analyzing plasma disappearance curves using a linear compartmental model. Greenway *et al.* (17) extended the parallel tube model to estimate V_{max} and K_m for hepatic uptake of galactose based on the logarithmic mean concentration. A recent publication by Geng *et al.* (18) has examined carrier-mediated hepatic transport using a parallel tube model.

In the present study, we have incorporated uptake-limited (by facilitated transport and by diffusion) and capacity-limited elimination into two hepatic clearance models which are similar to the conventional "well-stirred" and "parallel-tube" models (9). The purpose of the study was to derive equations for these models which would incorporate these concepts, and to illustrate the differences in the behavior of these models as pharmacokinetic parameters were varied. Additionally, in an accompanying paper, we have examined the influence of the heterogeneity of facilitated transport proteins on the hepatic disposition and clearance of substrates.

THEORY

Model I consists of three compartments: the central (reservoir), sinusoid, and hepatocyte, while Model II is composed of the sinusoid and hepatocyte compartments. The elimination of drug is assumed solely via nonlinear elimination and designated as "intrinsic clearance" by "metabolizing enzyme", an apparent unienzyme system, to simplify the model description. However, this elimination could represent hepatobiliary intrinsic clearance. The important elements and assumptions for these models are described as follows.

1. A unidirectional facilitated transport of substrate from the sinusoidal blood into hepatocytes by an evenly distributed carrier system as well as bidirectional passive diffusion.
2. A nonlinear intrinsic clearance by an evenly distributed metabolizing enzyme (or canalicular transport proteins).
3. Only unbound drug can traverse membranes and is subject to the intrinsic clearance.
4. Fractions of drug unbound to blood (f_b) and tissue components (f_t) are assumed constant throughout the liver.
5. There is no limitation on cofactor availability for both transporter and metabolizing enzyme.

Model I

Model I (Fig. 1), as in the conventional "well-stirred" model, assumes the instantaneous complete mixing of substrate within each compartment.

CL_1 represents the uptake clearance of substrate from the sinusoid compartment into the hepatocyte compartment via a carrier-mediated transport system and by passive diffusion. CL_2 is the diffusional efflux clearance from the hepatocyte into the sinusoidal compartment which can be expressed as a diffusional clearance (CL_d). CL_3 is the nonlinear intrinsic clearance. The maximum transport and intrinsic metabolism capacities are designated as $V_{max,1}$ and $V_{max,3}$, respectively. Michaelis-Menten constants for the transporter and metabolizing enzyme are designated $K_{m,1}$ and $K_{m,3}$, respectively. CL_1, CL_2 and CL_3 can be characterized as follows:

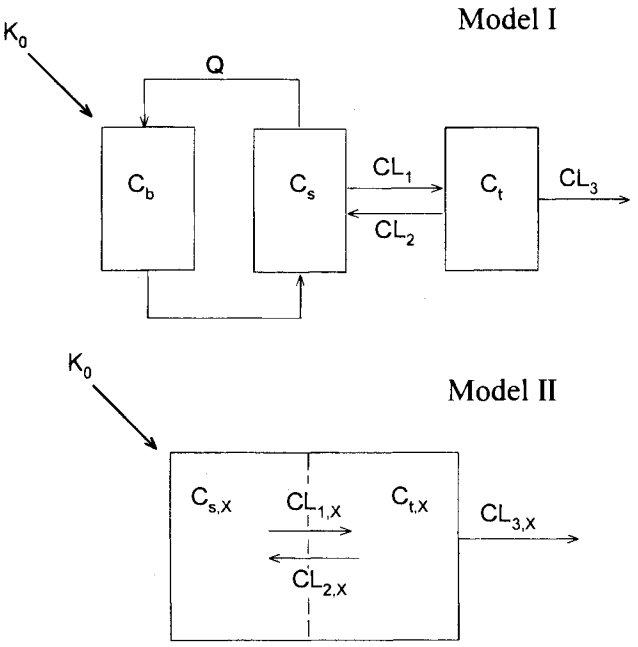

Fig. 1. Model I consists of three well-stirred compartments: central, sinusoid and hepatocyte. Q represents the blood flow rate. C_b, C_s and C_t are the concentrations of drug in the reservoir, sinusoid and hepatocyte compartments, respectively. CL_1, CL_2, and CL_3 represent the clearances for the uptake of drug from the sinusoid to hepatocyte (unidirectional facilitated transport and diffusion), the efflux of drug from the hepatocyte to sinusoid (diffusion), and the nonlinear intrinsic elimination of drug within hepatocytes, respectively.

