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Correlation between in-vitro microsomal enzyme activity and whole organ hepatic elimination kinetics: analysis with a dispersion model

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A new model, the *dispersion model* of hepatic elimination, is applied to the correlation between in-vitro microsomal data and corresponding rat isolated perfused liver data for a number of drugs reported in the literature, whose extraction ratio varies over the range of 0·01 to 0·995. The dispersion model described the data better than either the 'well-stirred' model or the 'parallel-tube' model, two other widely used models of hepatic elimination. The experimental data support the concept that elimination of solutes is affected by is a considerable dispersion on passage through the liver.

Hepatic drug clearance is mainly determined by the specific enzyme activity in individual hepatocytes and the delivery of drug and cosubstrates to the enzymatic sites. In recent years, considerable progress has been made in correlating the metabolic rate of drugs in microsomal enzymes in-vitro with observed in-vivo clearances (Yih 1976; Billings et al 1977; Rane et al 1977; Collins et al 1978; Blom et al 1982). However, good correlations are only apparent for drugs with low extraction ratios. At high extraction ratios, the elimination of drugs in-vivo and in the isolated perfused liver appears to be more rapid than would be predicted from in-vitro studies (Billings et al 1977; Rane et al 1977).

The agreement between predicted and observed clearances is highly dependent on the model used to extrapolate in-vitro data to an in-vivo situation accounting for the many determinants of drug delivery to the enzyme system. These determinants include hepatic blood flow (Goresky 1963; Wilkinson & Shand 1975; Pang & Rowland 1977a), fraction of drug unbound in blood (Pang & Rowland 1977a), transport of drug across the sinusoidal membrane into the cell (Goresky et al 1983) and the nature of the vasculature in the liver (Roberts & Rowland 1985). Only one model, the 'well-stirred' model, appears to have been used to correlate in-vitro metabolism data to in-vivo clearance data (Rane et al 1977; Collins et al 1978; Blom et al 1982). The 'well-stirred' model assumes a uniform concentration of drug in a single well mixed compartment in which cellular barriers and structural hepatic morphology do not exist. Deviations between predicted and observed in-vivo observations are therefore more likely when elimination is influenced by the organization of cells and microvasculature in the liver. Another model of hepatic elimination is the 'parallel-tube' or 'sinusoidal perfusion' model, which assumes that the liver is composed of a series of cylinders with movement of material down the tube in a plug-flow behaviour (Brauer et al 1956; Pang & Rowland 1977a). This model too, does not accord with the complex events occurring in the liver.

One model which accounts for the effects of hepatic morphology on drug extraction is the dispersion model (Roberts & Rowland 1985). This model is based on the variation in residence times of blood in the liver as evidenced by the dispersed output concentration-time profile of labelled red blood cells injected as a bolus into the liver. The distributed tube model (Bass et al 1978) also predicts that extraction can be affected by variation in blood velocities among sinusoids. In this paper, we evaluate the ability of the dispersion model to accommodate published rat microsomal and isolated perfused liver data, for drugs whose hepatic extraction ratio extends over the entire range of 0·01 to 0·995.

THEORY

Hepatic drug metabolism is determined by intrinsic enzyme activity and the concentration of drug delivered to the enzyme. Intrinsic enzyme activity can be assessed from the metabolism rates of drugs incubated with microsomal enzymes or hepatocytes in-vitro. The intrinsic enzyme activity (intrinsic clearance, CL_{int}) can be expressed in terms of the

* Correspondence.

Michaelis-Menten constants for a given drug. At low concentrations (Gillette 1971),

$$CL_{int} = \sum_{i=1}^{n} \frac{V_{m,i}}{K_{m,i}} \quad (1)$$

where $V_{m,i}$ and $K_{m,i}$ are the Michaelis-Menten constants for the ith enzyme type present.

