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CURRENT MODELS OF HEPATIC PHARMACOKINETICS: FLOW EFFECTS ON KINETIC CONSTANTS OF ETHANOL ELIMINATION IN PERFUSED RAT LIVER

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Abstract—At present two different pharmacokinetic models of the enzymic elimination of substances from the blood flowing through the liver are used: the sinusoidal perfusion model assuming the elimination to take place at the local sinusoidal blood substrate concentration, falling continuously from the inlet to the outlet of the sinusoid, and the venous equilibration model assuming elimination at the hepatic outlet concentration. It is an ultimate requirement of such models that the estimates of the enzymic parameters V_{max} and K_m be independent of variations in hepatic blood flow rate. This was used to compare the two models experimentally.

Ethanol was given at two successive constant infusion rates (7 and 10 μmol/min) to 11 livers from rats (200 g) perfused by a recirculating medium. In each of the infusion periods the hepatic blood flow rate was varied experimentally (10 and 17 ml/min, respectively). From the measured steady-state ethanol concentrations in the hepatic inlet and outlet, K_m and V_{max} were calculated from both models at both low and high blood flow rates. The K_m calculated according to the venous equilibration model was significantly lower in the low-flow than in the high-flow periods ($P < 0.05$); this model is thus not consistent with data. V_{max} values were not influenced by hepatic blood flow.

The K_m calculated according to the sinusoidal perfusion model were not influenced by flow ($P < 0.5$) and V_{max} was also unchanged. The sinusoidal perfusion model is thus not inconsistent with data.

Models of hepatic elimination kinetics, formulated on a physiological basis, may be useful in clinical pharmacology, e.g. for the dosage of drugs.

Most hepatic processes are enzymic reactions, and most models assume that the elimination follows Michaelis–Menten kinetics with a maximal elimination rate V_{max} and a half saturation blood concentration K_m. It is an ultimate requirement of such models that the estimates of the enzymic parameters be independent of variations of the hepatic blood flow rate [1–4]. This was used to test two commonly used models experimentally.

In the sinusoidal perfusion model the substrate is taken up from the blood perfusing the sinusoid by a process which follows Michaelis–Menten kinetics at the local sinusoidal blood substrate concentration at every location along the sinusoid. This elimination creates a continuously falling blood substrate concentration from the inlet to the outlet of the sinusoid [4]. At time-independent blood concentration mathematical treatment of this idea gives [2, 5]

$$v = Q \cdot (C_i - C_o) = \frac{V_{max} \cdot \hat{C}}{K_m + \hat{C}} \qquad (1a)$$

where v is the elimination rate, C_i the concentration in the inlet, C_o in the outlet, Q the hepatic blood flow rate, the elimination rate $v = Q \cdot (C_i - C_o)$ and $\hat{C}$ is a logarithmic average sinusoidal substrate concentration

$$\hat{C} = \frac{C_i - C_o}{\ln C_i - \ln C_o} \qquad (1b)$$

According to the "well-stirred model" [3], also called the venous equilibration model [6] the elimination of substrates from the blood by the liver cells takes place at a concentration equal (or proportional) to the concentration in the hepatic outlet,

$$v = \frac{V_{max} \cdot C_o}{K_m + C_o} \qquad (2)$$

Thus the fall in blood concentration takes place at the inlet of the sinusoids [7].

In the present study the flow-independence of the enzymic parameters estimated according to either of the models was tested by means of ethanol elimination: V_{max} and K_m were calculated by the linear regressions of $1/v$ on $1/\hat{C}$ (equation 1) and $1/v$ on $1/C_o$ (equation 2). Since V_{max} is calculated by extrapolation to infinite concentration it is *a priori* flow-independent according to both models. K_m, however, is a concentration in a range where extraction is flow-dependent; therefore the effect of flow on K_m may be used to test the two models experimentally.

The study design included two successive constant ethanol infusion rates given into a perfusate recirculated through isolated rat livers. During each steady-state infusion period, the flow rate of the perfusate was changed, and the effect on the ethanol concentrations in the hepatic inlet and outlet medium measured.

MATERIALS AND METHODS

Liver perfusions. Livers from female Wistar rats (body weight 185–206 g, liver weight 5.4–7.7 g) were perfused with a recirculating medium via the portal vein with the liver remaining *in situ* in the animal.

