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Report

Influence of Unbound Fraction and Perfusate Flow Rate on Taurocholate Elimination by Perfused Rat Liver: Applicability of Three Distributed Models

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The applicability of three different distributed models to the kinetics of elimination of taurocholate by isolated perfused rat liver was examined by fitting each model to literature data. Each of the models was able to predict the effect of changing hepatic blood flow on elimination, but only the model which incorporates separate density functions describing the degree of sinusoidal heterogeneity of blood flow and intrinsic clearance was able to predict the effect of changing unbound fraction on elimination.

KEY WORDS: distributed model; isolated perfused rat liver; protein binding; hepatic blood flow rate; taurocholate.

INTRODUCTION

Numerous advances have been made in recent years toward our understanding of the kinetics of hepatic elimination. The first models developed were the undistributed sinusoidal, or parallel-tube, model and the venous equilibrium, or well-stirred, model (1). These models are operationally accurate in certain circumstances but are not very realistic physiologically (2). More recently, several distributed sinusoidal models have been advanced which represent the hepatic sinusoids as a collection of segregated tubes with different properties (3–5). These distributed models show promise but their compatibility with experimental observations has not been investigated extensively.

Discrimination among models of hepatic elimination can, in theory, be achieved in the isolated perfused rat liver preparation by measuring the hepatic availability of test substance (i.e., ratio of hepatic venous/portal venous concentrations) at different perfusate flow rates (Q) or at different unbound fractions (f_u) of the substance in the perfusate (1,6). Using the latter experimental design, the hepatic elimination of the bile salt, taurocholate, was best described by a distributed model in which the radial or transverse heterogeneity of both sinusoidal Q and sinusoidal intrinsic clearance (CL_{int}) was very large (7). This model, proposed by Sawada *et al.* (5), assumes that the transverse heterogeneity of sinusoidal Q is independent from that of sinusoidal CL_{int}. The distributed model of Forker and Luxon (4), in which transverse heterogeneity of only sinusoidal Q is assumed, did not fit the taurocholate data well. The distributed model of Bass *et al.* (3), in which transverse heterogeneity of the ratio of sinusoidal CL_{int}/Q is assumed, was not fitted to the taurocholate data by Smallwood *et al.* (7). Because earlier it had been shown that this model was appropriate only for small degrees of heterogeneity (8).

Using the alternate experimental design, Pries *et al.* (9) measured the hepatic availability of taurocholate at different values of Q in the isolated perfused rat liver preparation. Bass *et al.* (10,11) fitted their distributed model to these data and, in contrast to the results of Smallwood *et al.* (7), obtained a good fit with a low degree of transverse heterogeneity of CL_{int}/Q. In this communication, these apparently conflicting findings are reconciled.

MATERIALS AND METHODS

Distributed Model of Forker and Luxon (4) (Model 1). In this model m sinusoids with identical CL_{int}, but with a range of flows between zero and twice the mean flow, are grouped in n equally spaced flow classes. The fraction α_i of sinusoids in the ith class is calculated from the normal density function as approximately the product of midpoint frequency and class width,

$$\alpha_1 = \exp^{-1/2}\left[\frac{2i-1-n}{n \cdot cv'}\right]^2 \Big/ \sum_{i=1}^{n} \exp^{-1/2}\left[\frac{2i-1-n}{n \cdot cv'}\right]^2 \quad (1)$$

where cv' is the coefficient of variation for the normal distribution. In the single-pass, isolated perfused rat liver preparation, the steady-state availability (F) for a constant portal venous drug concentration is given by

$$F = \sum_{i=1}^{n} \frac{\alpha_i(2i-1)}{n} \exp\left[\frac{-f_u \cdot CL_{int} \cdot n}{Q \cdot (2i-1)}\right] \quad (2)$$

Distributed Model of Sawada et al. *(5) (Model 2).* This model incorporates transverse heterogeneity in both Q and

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CL_{int} in the sinusoids by grouping sinusoids with capillary transit time in the ith class and CL_{int} in the jth class. F for this model is given by

$$F = \sum_{i=1}^{n} \left\{ \sum_{j=1}^{n} \left[\frac{\alpha i (2i-1)\epsilon_i}{n} \exp \frac{-(2j-1) f_u \, CL_{int}}{(2i-1) \; Q} \right] \right\} \quad (3)$$

where αi is given by Eq. (1) and ϵ_i is the fraction of sinusoids in the ith flow class having that fraction of CL_{int} in the jth class, according to

$$\epsilon_j = \exp^{-1/2} \left[\frac{2i-1-n}{n \cdot cv''} \right]^2 \Big/ \sum_{i=1}^{n} \exp^{-1/2} \left[\frac{2i-1-n}{n \cdot cv''} \right]^2 \quad (4)$$

where cv'' is the coefficient of variation for the normal distribution describing the frequency distribution of CL_{int}.

