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Models of hepatic drug clearance: discrimination between the 'well stirred' and 'parallel-tube' models

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The predictive ability of two models of hepatic drug clearance are compared. The 'parallel-tube' model predicts that the steady-state drug concentration following constant rate oral administration increases with increase in hepatic blood flow. The 'well-stirred' model predicts that this parameter is not sensitive to changes in hepatic blood flow. Using the steady-state reservoir drug concentration as the discriminatory index, the predictions of the models were tested in a recirculating isolated perfused rat liver system with lignocaine and pethidine, both of which are highly extracted, as test drugs. The steady-state reservoir concentration of both drugs was found to be constant when flow through the liver was increased from 10 ml min^{-1} to 15 ml min^{-1}. The experimental findings indicate that the 'well-stirred' model more accurately describes the elimination of highly cleared drugs with perturbations of flow than does the 'parallel-tube' model.

The general equation describing the steady-state blood drug concentration following constant oral administration ($C_{b,ss,oral}$) is as follows (Wagner et al 1965),

$$C_{b,ss,oral} = \frac{F.R_0}{CL} \quad (1)$$

where F is the availability, R_0 is the rate of administration and CL is the clearance. When the sole cause of loss of availability and of elimination is hepatic extraction, $F = 1 - E$, where E is the hepatic extraction ratio. Clearance is related to E by $CL = Q.E.$, where Q is the hepatic blood flow.

Two models of hepatic drug clearance incorporating the physiological variables of hepatic blood flow, drug binding and activity of drug metabolizing enzymes have been used in recent years. Rowland et al (1973) assumed that the liver is a single well-stirred compartment ('well-stirred' model), and that the concentration of unbound drug in the emergent blood is in equilibrium with unbound drug within the liver. Under these conditions, following portal vein infusion of drug, equation 1 can be expressed as (Pang & Rowland 1977a),

$$C_{b,ss,oral} = \frac{R_0}{f_u.CL_{int}} \quad (2)$$

where f_u is the fraction of drug in the blood unbound, and CL_{int} is the intrinsic clearance of the drug by the system, which describes the ability of the liver to eliminate the drug in the absence of any flow limitations. Winkler et al (1973) assumed that the liver is composed of a number of identical and parallel tubes ('parallel-tube' model), along which drug concentration decreases progressively in the direction of blood flow, due to its elimination by enzymes in the hepatocytes. Under the preceding conditions, for portal vein infusion of drug, equation 1 can be expressed as:

$$C_{b,ss,oral} = \frac{R_0.e^{-(f_u.CL_{int}/Q)}}{Q.(1 - e^{-f_u.CL_{int}/Q})} \quad (3)$$

where the indices are as previously defined. While CL_{int} has the same meaning in both models, the numerical value differs between the models for given values of clearance and flow. Comparison of equations 2 and 3 indicates that the steady-state drug concentration following constant rate oral administration is a useful discriminator between the two models, since equation 2 is independent of flow whereas equation 3 describes a flow-dependent steady-state drug concentration particularly for drugs of high hepatic extraction ratio (Pang & Rowland 1977a).

A number of pharmacokinetic analyses of drugs that exhibit high first-pass effects assume the validity of the 'well-stirred' model (Bishoff et al 1971; Benowitz et al 1974; Branch et al 1974; Shand et al 1976). Although the investigations of Shand et al

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(1975) showed that, in a recirculating isolated perfused liver system, the 'well-stirred' model was more successful at predicting the pharmacokinetics of lignocaine, there is also evidence that the elimination of galactose in similar systems was more accurately described by the 'parallel-tube' model (Keiding & Chiarantini 1978). Experiments on lignocaine elimination in the isolated perfused rat liver indicated that, (a), its extraction ratio is 0·99 (Pang & Rowland 1977b) and thus exceeds the value of 0·95 reported by Shand et al (1975) and (b), the clearance of lignocaine in a recirculating system decreases with time (Lennard et al 1978). The predictions of the 'well-stirred' and 'parallel-tube' models for change in flow, differ most markedly for drugs that have extraction ratios in excess of 0·95 (Pang & Rowland 1977a). Since we confirmed that the extraction ratio of lignocaine in the perfused rat liver is 0·99 and that extraction is constant for 90 min, we used this drug to distinguish between the predictive capacities of the two models. Pethidine which has an extraction ratio of 0·98 in the rat liver was also studied.