The medium consisted of 0.30 l/l washed bovine erythrocytes in a Krebs–Henseleit buffer with 20 g/l bovine albumin (fraction V, Sigma). It was recirculated through the liver by means of a calibrated roller pump (O. Dick, Copenhagen) and oxygenated by perfusion through a thin-walled tube located in a bottle through which air containing 5% CO_2 was flowing. The total volume of the medium was about 70 ml. The perfusion set up has been described in detail [8–10].

Experimental design. The data comprise 11 experiments each consisting of five steady-state periods (A–E) lasting for 15 min. Ethanol was given at a constant infusion into the reservoir during periods A and B (mean ± S.E.M.: 6.76 ± 0.13 μmol/min), and at another infusion rate during period C and D (9.67 ± 0.24 μmol/min); the infusion rate during period E was identical with that of period A. The perfusion rate of the medium (the hepatic blood flow rate) was varied at two levels between periods A and B, and at the same two levels between periods C and D; period E had the same flow rate as period A; in periods A, D and E, it was on the average 10.5 ± 0.5 ml/min, in periods B and C 17.3 ± 0.7 ml/min. Figure 1 gives an example of the experimental design.

Samples of the medium in the hepatic inlet and outlet were taken for analysis every second min during the last 10 min of each period.

Analyses. Ethanol in the medium was measured enzymically [11].

The oxygen saturation (Osm 1, Radiometer, Copenhagen), oxygen tension (ABL 2, Radiometer) and haemoglobin concentration were measured once in each period, and the oxygen uptake calculated as blood flow rate multiplied by the inlet–outlet concentration difference.

Evaluation of steady-state of the perfusions. There were no visible changes of the livers during the experimental periods. The oxygen uptake did not change significantly or systematically from the first to the last period, nor with the flow variations. The mean was 3.6 μmol/min per g liver (range 2.6–4.3), equal to previous findings [9]. Also the perfusion pressure, measured by an open-air tube at the inlet, did not change significantly or systematically from the first to the last period, being on average 17 cm H_2O (range 12–24) in the high-flow periods and 12 cm H_2O (8–15) in low-flow periods.

There were no systematic changes of the elimination rate of ethanol, calculated as the hepatic blood flow rate multiplied by the inlet–outlet concentration difference, from the first to the last period ($P > 0.2$). The infusion rates and the calculated elimination rates showed no systematic deviations, and the perfusion system was thus internally consistent.

In each of the experiments, however, the ethanol blood concentration increased between the otherwise identical periods A and E. This is attributable to a continuous small loss of functioning liver cells during the experimental period [9]. A total of 16 experiments was performed, but five experiments were excluded because of non-steady-state, i.e. an increment of the inlet concentration larger than 0.16 mmol/l (equal to two times the standard error of the ethanol concentration measurements).

Statistics. Comparisons were made by Wilcoxon two-tailed test for pair differences.

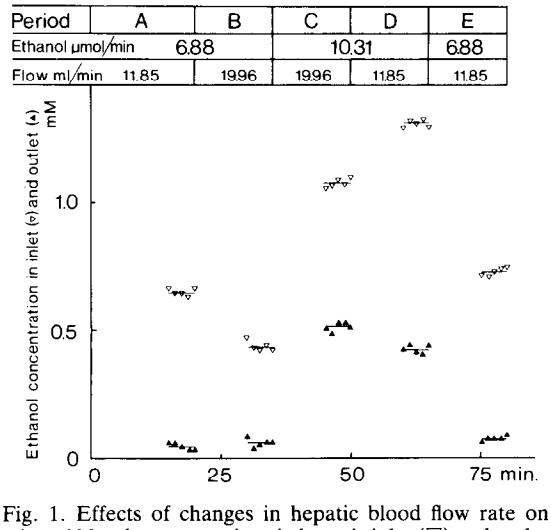

Fig. 1. Effects of changes in hepatic blood flow rate on ethanol blood concentrations in hepatic inlet (▽) and outlet (▲) during constant ethanol infusion rates given into a medium (70 ml) recirculated through a perfused rat liver (Exp. no. 4, liver weight 6.57 g). Blood flow and ethanol infusion rates were varied as indicated.