Model II consists of two compartments: sinusoid and hepatocyte. $C_{s,x}$ and $C_{t,x}$ are the concentrations of the drug in the sinusoid, and hepatocyte compartments at any point x ($0 \leq x \leq L$) along the sinusoid flow path, L, respectively. The uptake and efflux of the drug between the sinusoid and hepatocyte compartments at x are characterized by $CL_{1,x}$ and $CL_{2,x}$, respectively. Nonlinear intrinsic elimination within the hepatocyte at x is denoted by $CL_{3,x}$.

$$CL_1 = \frac{V_{max,1}}{K_{m,1} + f_b C_s} + CL_d \tag{1}$$

$$CL_2 = CL_d \tag{2}$$

$$CL = \frac{V_{max,3}}{K_{m,3} + f_t C_t} \tag{3}$$

C_s and C_t are concentrations of drug in the sinusoid and hepatocyte compartments, respectively. The rate of change of drug amount in the hepatocyte compartment is zero at steady state, where V_t is the volume of the hepatocyte compartment (equation 4).

$$V_t \frac{dC_t}{dt} = CL_1 f_b C_s - (CL_2 + CL_3) f_t C_t = 0 \tag{4}$$

Combining equations 1, 2 and 3 into equation 4 results in a quadratic equation of C_s, which can be expressed as follows:

$$C_s = \frac{-b + \sqrt{b^2 - 4ac}}{2a} \quad (5)$$

where a, b and c are

$$a = f_b^2\, CL_d$$

$$b = f_b\left(V_{\max,1} + CL_d K_{m,1} - \left(CL_d + \frac{V_{\max,3}}{K_{m,3} + f_t C_t}\right) f_t C_t\right)$$

$$c = -K_{m,1}\left(CL_d + \frac{V_{\max,3}}{K_{m,3} + f_t C_t}\right) f_t C_t$$

At steady state, the infusion rate (k_o) into the central compartment is equal to the intrinsic elimination rate from the hepatocyte compartment (equation 6).

$$k_o = CL_3 f_t C_t = \frac{V_{\max,3}}{K_{m,3} + f_t C_t} f_t C_t \quad (6)$$

C_t can be expressed as follows.

$$C_t = \frac{k_o K_{m,3}}{f_t(V_{\max,3} - k_o)} \quad (7)$$

The rate of change of drug amount in the central compartment is zero at steady state.

$$V_b \frac{dC_b}{dt} = k_o + QC_s - QC_b = 0 \quad (8)$$

where V_b, C_b, and Q represent the volume and the concentration of drug in the central compartment, and the hepatic blood flow rate, respectively. The hepatic clearance (CL) and extraction ratio (E), at an infusion rate (k_o), can be estimated with equation 9, after incorporating equation 8.

$$CL = QE = \frac{Q(C_b - C_s)}{C_b} = \frac{k_o}{C_b} \quad (9)$$

Model II

In model II (Fig. 1), the liver is viewed as a series of identical parallel tubes with bulk blood flow along a common sinusoidal flow path. The drug is infused at a constant concentration into the liver. The rate of change of drug amount in the sinusoid compartment at any point x ($0 \le x \le L$) along the flow path, L, at steady state can be expressed as the following equation under steady state conditions.

$$\frac{V_s}{L}\frac{dC_{s,x}}{dt} = -Q\frac{dC_{s,x}}{dx} + \frac{1}{L}(CL_{2,x} f_t C_{t,x} - CL_{1,x} f_b C_{s,x}) = 0 \quad (10)$$

V_s is the volume of the sinusoid compartment, and $C_{s,x}$ and $C_{t,x}$ are the drug concentrations in the sinusoid and hepatocyte compartments at x, respectively.