MATERIAL AND METHODS

(i) Experimental system

A recirculating isolated perfused rat liver in-situ system similar in design to that described by Pang & Rowland (1977b) was used. Male Wistar rats (400–450 g, University of Bath strain) anaesthetized with pentobarbitone (Sagatal, May & Baker, 60 mg kg^{-1}), were used as liver donors. The operative procedure for preparation of the perfused rat liver in-situ has been described (Pang & Rowland 1977b). Essentially it consisted of cannulating the portal and hepatic veins with a 16 G and 14 G catheter respectively (Venflon, Viggo) without interrupting flow to the liver. All operative procedures were completed within 10 min. The perfusion medium used consisted of Krebs-bicarbonate solution with the addition of 300 mg/100 ml glucose, 0·41% sucrose and 20% v/v out-dated human red blood cells. A reservoir of 117 ml of the perfusion medium was used for each experiment.

(ii) Determination of half-life ($t_{1/2}$) and time to reach steady-state

The isolated perfused liver was set up as described in (i) above. For 45 min, drug dissolved in isotonic saline was infused (Perfusor, Braun) into the portal vein cannula. One–3 ml samples of the medium in the reservoir, taken at suitable times during 90 min, were assayed for lignocaine or pethidine by a gas-liquid chromatographic method using methyl-lignocaine (Astra) as the internal standard (Mather & Tucker 1974). The extraction ratio of the drug was determined at the 40th min by sampling the drug concentration in the inflow and outflow cannulae.

The rate constants for elimination (k) during the portal vein infusions were calculated from the regression slopes (−k/2·303) of the semilogarithmic plots of the difference between the plateau reservoir drug concentrations and the value at each sampling time during the infusions (Rowland & Tozer 1980). The rate constants for elimination after infusion were calculated from the slopes (−k/2·303) of the regression of the semilogarithmic plots of the reservoir drug concentration against time. The $t_{1/2}$ values were calculated from the estimated values of k.

(iii) Influence of flow rate on the steady-state reservoir drug concentration

The recirculating isolated perfused liver system was set up as previously described. Flow was adjusted to 10 ml min^{-1}, with drug being perfused into the portal vein cannula. The reservoir drug concentrations were sampled at the 20th, 25th and 30th min. Flow was then increased to 15 ml min^{-1} over 2 min. Further reservoir samples were withdrawn as before. Flow was then readjusted to 10 ml min^{-1} and sampling was carried out at the above times.

The extraction ratio across the liver at each blood flow was also determined by sampling at the inflow and outflow cannulae at the 30th min after each flow setting.

RESULTS

(i) Viability and steady-state conditions

Preliminary experiments indicated that stability of the recirculating isolated perfused liver system, as demonstrated by the constancy of extraction ratio of the drug with time, was enhanced by the addition of sucrose 0·41% to the medium, confirming the observation of Beaubein et al (1979), and by using relatively large donor rats (400–450 g). Preliminary work also indicated that steady-state conditions could not be maintained sufficiently long to complete the experiments if the rate of drug infusion exceeded 30 μg min^{-1}. Drug infusion rates below 10μg min^{-1} necessitated the removal of amounts of fluid sufficient to deplete reservoir volume significantly, in order to obtain enough drug to assay. Drug infusion rates between 10 and 20 μg min^{-1} gave experimental conditions sufficiently stable for the conduct of the experiments and this infusion range was used throughout.