Distributed Model of Bass et al. *(3) (Model 3).* In this model, sinusoids with identical values of CL_{int}/Q are classified into n groups and F is given by

$$F = \exp \left[-\frac{f_u \, CL_{int}}{Q} + 0.5 \, (cv)^2 \left(\frac{f_u \, CL_{int}}{Q} \right)^2 - R \right] \quad (5)$$

where cv is the coefficient of variation of the distribution of CL_{int}/Q among the n groups of sinusoids. The term R in Eq. *(5)* is a remainder term for which upper and lower bounds have been defined provided that cv is small enough for the quantity v_o to be positive, where v_o is given by (8)

$$v_o = \frac{1}{cv} - \frac{cv \, f_u \, CL_{int}}{Q} \quad (6)$$

For Eq. (5) to be valid, the value of R must also be small compared with the preceding term $[0.5(cv)^2(f_u \, CL_{int}/Q)^2]$ in Eq. *(5) (8).*

The report of Pries *et al.* (9) contained a single value of F for taurocholate for each of 31 isolated perfused rat liver preparations, in which Q ranged from 0.66 to 3.57 ml/min/g liver and the perfusate albumin concentration was 30 g/liter. This albumin concentration corresponds to a taurocholate f_u of approximately 0.13 (7). In fitting model 3 to these data, Bass *et al.* (10,11) excluded the 12 experiments in which Q was less than 1.1 ml/min/g liver. This was because of concern that possible closure of sinusoids at low flow could compromise the physiological stability of the preparation. In the present study, the three distributed models [Eqs. *(2),* (3), and *(5)*] were each fitted to these 24 pairs of F versus Q data from Pries *et al.* (9). Fitting was by nonlinear least-squares regression using the Funfit program (12). The fitted parameter values were then used in Eqs. *(2), (3),* and *(5)* to produce a simulation for each model describing the effect of changing f_u on F at constant Q (32 ml/min) for a 7.2-g liver. These simulations were then compared with the mean data of Smallwood *et al.* (7).

RESULTS AND DISCUSSION

The fits of the three distributed models to the data of Pries *et al.* (9) are shown in Fig. 1 and the minimum residual sums of squares and parameter values are shown in Table I.

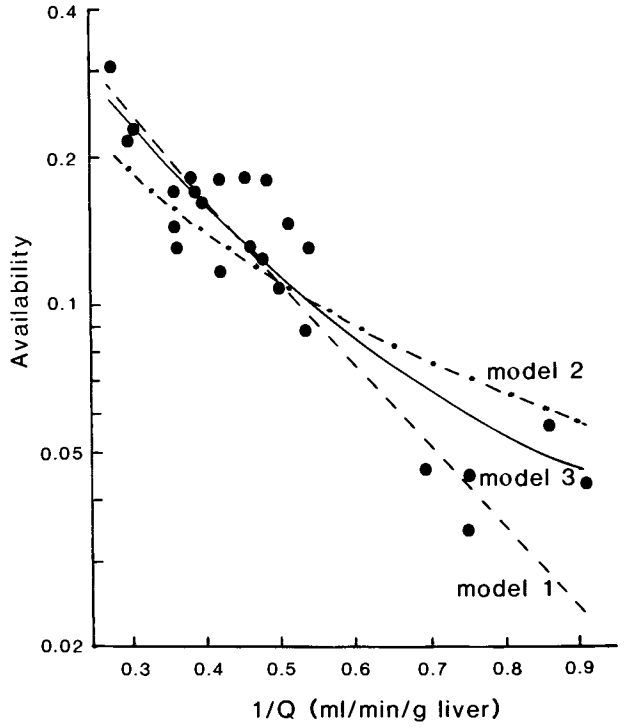

Fig. 1. Effect of perfusate flow rate (Q) on availability of taurocholate in the isolated perfused rat liver preparation from Pries *et al.* (9) (●). Also shown are the fits for the distributed models of Forker and Luxon (4) [Eq. (2), model 1], Sawada *et al.* (5) [Eq. (3), model 2], and Bass *et al.* (3) [Eq. (5), model 3].

A weighting factor of unity was used in fitting models 1 and 3, whereas a weighting factor of $1/F$ gave the best fit for model 2. In Fig. 1, log$F$ was plotted against $1/Q$ because the lower the degree of heterogeneity, the closer this relationship will be to a straight line. The fitted value of cv for model 3 of 0.39 is comparable to that obtained by Bass *et al.* (10,11) (0.35) with these same data. All three models fitted the data well (Fig. 1), but using the criterion of sums of squares, model 3 gave a slightly better fit.