In the hepatocyte compartment, the rate of change of drug amount can be expressed as in equation 11, where $CL_{3,x}$ is the intrinsic clearance at x.

$$\frac{V_t}{L}\frac{dC_{t,x}}{dt} = \frac{1}{L}(CL_{1,x} f_b C_{s,x} - CL_{2,x} f_t C_{t,x} - CL_{3,x} f_t C_{t,x}) = 0 \quad (11)$$

Combining equations 10 and 11 results in equation 12.

$$\frac{dC_{s,x}}{dx} = \frac{-CL_{3,x} f_t C_{t,x}}{QL} \quad (12)$$

$C_{s,0}$ (inlet concentration) and $C_{s,L}$ (outlet concentration) correspond to C_b and C_s in equation 9 for model I, respectively, for the estimation of CL.

METHODS

Simulations were performed, assuming constant infusion of drugs into the central compartment for model I or into the sinusoid compartment for model II. Numerical approximation of drug disappearance in the sinusoidal compartment for model II was performed using Euler's method (19) with 200 incremental steps for the numerical approximation (dx = 1/200 of flow path) at $C_{s,0}$ equivalent to C_b in model I over a range of infusion rates. Differences in the estimated values between using this method and PCNONLIN (Statistical Consultant Inc. Lexington, KY) were negligible.

For the reference conditions (Table 1), the primary uptake mechanism of drug into hepatocytes at low concentrations was assumed to be carrier-mediated, by setting a higher carrier-mediated uptake clearance (10 ml/min/g liver, $V_{max,1}$ and $K_{m,1}$ of 1 mg/min/g liver and 0.1 mg/ml, respectively), compared with CL_d (0.1 ml/min/g liver). A higher $V_{max,3}$ (10 mg/min/g liver) than $V_{max,1}$, with $K_{m,3}$ (0.1 mg/ml) equal to $K_{m,1}$ was assigned to assure that intrinsic clearance is not a rate-limiting step at low substrate concentrations. The reference blood flow rate was assigned a value of 1 ml/min/g liver, which is 10 fold slower than CL_1 at low concentrations. These reference values are similar to those estimated for hepatic transport of taurocholic acid in rats from the literature (16,20), except for $V_{max,3}$ (Table 1). The relatively high $V_{max,3}$ was used for the present simulations to allow illustration of gradual changes in the rate-limiting step in CL from blood flow to the membrane transport processes and eventually to intrinsic elimination. No binding of drug to blood and tissue components was assumed. Each simulation study was designed to illustrate the effects of changes in a particular pharmacokinetic parameter ($V_{max,1}$ (or $K_{m,1}$), CL_d, Q, $V_{max,3}$ (or $K_{m,3}$)) on CL over a range of infusion rates.

RESULTS

Figure 2 illustrates the change in rate-limiting steps in CL from Q, to CL_1 (carrier-mediated transport and then Cl_d once carrier-mediated transport became saturated), and eventually to CL_3 as k_o increased, under the designated reference conditions.

Figure 3 shows the effects of changes in $V_{max,1}$ on CL. When $V_{max,1}$ was small (0.01 mg/min/g liver), little change in

Table 1. Reference Parameters for a Hypothetical Substrate

Q ml/min/ g liver	$V_{max,1}$ mg/min/ g liver	$K_{m,1}$ mg/ml	CL_d ml/min/ g liver	$V_{max,3}$ mg/min/ g liver	$K_{m,3}$ mg/ml	f_b	f_t
1	1	0.1	0.1	10	0.1	1	1
	0.2	0.04	0.01	0.2	0.3		

Note: Values in the second row are the literature values for the hepatic transport of taurocholic acid in rats (16,20).