The dispersion model is characterized by two dimensionless parameters, the *dispersion number*, D_N, and the *efficiency number*, R_N. The dispersion number is a measure of the dispersion or spread in residence times of solute molecules moving through the liver, caused by variations in velocity and path lengths travelled by elements of blood, and by both branching and interconnection of sinusoids. The efficiency number is a measure of the rate at which solute is irreversibly removed from the blood passing through the liver. We define the relationship between R_N and the permeability coefficient of the hepatocyte wall to drug (P), the fraction of drug in blood unbound (fu_b), and the blood or perfusate flow rate to the liver (Q), by the equation:

$$R_N = \frac{fu_b P CL_{int}}{Q(P + CL_{int})} \quad (2)$$

Consider now the liver being perfused with a constant concentration of solute. The dispersion model expresses the transport of solute molecules in the liver in terms of bulk (convective) flow, axial dispersion (mixing of blood) and disappearance by elimination. At steady state, for a first-order reaction, the following rate equation holds (Levenspiel 1972):

$$\underset{\text{Axial dispersion}}{D_{ax}\frac{d^2C}{dx^2}} - \underset{\text{Convective flow}}{v\frac{dC}{dx}} - \underset{\text{Elimination}}{kC} = 0 \quad (3)$$

where D_{ax} is the axial mixing or dispersion coefficient, v is the mean velocity of blood in the liver, C is the concentration of solute at distance x in the liver (of length L) and k is the first-order rate constant for irreversible removal of solute. Equation 3 can be restated in dimensionless terms $C = C/C_{in}$ (where C_{in} is the input concentration), $z = x/L$, $D_N = D_{ax}/vL$, and $R_N = kL/v$:

$$\frac{d^2C}{dz^2} - \frac{1}{D_N}\frac{dC}{dz} - \frac{R_N}{D_N}C = 0 \quad (4)$$

where z is the fraction of the distance along the liver, and C is the concentration of solute at point z, normalized to the entering solute concentration. Equation 4 has been solved analytically by Wehner & Wilhelm (1956) using boundary conditions which ensure a continuous concentration-distance profile at the entrance and exit of the liver. The complete solution is

$$F = \frac{4a}{(1+a)^2 \exp[(a-1)/2D_N] - (1-a)^2 \exp[-(a+1)/2D_N]} \quad (5)$$

where F is the ratio of output to input concentrations of solute, i.e. the availability of substance across the liver, and $a = (1 + 4R_N D_N)^{\frac{1}{2}}$.

For $D_N < 1$ and all likely values of R_N (0–20), equation (5) may be approximated to

$$F \simeq \frac{4a}{(1+a)^2} \exp\left[\frac{(1-a)}{2D_N}\right] \quad (6)$$

To use microsomally determined intrinsic clearances in our estimation of F we also assume that membrane permeability is not rate-limiting i.e. $P \gg CL_{int}$, so that the efficiency number becomes

$$R_N = \frac{fu_b CL_{int}}{Q} \quad (7)$$

The 'well-stirred' model referred to in earlier work (Rane et al 1977) is an asymptotic form of equation 5 corresponding to infinite dispersion ($D_N \rightarrow \infty$):

$$F = \frac{Q}{Q + fu_b CL_{int}} \quad (8)$$

The other asymptotic form, when no dispersion occurs ($D_N \rightarrow 0$), is the 'parallel-tube' model (Brauer et al 1956; Pang & Rowland 1977a) according to which

$$F = e^{-(fu_b CL_{int}/Q)} \quad (9)$$

Equations 5, 6, 8 and 9 can also be expressed in terms of extraction ratio (E) and clearance (CL), by recognizing their equivalent forms:

$$CL = QE = Q(1 - F) \quad (10)$$

METHODS

The data used were gathered from the literature and apply exclusively to the rat. The data, for drugs whose extraction ratio varied over the range 0·01–0·995, were obtained from liver microsome studies and from studies in rat isolated perfused livers.

Values for Michaelis Menten constants (V_m, K_m) and hence CL_{int} were determined from the liver microsome studies. The V_m values are usually reported in terms of rate of metabolism (mg microsomal protein)$^{-1}$. These values were corrected for an estimated 30% loss of microsomal activity in the supernatant and multiplied by 50 to give the metabolic rate g^{-1} of liver, as suggested by Rane et al

(1977). In the isolated perfused liver studies, the fraction of drug unbound in perfusate was assumed to be unity if no protein was present and the further assumption made was that a total perfusate flow of 10 ml min^{-1} to the liver was equivalent to 1·0 ml min^{-1} g^{-1} liver. A value of R_N for each drug was calculated from CL_{int} (from the microsomal data), fu_b, and Q.

The dispersion parameter, D_N, was estimated by regressing the set of observed bioavailability values against equation 6, using the non-linear regression program ELSFIT and a full variance model (Sheiner 1983). The predicted values of F for each drug associated with the 'well-stirred' and 'parallel-tube' models were calculated from equations 8 and 9, respectively.