An important property of a mathematical model is its ability to make accurate predictions. The predictive ability of the three distributed models was examined by using the parameter values in Table I as input for simulations of the relationship between taurocholate F and f_u. These simulations are shown in Fig. 2 together with mean data from seven single-pass experiments with taurocholate in the isolated perfused rat liver from Smallwood *et al.* (7).

In Fig. 2, the predictions of model 2 were in reasonable

Table I. Fits of Distributed Models to Taurocholate Data of Pries *et al.* (9)

Model	MRSS[a]	CL_{int} (ml/min/g liver)	cv'	cv''	cv
1	0.0227	46.6	11.1	—	—
2	0.0236	85.3	10^4	1.32	—
3	0.0205	41.8	—	—	0.39

[a] Minimum residual sum of squares.

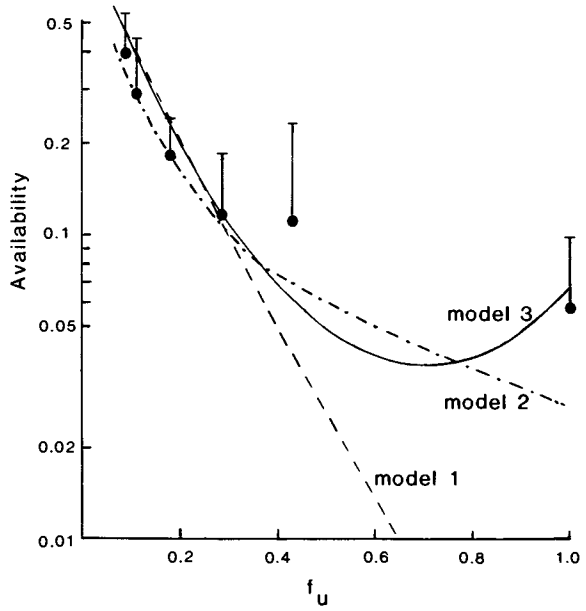

Fig. 2. Effect of unbound fraction (f_u) on availability of taurocholate in the isolated perfused rat liver preparation, showing mean data (●) and 95% confidence limit, from Smallwood *et al.* (7). Also shown are the curves predicted by model 1 [Eq. (2)], model 2 [Eq. (3)], and model 3 [Eq. (5)], using the parameters in Table I.

agreement with the experimental data. In contrast, neither model 1 nor model 3 predicted the curvilinear decrease of log$F$ with increasing f_u (Fig. 2). Model 1 predicted an almost linear decline, similar to that expected from the undistributed sinusoidal model, and model 3 predicted an increase in F at high f_u.

The anomolous behavior of model 3 in Fig. 2 is due to the violation of the requirement that, for this model to be valid, the quantity v_o in Eq. (6) must be positive. According to Eq. (6), v_o is positive only for $f_u < 0.7$. Therefore, model 3 is incompatible with the experimental data shown in Fig. 2. The calculation of f_u for the study by Pries *et al.* (9) and differences in experimental conditions (e.g., flow rates) between the two studies may add some uncertainty to the present analysis. However, the anomolous behavior of model 3 has been identified previously (18), where it was shown that in addition to this behavior becoming more marked as cv increases (8), it also becomes more marked as CL_{int} increases (18).

An underlying assumption of the three models is that hepatic elimination is proportional to f_u [Eqs. (2), (3), and (5)]. However, there is evidence of a greater apparent uptake rate of unbound ligand in the presence of tight binding of ligand to albumin than in the absence of albumin. This has led to the hypothesis that albumin may facilitate the hepatic uptake of unbound ligands (13–16), which would invalidate the assumption of models 1–3 that elimination is proportional to f_u. It should be noted that the hypothesis of albumin-facilitated uptake was originally put forward, not on the basis of direct experimental observation, but because of the incompatibility of model 1 with F versus f_u data, as in Fig. 2 (13) and the finding of a nonlinear relationship between hepatic uptake rate and unbound ligand concentration entering the liver (14). However, the need to invoke a mechanism involving the facilitation of uptake by albumin was diminished when it was found that model 2 fitted F versus f_u data very well (7) and that a nonlinear relationship between uptake rate and influent unbound ligand concentration is also consistent with uptake in which albumin plays no special role (17). The question of albumin-facilitated uptake is therefore controversial.

In conclusion, models 1–3 were all compatible with the experimental data in which Q was changed (Fig. 1), i.e., this experimental design was not able to discriminate among the models. In contrast, changing f_u provided much better discrimination among the models (Fig. 2) and showed that models 1 and 3 were inappropriate. Until more direct measurements of events within the sinusoids become available, model 2 appears to remain a viable model of hepatic elimination.

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