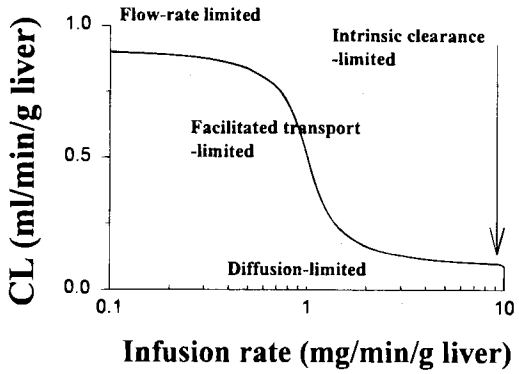

Fig. 2. Relationship between CL and k_o at the reference conditions (Table 1).

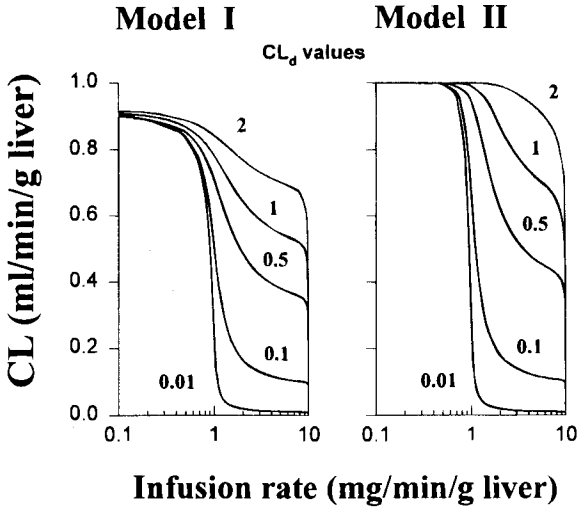

Fig. 4. Effects of varying Cl_d on the relationship between CL and k_o.

CL was observed as k_o was increased. As $V_{max,1}$ was increased, CL increased at low k_o, but there were distinct differences between the two models, with model II demonstrating higher clearance values than model I at $V_{max,1}$ values of 0.1 mg/min/g liver or greater. In both models, significant decreases in CL occurred when the transporter and metabolizing enzyme were saturated at high k_o. When $K_{m,1}$ was varied (from 0.01 to 10 mg/ml) so that CL_1/Q ranged from 0.1 to 100, similar CL profiles were obtained for the two models at the highest and lowest $K_{m,1}$ values (0.01 and 10 mg/ml), but at intermediate values (0.1 and 1 mg/ml) model II predicted higher CL values. Large changes in CL occurred as the sinusoidal membrane transporter became saturated (data not presented).

Figure 4 shows the effects of changes in CL_d (from 0.01 to 2 ml/min/g liver) on CL. At low k_o, CL was relatively unaffected by the changes in CL_d in both models. At higher k_o, CL precipitously declined at the lower values for CL_d, as CL became rate-limited by CL_d. Changes in CL at higher values for CL_d were more gradual.

Figures 5 shows the effects of Q changes on CL. At lower Q, Q represented a major rate-limiting step throughout the entire range of k_o except at very high k_o. At higher Q values, the rate-limiting step was influenced by CL_1 (reference value of $V_{max,1}$/$K_{m,1}$ is 10 ml/min/g liver) as well. Only small differences between the two models were apparent.

Figure 6 shows the effects of $V_{max,3}$ on CL. Model II estimated higher CL values for the same $V_{max,3}$ at low k_o than model I. At $V_{max,3}$ smaller than 1 mg/min/g liver, CL decreased abruptly as k_o approached $V_{max,3}$, the limit necessary to achieve a steady state condition (equation 10). With higher $V_{max,3}$, CL became linear over a range of k_o after a sharp decrease just prior to a k_o of 1 mg/min/g liver (the $V_{max,1}$ value). In both models, CL with $V_{max,3}$ of 100 mg/min/g liver overlapped that with a lower $V_{max,3}$ (10 mg/min/g liver) up to k_o of 10 mg/min/g liver, indicating that CL_3 was not rate-limiting.