Typical changes in reservoir drug concentration in

two experiments during and after a 45 min infusion into the portal vein appear in Fig. 1a for lignocaine and Fig. 1b for pethidine. Table 1 gives the mean values for k and t½ for both drugs. In the case of lignocaine the mean values of k and t½ determined during the 45 min portal infusion did not differ significantly from the corresponding values determined during the subsequent 45 min indicating that the preparation is stable for 90 min. However, for pethidine, the mean value of k determined at the infusion period was significantly greater ($P<0.05$) than that determined during the second half of the experiment, indicating that for this drug, the system was not completely stable over 90 min.

Table 1. Mean ± s.e.m. values of the elimination rate constants (k) and half-lives (t½) of lignocaine and pethidine determined in the recirculating isolated perfused rat liver in-situ system (flow rate = 10 ml min^{-1}, n = 5).

	Mean ± s.e.m.	
Parameters	Lignocaine	Pethidine
k-upcurve (0–45 min)	0·0818 ± 0·0032 min^{-1}	0·0803 ± 0·0029 min^{-1}
k-downcurve (45–90 min)	0·0818 ± 0·0030 min^{-1}	0·0719 ± 0·0024 min^{-1}
t½-upcurve (0–45 min)	8·52 ± 0·33 min*	8·67 ± 0·34 min**
t½-downcurve (45–90 min)	8·52 ± 0·34 min*	9·68 ± 0·33 min**

* denotes no significant difference, and ** denotes significant difference (Student's t-test at $P = 0.05$).

(ii) Influence of varying perfusion rates on the drug reservoir concentration

Data on the consequence of changing the hepatic perfusion rate from an initial value of 10 to 15 ml min^{-1} on the reservoir drug concentrations are given in Tables 2 and 3 for lignocaine and pethidine respectively. Table 2 indicates that, once a steady-state condition was achieved, the reservoir concentration of lignocaine was insensitive to changes in hepatic blood flow, even though the flow was changed to 1½ times the original value. Additional evidence of insensitivity to reservoir drug concentration to changes in flow can be seen with pethidine (Table 3). Although there is evidence of instability of the system at longer perfusion times (during the 2nd control period with flow at 10 ml min^{-1}), data collected at the beginning of the experiment, in which the perfusion rate was maintained at 10 ml min^{-1}, when compared with the higher per-

Table 2. Data on the influence of perfusion flow rate on the reservoir concentration (C_{res}) of lignocaine in recirculating isolated perfused liver in-situ systems. The effluent lignocaine concentration (C_{out}) was determined at the 30th min of each steady-state condition.

Flow rate (ml min^{-1})	Time (min)	C_{res} (mg litre^{-1})				
		1	2	3	4	5
10	20	0·012	0·012	0·007	0·010	0·015
	25	0·012	0·013	0·008	0·012	0·016
	30	0·013	0·014	0·009	0·012	0·016
	C_{out}	0·012	0·014	0·010	0·012	0·016
15	20	0·013	0·013	0·009	0·012	0·016
	25	0·012	0·013	0·009	0·012	0·017
	30	0·012	0·014	0·009	0·012	0·017
	C_{out}	0·013	0·014	0·010	0·013	0·016
10	20	0·011	0·014	0·010	0·011	0·018
	25	0·010	0·014	0·010	0·012	0·017
	30	0·012	0·014	0·010	0·012	0·017
	C_{out}	0·012	0·014	0·010	0·013	0·018