RESULTS AND DISCUSSION

Table 1 shows the data used to estimate values of R_N for a number of drugs and gives the corresponding observed availabilities in rat perfused liver preparations. Availability, rather than extraction ratio, was chosen as the dependent variable because it is directly proportional to the actual measurement, C_{out} ($F = C_{out}/C_{in}$) and because it is more sensitive to values of R_N in the important region of high R_N values, when the extraction ratio reaches the limiting value of 1. Only at high R_N values is discrimination between the various models of hepatic elimination possible and appropriate. Fig. 1 shows the relationship between observed availability (F) and efficiency number (R_N), and the predictions of the 'well-stirred', dispersion and 'parallel-tube' models. It is apparent that for a given efficiency number the 'parallel-tube' model predicts the lowest availability, i.e. the system is most efficient operating under these conditions. In practice, it is seen that the data are best fitted by the dispersion model, the 'well-stirred'

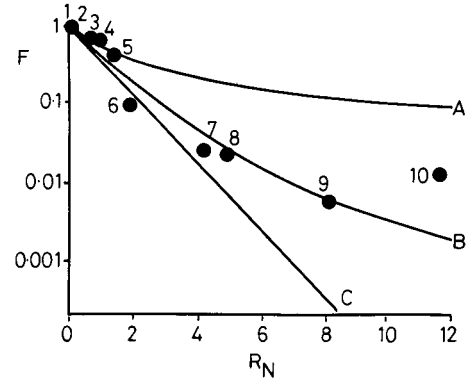

FIG. 1. Availabilities (F) observed after perfusion of drugs through rat isolated perfused livers versus the estimated efficiency number (R_N) determined from in-vitro microsomal data. Key: 1, antipyrine; 2, carbamazepine; 3, hexobarbitone; 4, phenytoin; 5, 5-hydroxytryptamine; 6, phenacetin; 7, pethidine; 8, propranolol; 9, lignocaine; 10, alprenolol. A, 'well-stirred' model; B, dispersion model; C, 'parallel-tube' model.

Table 1. Availabilities for various drugs (F) and estimated efficiency numbers (R_N) determined from in-vitro enzyme data and perfusions of isolated rat livers.

	Microsomes				Perfused liver				
Drug	CL_{int} ml min^{-1} g^{-1} liver	K_m mM	Ref	fu	Q ml min^{-1} g^{-1} liver	C_{in} mM	F	Ref	R_N
Alprenolol	23·5	0·017	(1,2)*	1**	2·00	0·015	0·02	(1,2)*	11·75
Antipyrine	0·08	22	(1)	1·0	2·00	0·04	0·99	(1)	0·04
Carbamazepine	0·11	0·73	(1)	1·0	2·00	0·042	0·96	(1)	0·055
Hexobarbitone	1·60	0·105	(1)	1·0	2·00	0·053	0·67	(1)	0·8
5-Hydroxytryptamine	1·70	0·07	(3)	0·93	1·12	0·0001	0·41	(3)	1·41
Lignocaine	8·21	0·058	(1)	1·0	1·0	0·004–0·008	0·005 0·0095	(4) (5)	8·2
Pethidine	4·25	0·28	(6)	1·0	1·0	0·004–0·008	0·024	(5)	4·25
Phenacetin	1·89	0·455, 0·005	(5)	1·0	1·0	0·0001	0·09	(7)	1·89
Phenytoin	1·99	0·031	(1, 8)	1·0	2·0	0·02	0·67 0·63	(1) (9)	0·995
Propranolol	10·00	0·005	(1, 10)	1·0	2·0	0·039	0·02	(11)	5

* 1. Rane et al (1977)
2. Borg et al (1974)
3. Wiersma & Roth (1980)
4. Pang & Rowland (1977b)
5. Ahmad et al (1983)
6. Freeman et al (1977)
7. Pang & Gillette (1978)
8. Kutt & Fouts (1971)
9. Shand et al (1975)
10. Shand & Oates (1971)
11. Wood et al (1979)

** Binding unknown, as assumed by Rane et al (1977).

model predicting too high an availability for a given value of R_N, especially at high values of R_N. At low values of R_N ($0 < R_N < 0{\cdot}5$), the corresponding high availability of solutes is virtually independent of the dispersion number and hence the availabilities of solutes in this region are adequately described by virtually any model of hepatic elimination. According to the 'well-stirred' model, the clearance of drugs with availabilities less than about 0·5 can be classified as flow-dependent (Wilkinson & Shand 1975) and it is in this range of availabilities that the 'well-stirred' model appears to be inappropriate for in-vitro–in-vivo correlations. If the 'well-stirred' model were operative at these low availabilities, enormous enzyme quantities would be required to achieve the observed availabilities. This model is therefore inconsistent with the concept of high efficiency of the liver as a detoxifying organ with small expenditure of energy for yield.