Increases in $K_{m,3}$ had significant effects on CL over the entire range of k_o. The overall CL profiles at the different $K_{m,3}$ values were similar in both models with Model II estimates of CL being higher than those of Model I at low k_o. At a high $K_{m,3}$ (100 mg/ml), the low intrinsic clearance represents the rate-limiting step responsible for the pronounced decrease in CL (data not presented).

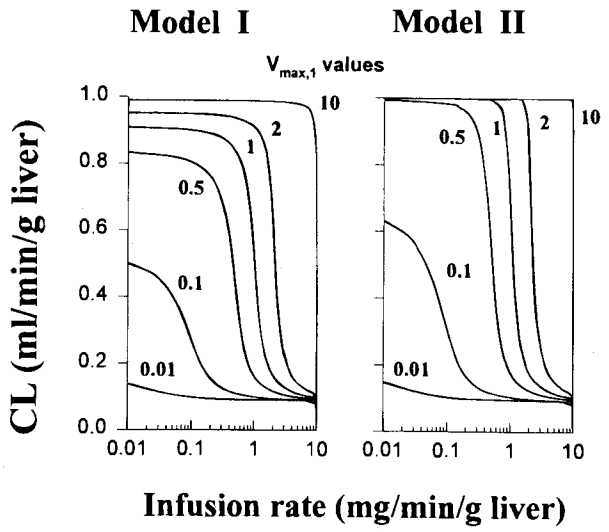

Fig. 3. Effects of varying $V_{max,1}$ on the relationship between CL and k_o.

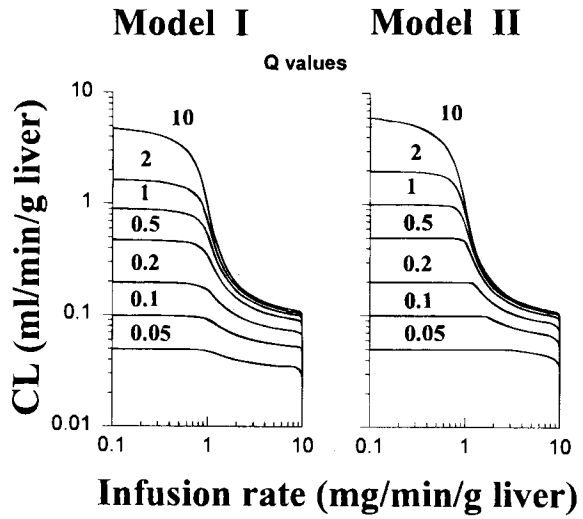

Fig. 5. Effects of varying Q on the relationship between CL and k_o.

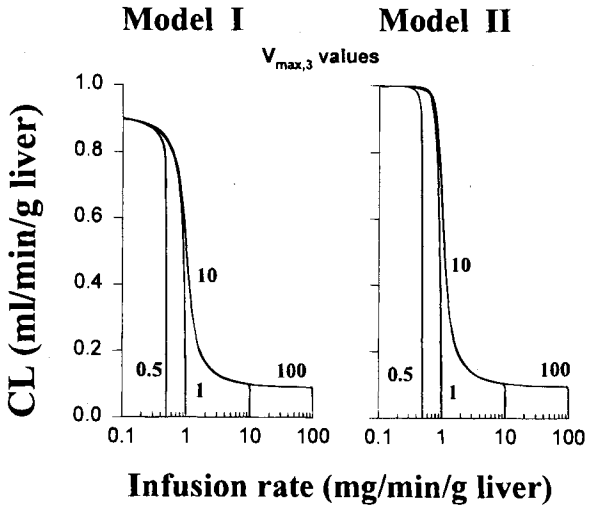

Fig. 6. Effects of varying $V_{max,3}$ on the relationship between CL and k_o.