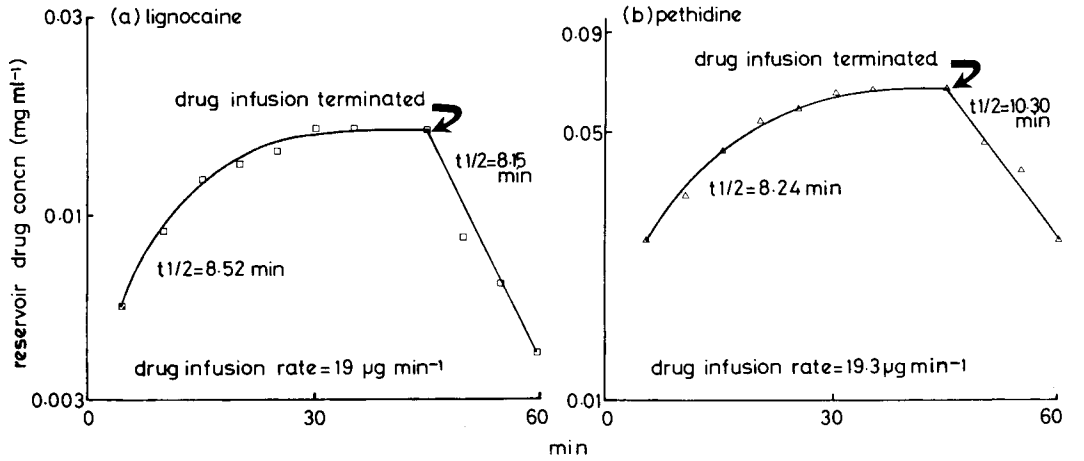

FIG. 1. Semilogarithmic plot of changes in the drug-reservoir concentration with time during and after a constant infusion of drug for 45 min in a recirculating isolated perfused liver in-situ system.

Table 3. Data on the influence of perfusion flow rate on the reservoir concentration (C_{res}) of pethidine in recirculating isolated perfused liver in-situ systems. The effluent pethidine concentration (C_{out}) was determined at the 30th min of each steady-state condition.

Flow rate (ml min^{-1})	Time (min)	C_{res}(mg litre^{-1}) 1	2	3	4
10	20	0·044	0·031	0·038	0·022
	25	0·047	0·036	0·042	0·026
	30	0·048	0·039	0·042	0·028
	C_{out}	0·048	0·040	0·043	0·029
15	20	0·050	0·039	0·042	0·029
	25	0·049	0·040	0·044	0·028
	30	0·049	0·039	0·043	0·029
	C_{out}	0·049	0·040	0·045	0·030
10	10	0·049	0·040	0·046	0·030
	15	0·049	0·045	0·051	0·034
	20	0·050	0·049	0·063	0·035
	C_{out}	0·054	0·062	0·084	0·040

fusion rate of 15 ml min^{-1}, suggest that reservoir pethidine concentration is also insensitive to changes in the liver blood flow.

From the steady-state reservoir drug concentration observed during the initial control flow of 10 ml min^{-1}, the intrinsic clearance for the individual system as defined by the 'well-stirred' (eqn 2) and 'parallel-tube' (eqn 3) models can be calculated. On the assumption that the CL_{int} values for the same liver system are independent of flow, the corresponding steady-state reservoir drug concentration associated with both models at the higher flow rate can be predicted (Tables 4 and 5 for lignocaine and pethidine respectively). The data in Tables 4 and 5 showed that the range of steady-state reservoir drug concentration values predicted by the 'well-stirred' model accord with the experimentally observed values, in contrast to those of the 'parallel-tube' model which in each instance exceed the observed values.

Predictions of the two models in two typical experiments appear in Fig. 2a for lignocaine and Fig. 2b for pethidine. Since the clearance of both drugs is virtually equal to the perfusion rate, the t½ of the drug at 15 ml min^{-1} can be calculated from the volume of distribution (volume of reservoir) and the flow rate. The anticipated reservoir concentration on approach to steady-state for both models, and also when perfusion is returned to 10 ml min^{-1} can then be calculated. Fig. 2a,b clearly demonstrates that the observed data are consistent with the predictions of the 'well-stirred' model.

DISCUSSION

Theoretical considerations by Pang & Rowland (1977a) indicate that discrimination between the 'well-stirred' and 'parallel-tube' models is best performed under steady-state and first order conditions, since all pharmacokinetic parameters are then independent of concentration and time. Their analysis showed that certain parameters—availability, steady-state drug concentration in the hepatic venous blood, area under the curve (AUC) following a single oral dose and steady-state drug concentration in blood following constant oral administration —are excellent discriminators when the behaviour of highly extracted drugs is examined under conditions in which either flow or the degree of binding within blood are perturbed.