According to the dispersion model evaluation of hepatic extraction data, dispersion numbers in the order of 0·1–0·2 correspond best to the observed hepatic output concentration-time profile after bolus injections of labelled solutes into the liver (unpublished data). This range implies a reasonable degree of dispersion of blood elements within the liver. The dispersion number obtained from the regression of the data in Fig. 1 was 0·17 (CV = 68%), again supporting the idea of considerable mixing of blood within the liver.

This dispersion number corresponds to a minimum dispersion coefficient of $1{\cdot}1 \times 10^{-4}$ cm^2 s^{-2} assuming a minimum blood velocity of 0·027 cm s^{-1} (Koo et al 1975) and a sinusoidal length of 0·05 cm (Bass et al 1976). The value of the dispersion coefficient is more than an order of magnitude greater than the diffusion coefficient of molecules in plasma (5×10^{-6} cm^2 s^{-1}, Bass et al 1976) and is consistent with a dispersion coefficient determined by the anatomy and haemodynamics of the liver rather than by physicochemical properties of the substrates.

The dependence of availability on R_N for the distributed model is of the same mathematical form as the dispersion model for a given small D_N (Roberts & Rowland 1985) and hence a similar evaluation could apparently be made in terms of the distributed tube model. However, at very high values of R_N, the convergence of the requisite expansions in the distributed tube model equations may fail (Bass et al 1978).

In analysing data from many different sources, simplifying assumptions are inevitable. In particular, the above analysis assumes the complete absence of intrahepatic shunts, the same fractional loss (30%) of R_N for all drugs in the transition from intact liver to the homogenate, and the common value of 10 g for the rat liver weight.

The present analysis has assumed that enzymatic degradation of drug and not transport across the hepatocyte cell wall is rate limiting. The validity of this assumption can be assessed by substantial differences in the metabolism of drugs in hepatocytes and in microsomal suspensions. A number of recent studies have compared the metabolism of drugs in microsomal enzyme systems to metabolism in intact cells with equivocal results. Of seven compounds studied by Billings et al (1977), four were metabolized more rapidly in microsomal supernatant than in hepatocytes. Grundin et al (1975) have suggested that cell entry of alprenolol is not rate limiting in its overall metabolism, whereas Tsuri et al (1982) suggested a diffusion barrier for phenytoin metabolism. Jones & Mason (1978) reported similar metabolism for hexobarbitone, phenyramidol and alprenolol in cells and microsomal suspensions. An active transport mechanism may exist in the hepatocyte wall as reported for ethyl morphine by Erikson et al (1982). As the maximal difference between the reported findings for microsomal and hepatocyte systems appears to be about 2-fold, it is difficult to validate the assumption made. It is apparent that the difference is insufficient to compensate for the divergence of the low availability drugs from the predictions of the 'well-stirred' or 'parallel-tube' models (Fig. 1).

The discrepancy between in-vitro determined intrinsic clearances and availabilities observed in the perfused liver preparation (Fig. 1) also has implications in assessing enzyme activity in-vivo. Pang & Rowland (1977a) have shown that the intrinsic clearance defined by the 'well-stirred' model may be estimated from the total area under blood drug concentration-time curves after oral dosing (AUC_{oral}):

$$CL_{int} = \frac{Dose}{fu_b\, AUC_{oral}} \qquad (11)$$

This relationship has been used by some workers to obtain intrinsic clearances from in-vivo studies. Thus, Van der Graaf et al (1983) have used equation 11 to obtain estimates of intrinsic clearance for hexobarbitone and heptabarbitone. As the availability of hexobarbitone is in the region in which availability is independent of dispersion number (Fig. 1), this approach appears reasonable. However, caution is required in interpreting intrinsic clearances estimated for drugs of low availability.

For these drugs, intrinsic clearances determined on the basis of a liver behaving as a 'well-stirred' compartment may be a poor and overestimate of the actual enzyme activity in the liver.

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