DISCUSSION

The present theoretical work extends the "well-stirred" model, designated as model I, and the "parallel tube" model, designated as model II, to include carrier-mediated unidirectional uptake in the sinusoidal membrane and nonlinear elimination processes. It also represents an extension of the theoretical evaluation of transport published recently by Geng *et al.* (18).

CL can be described as equation 13 in model I or II (see derivation in Appendix).

$$CL = \underbrace{\frac{Qf_bCL_{i,app}}{Q + f_bCL_{i,app}}}_{\text{(model I)}} \text{ or } \underbrace{Q\left(1 - e^{-f_bCL_{i,app}/Q}\right)}_{\text{(model II)}} \quad (13)$$

where

$$CL_{i,app} = \frac{CL_1CL_3}{CL_2 + CL_3}$$

Equation 13 can be further simplified to equations reflecting two limiting conditions. First, if CL_1 and CL_2 are the same and much larger than CL_3 (i.e., a high permeability and no carrier-mediated transport system for substrates), then CL can be described as in equation 14 for the models.

$$CL = \underbrace{\frac{Qf_bCL_3}{Q + f_bCL_3}}_{\text{(model I)}} \text{ or } \underbrace{Q\left(1 - e^{-f_bCL_3/Q}\right)}_{\text{(model II)}} \quad (14)$$

These equations are the same as the equations describing the "well-stirred" and "parallel tube" models when a transport barrier for unbound drug across hepatocytes is absent and the enzyme activity is distributed evenly along the hepatic flow path. Depending on the relative magnitudes between Q and CL_3, CL can be flow rate-limited or intrinsic clearance-limited. For ionized and hydrophilic compounds which exhibit low membrane permeabilities or undergo transport into hepatocytes via facilitated transport systems, such that CL_2 is much smaller than CL_3, CL can be described as in equation 15.

$$CL = \underbrace{\frac{Qf_bCL_1}{Q + f_bCL_1}}_{\text{(model I)}} \text{ or } \underbrace{Q\left(1 - e^{-f_bCL_1/Q}\right)}_{\text{(model II)}} \quad (15)$$

CL is now independent of CL_3 and can be further defined as being either flow rate-limited or uptake clearance-limited, depending on the relative magnitudes of Q and CL_1.

Literature data support the contention that uptake can be rate-limiting for the hepatic clearance of ionized or hydrophilic compounds which exhibit low membrane permeabilities or utilize facilitated transport systems for hepatic uptake. Examples include propranolol (21), 4-methylumbelliferone (12), taurocholate (16,20), enalaprilat (11), quinaprilat (22), pravastatin (4), bromosulfophthalein glutathione conjugate (3), 4-methyumbelliferone glucuronide and sulfate conjugates (23), and the cyclic metabolite of rilmazafone (24). For example, a recent publication by Yamazaki *et al.* (4) demonstrated that the carrier-mediated uptake of the hydrophilic drug, pravastatin, represents the rate-limiting step in the hepatic elimination of the drug. The hepatic uptake of 1-propranolol, a lipophilic compound, is also rate-limiting at low doses (21), and it has been demonstrated that alterations in the hepatic extraction of propranolol in cirrhotic rats (25), and in rats with experimentally-induced renal failure (26), is due to impaired cellular influx. These latter studies emphasize the importance of evaluating uptake processes in order to correctly interpret disease-induced effects on hepatic clearance.

The simulation studies with changing $V_{max,1}$ (Fig. 3) (or $K_{m,1}$) illustrate the expected effects of disease, hormonal influences, competitive or noncompetitive drug or nutrient interactions or induction on drug substrate uptake into hepatocytes. For example, phenobarbital can selectively induce the electrogenic transport of glutathione and organic anions in rats (27), and the uptake of tetraethylammonium, which is transported into hepatocytes by a membrane potential-dependent pathway, can be inhibited by a range of tertiary and monoquaternary ammonium compounds (28). Induction or inhibition of transport processes will result in a change in substrate flux, but can also result in changing the rate-limiting step in a substrate's hepatic clearance.