With these considerations in mind, lignocaine and pethidine are suitable test substances for discriminating between the two models, since the clearance of these drugs is virtually equal to the perfusion rate. The predictions of the models for perturbation of flow differ particularly for drugs with extraction ratios which approach unity. The difference in extraction ratio of lignocaine observed in the present study (0·99) compared with that reported by Shand et al (1975) (0·95), although small is therefore important. Also, since the clearance of lignocaine in the isolated perfused rat liver decreases with time (Lennard et al 1978), one would expect that the range of concentrations used by Shand et al (1975)

Table 4. Influence of perfusion rate on pharmacokinetic parameters determined in the recirculating isolated perfused liver in-situ system receiving a constant portal venous infusion of lignocaine. Values of steady-state reservoir drug concentration predicted from the 'well-stirred' ($C_{res\ I}$), and 'parallel-tube' models ($C_{res\ II}$) are listed.

	Perfusion rate of 10 ml min^{-1}				Perfusion rate of 15 ml min^{-1}					
Study no.	F	CL ml min^{-1}	F/CL	C_{res} mg litre^{-1} observed	F	CL ml min^{-1}	F/CL	C_{res} mg litre^{-1} observed	$C_{res\ I}$ mg litre^{-1} predicted	$C_{res\ II}$ mg litre^{-1} predicted
1	0·008	9·92	0·0008	0·012	0·013	14·81	0·0009	0·013	0·012	0·041
2	0·009	9·91	0·0009	0·014	0·013	14·80	0·0009	0·014	0·014	0·045
3	0·010	9·91	0·0010	0·010	0·014	14·80	0·0009	0·010	0·010	0·033
4	0·009	9·91	0·0009	0·012	0·014	14·78	0·0010	0·013	0·012	0·040
5	0·011	9·89	0·0011	0·016	0·017	14·75	0·0012	0·016	0·016	0·048

Table 5. Influence of perfusion rate on pharmacokinetic parameters determined in the recirculating isolated perfused liver in-situ system receiving a constant portal venous infusion of pethidine. Values of steady-state reservoir drug concentration predicted from the 'well-stirred' ($C_{res\ I}$), and 'parallel-tube' model ($C_{res\ II}$) are listed.

	Perfusion rate of 10 ml min^{-1}				Perfusion rate of 15 ml min^{-1}					
Study no.	F	CL ml min^{-1}	F/CL	C_{res} mg litre^{-1} observed	F	CL ml min^{-1}	F/CL	C_{res} mg litre^{-1} observed	$C_{res\ I}$ mg litre^{-1} predicted	$C_{res\ II}$ mg litre^{-1} predicted
1	0·024	9·77	0·0024	0·048	0·035	14·74	0·0024	0·049	0·048	0·114
2	0·022	9·79	0·0022	0·040	0·032	14·52	0·0022	0·040	0·040	0·097
3	0·030	9·70	0·0031	0·045	0·046	14·31	0·0032	0·045	0·043	0·095
4	0·021	9·79	0·0021	0·029	0·032	14·53	0·0022	0·030	0·029	0·072

would affect the linearity of the system. Preliminary experiments with the present system confirms the observation of Lennard et al (1978), since there was evidence of a decrease in lignocaine clearance with time when the drug infusion rate exceeded 20 μg min^{-1}. Even with a drug infusion rate of 10 μg min^{-1} a relatively stable situation can only be maintained for 90 min. Although it may be possible to maintain a relatively longer period of stability by using drug infusion rates well below 10 μg min^{-1}, this would be limited by the sensitivity of the drug assay procedure. The same constraints also apply to the choice of infusion rates of pethidine.

The addition of sucrose to the perfusion medium helps to minimize oedema formation since preliminary experiments with a sucrose-free medium resulted in a visibly engorged liver. The choice of reservoir size (117 ml) was a compromise between a volume which would not be so small as to be affected significantly by sampling during the period of the experiment and one so large that the volume of distribution of the system and drug t½ would result in too long a time to reach steady-state. The fact that for both drugs the extraction ratio is virtually one, and hence clearance is essentially equal to perfusion flow, means that the t½ of drug in the system is related only to the volume of the reservoir and perfusion rate. It follows that the t½ is essentially the same for both drugs (Table 1).