In summary, the present simulation studies were able to illustrate the effects of the membrane transport processes, unidirectional carrier-mediated transport and diffusion in the hepatic sinusoidal membrane, on the hepatic clearance of drugs in the extended "well-stirred" and "parallel-tube" models. In addition, the effects of other physiological processes, including blood flow, protein binding and nonlinear elimination, on hepatic clearance were also investigated in the presence of a transport barrier for substrates. Overall, estimated CL values obtained using the two models were either similar, or were higher in model II compared with model I. The higher values predicted by model II may be due to the differences in drug concentrations estimated in the sinusoid and hepatocyte compartments between the models, and perhaps more importantly, due to the presence of the sinusoidal concentration gradient in model II. These more generalized models for the description of the drug hepatic clearance would be most applicable for hydrophilic organic anions and cations for which facilitated transport processes and limited membrane transport have been described.

APPENDIX

In model I, the mass balance in sinusoid compartment at steady state results in the following equation:

$$V_s \frac{dC_s}{dt} = QC_p - QC_s - CL_1 f_b C_s + CL_2 f_t C_t = 0 \quad (16)$$

V_s is the volume of sinusoid compartment. Solving equation 4 for steady state, C_t gives

$$C_t = \frac{CL_1 f_b C_s}{f_t(CL_2 + CL_3)} \tag{17}$$

Substituting equation 17 into equation 16 gives,

$$C_s = \frac{Q(CL_2 + CL_3)C_b}{Q(CL_2 + CL_3) + f_b CL_1 CL_3} \tag{18}$$

From equations 9 and 18, *CL* can be expressed as;

$$CL = \frac{Qf_b CL_1 CL_3}{Q(CL_2 + CL_3) + f_b CL_1 CL_3)} \tag{19}$$

In model II, at steady state, the rate of drug disappearance over an increment dx from x is represented by equation 20.

$$Q\frac{dC_{s,x}}{dx} = \frac{1}{L}(CL_{2,x} f_t C_{t,x} - CL_{1,x} f_b C_{s,x}) \tag{20}$$

At steady state the uptake rate of drug from sinusoidal blood into hepatocytes is equal to the sum of the efflux and intrinsic elimination rates from hepatocytes (equation 21)

$$CL_{1,x} f_b C_{s,x} = CL_{2,x} f_t C_{t,x} + CL_{3,x} f_t C_{t,x} \tag{21}$$

From equation 21, $C_{t,x}$ can be expressed as in equation 22.

$$C_{t,x} = \frac{CL_{1,x} f_b C_{s,x}}{f_t(CL_{2,x} + CL_{3,x})} \tag{22}$$

Substituting equation 22 into equation 20 and rearranging gives equation 23.

$$\frac{dC_{s,x}}{C_{s,x}} = \frac{-f_b CL_{1,x} CL_{3,x}}{QL(CL_{2,x} + CL_{3,x})} dx \tag{23}$$

Assuming that the uptake, efflux, and intrinsic clearances at x are equal to their respective length-averaged clearances, equation 24 can be obtained after a definite integration of equation 23 from 0 to L for x.

$$\ln\left(\frac{C_{s,L}}{C_{s,0}}\right) = \frac{-f_b CL_1 CL_3}{Q(CL_2 + CL_3)} \tag{24}$$

From equation 24, E and CL can be expressed as in the following equations.

$$E = \frac{C_{s,0} - C_{s,L}}{C_{s,}} = 1 - e^{f_b CL_1 CL_3/Q(CL_2 + CL_3)} \tag{25}$$