Examination of the mathematics of such a system (Rowland et al 1973; Pang & Rowland 1977a) indicates that at steady-state following a portal infusion, the effluent hepatic concentration is equal to the reservoir concentration. In the present experimental system, data obtained from direct sampling of the effluent drug concentration and the

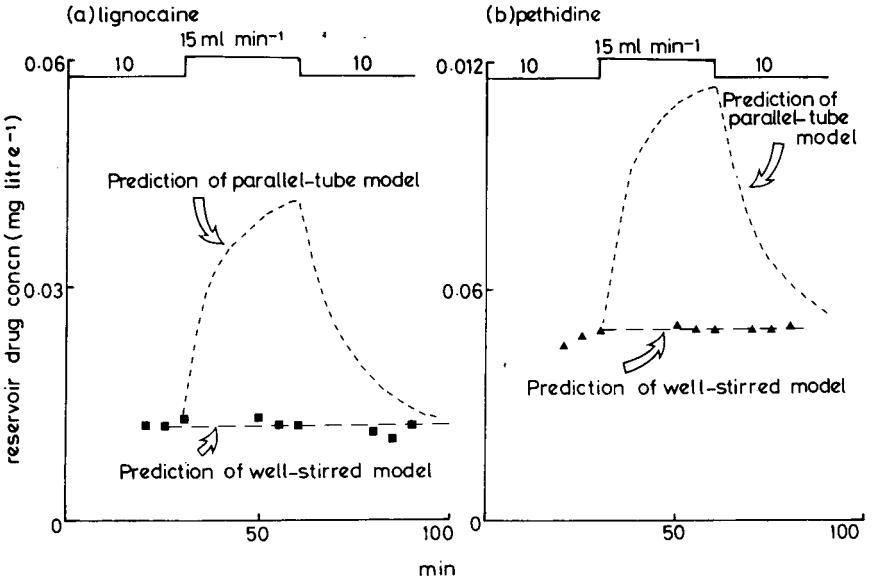

FIG. 2. Influence of perfusion flow rate on the reservoir drug concentration in the recirculating isolated perfused rat liver in-situ system. Predictions of the 'well-stirred' and 'parallel-tube' models are superimposed.

reservoir drug concentration at the 30th min, are virtually identical, confirming that steady-state conditions are achieved within the period (Tables 2 and 3 for lignocaine and pethidine respectively).

Using the steady-state reservoir drug concentration in blood following constant rate oral (portal) administration as a discriminator between the two models, the 'parallel-tube' model predicts that this value increases with increase in hepatic blood flow. The 'well-stirred' model predicts that, under the same conditions, the parameter is not sensitive to changes in hepatic blood flow. Experimental data using lignocaine as a test drug indicate that, once steady-state conditions are achieved, the concentration of the drug in the reservoir is insensitive to changes in the hepatic flow rate (Table 2, Fig. 2a). Also, within the constraints of the stability of the system, the same conclusion can be drawn from the pethidine data (Table 3, Fig. 2b).

The independence of the steady-state drug concentration, following a constant rate portal infusion, on hepatic blood flow in the 'well-stirred' model is reflected by the absence of Q in equation 2. It is also apparent (eqn 1) that the major determinant of the steady-state drug concentration following a constant rate portal perfusion is the ratio F/CL for both models. On determining the values of F and CL for each steady-state condition (Tables 4 and 5 for lignocaine and pethidine respectively), it can be seen that the change in F caused by change in Q is always accompanied by a corresponding change in clearance, so that F/CL values are similar at both flow rates. This is predicted from the equations of the 'well-stirred' model since F and CL are directly related to flow (Pang & Rowland 1977a). With the 'parallel-tube' model, F is exponentially, while CL is directly, related to flow and in consequence F/CL values change at different flow rates. The data in Tables 4 and 5 for lignocaine and pethidine respectively are clearly consistent with the predictions of the 'well-stirred' model.

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