$$CL = Q(1 - e^{-f_b CL_1 CL_3/Q(CL_2 + CL_3)} \tag{26}$$

REFERENCES

1. S. Iida, T. Mizuma, N. Sakuma, M. Hayashi, and S. Awazu. *Drug Metab. Disp.* **17**:341–344 (1989).
2. H. M. Solomon and L.S. Schanker. *Biochem. Pharmacol.* **12**:621–626 (1963).
3. W. P. Geng, A. J. Schwab, C. A. Goresky, and K. S. Pang. *Hepatology* **22**:1188–1207, 1995.
4. M. Yamazaki, S. Akiyama, R. Nishigaki, and Y. Sugiyama. *Pharm. Res.* **13**:1559–1564, 1996.
5. R. P. J. Oude Elferink, D. K. F. Meijer, F. Kuipers, P. L. M. Jansen, A. K. Groen, and G. M. M. Groothuis. *Biochim. Biophy. Acta* **1241**:215–268 (1995).
6. M. Vore. In F. C. Kauffman (ed), *Conjugation-Deconjugation Reactions in Drug Metabolism and Toxicity,* Springer-Verlag, New York, 1994, pp. 311–338.
7. L. Bass, S. Keiding, K. Winkler, and N. Tygstrup. *J. Theor. Biol.* **61**:393–409 (1976).
8. M. Yokota, T. Iga, Y. Sugiyama, A. Suyama, S. Awazu, and M. Hanano. *J. Pharm. Dyn.* **4**:287–293, (1981).
9. B. A. Saville, M. R. Gray, and Y. K. Tam. *Drug Metab. Rev.* **24**:49–88 (1992).
10. R. Kroker, M. S. Anwer, and D. Hegner. *Naunyn-Schmiedebergs Arch. Exp. Pathol. Pharmakol.* **303**:287–293 (1978).
11. I. A. M. deLannoy and K. S. Pang. *Drug. Metab. Dispos.* **14**:513–520 (1986).
12. S. Miyauchi, Y. Sugiyama, Y. Sawada, K. Morita, T. Iga, and N. Hanano. *J. Pharmacokin. Biopharm.* **15**:25–38 (1987).
13. S. Miyauchi, Y. Sugiyama, T. Iga, and M. Hanano. *J. Pharm. Sci.* **77**:688–692 (1988).
14. E. L. Forker and B. A. Luxon. *Am. J. Physiol.* **244**:G573–G577 (1983).
15. M. S. Roberts and M. Rowland. *J. Pharmacokin. Biopharm.* **14**:227–260 (1986).
16. X. Deroubaix, T. Coche, E. Depiereux, and E. Feytmans. *Am. J. Physiol.* **260**:G189–G196 (1991).
17. C. V. Greenway and F. J. Burczynski. *Can. J. Physiol. Pharmacol.* **65**:1193–1199 (1987).
18. W. P. Geng, K. Poon, and K. S. Pang. *J. Pharmacokin. Biopharm.* **23**:347–378 (1995).
19. R. B. Hornbeck. *Numerical Methods,* Quantum Publishers, Inc., New York, 1975, pp. 185–226.
20. X. Deroubaix, T. Coche, Depiereux, and E. Feytmans. *Am. J. Physiol.* **257**:G210–G220 (1989).
21. S. Miyauchi, Y. Sawada, T. Iga, M. Hanano, and Y. Sugiyama. *Biol. Pharm. Bull.* **16**:1019–1024, 1993.
22. M. E. Morris and Y. Li. *Pharm. Res.* **8**:S-322, 1991.
23. S. Miyauchi, Y. Sugiyama, T. Iga, and M. Hanano. *J. Pharm. Sci.* **77**:688–692, 1988.
24. N. Muranushi, S. Miyauchi, H. Suzuki, Y. Sugiyama, M. Hanano, H. Kinoshita, T. Oguma, and H. Yamada. *Biopharm. Drug Dispos.* **14**:279–290 (1993).
25. L. Gariepy, D. Fenyves, I. Kassissia, and J.-P. Villenueve. *Hepatology* **18**:823–831 (1993).
26. R. Hori, K. Okumura, M. Yasuhara, and H. Katayama. *Biochem. Pharmacol.* **34**:2679–2683 (1985).
27. J. C. Fernandez-Checa, M. Ookhtens, and N. Kaplowitz. *J. Biol. Chem.* **268**(15):10836–10841 (1993).
28. Y. Kwon and M. E. Morris. *Pharm. Res.* **12**:1109–1114 (1995).