RESULTS

Figure 1 gives an example of the concentration measurements, and Table 1 V_{max} and K_m estimated according to the two models, at high and low flow rates respectively.

K_m estimated according to the venous equilibration model (equation 2) at low and high blood flow rates was significantly lower in the low-flow periods than in the high-flow periods ($P < 0.05$). The requirement of a flow-independent estimate of K_m is thus not fulfilled experimentally by this model. V_{max} was not significantly different between low-flow periods and high-flow periods ($P > 0.2$), the mean being 10.6 μmol/min.

K_m estimated according to the sinusoidal perfusion model (Table 1) showed no significant difference between low-flow periods and high-flow periods ($P > 0.5$). This is in agreement with the sinusoidal perfusion model. The mean K_m (±S.E.M., N = 11) was 0.26 ± 0.04 mmol/l. Also V_{max} was not influenced by flow variations ($P > 0.5$); the mean (±S.E.M.) was 12.5 ± 1.0 μmol/min.

DISCUSSION

The choice of the perfused rat liver as experimental model in the present study implies that K_m and V_{max} were calculated from only two sets of elimination rate and concentration measurements at each flow rate, because the perfused rat liver preparation, at least in our hands, is not reasonably steady for more than a few hours. Accordingly it is not possible to test if Michaelis–Menten kinetics was obeyed but this was assumed to be the case, in agreement with other studies (e.g. [12] and references therein). Like-

Table 1. Test of predicted flow-independence of the Michaelis constant K_m calculated according to the sinusoidal perfusion model ($\hat{C}$-model) and the venous equilibration model (C_o-model)

	$\hat{C}$-model				C_o-model			
	K_m†		V_{max}†		K_m†		V_{max}†	
Flow*: Exp.	high	low	high	low	high	low	high	low
	(mmoles/l)		(μmoles/min)		(mmoles/l)		(μmoles/min)	
1	0.043	0.095	9.1	10.8	0.003	0.003	8.4	9.1
2	0.096	0.281	11.6	15.2	0.029	0.040	10.9	12.2
3	0.218	0.451	12.4	17.4	0.085	0.063	11.3	12.6
4	0.153	0.188	13.5	12.9	0.037	0.026	12.0	11.1
5	0.339	0.293	12.4	10.8	0.146	0.096	10.7	9.3
6	0.368	0.438	13.1	14.1	0.141	0.069	11.3	10.4
7	0.115	0.279	11.5	13.7	0.034	0.052	10.8	11.7
8	0.347	0.194	16.6	10.7	0.075	0.019	12.8	8.5
9	0.114	0.204	9.0	9.5	0.044	0.039	8.5	8.2
10	0.253	0.261	11.4	11.8	0.106	0.052	10.3	9.7
11	0.563	0.459	14.6	13.7	0.236	0.085	12.2	10.3

* High flow rate: mean 17 ml/min, low: 10 ml/min.

† Wilcoxon two-tailed test for pair differences: K_m ($\hat{C}$-model): $P > 0.5$; K_m (C_o-model): $P < 0.05$. V_{max} ($\hat{C}$-model): $P > 0.5$; V_{max} (C_o-model): $P > 0.2$.

wise it is not possible to calculate experimental uncertainties of the individual K_m and V_{max} values, and comparison of individual values at high and low flow rates respectively could not be performed. We therefore compared the K_m and V_{max} values within the group of experiments by means of Wilcoxon test for pair differences, whereby the hypothesis tested is "no change".

K_m estimated according to the venous equilibration model was flow-dependent, whereas K_m estimated according to the sinusoidal perfusion model did not depend significantly on flow. Although the data thus clearly reject the venous equilibration model for ethanol elimination in perfused rat liver and the data are consistent with the sinusoidal perfusion model, this of course does not verify the latter model.

In the study five experiments were excluded from the material due to non-steady-state (see Methods). If these experiments had been included, the conclusion was, however, the same.

It may be noticed that K_m according to the sinusoidal perfusion model is about the same as found previously in perfused rat liver and that using the venous equilibration model on the present data gives K_m values that are about 25% of the values from the sinusoidal perfusion model (cf. Table 1).

The study emphasizes that when considering quantitative hepatic pharmacokinetics, one has to take into account both the continuous fall in the sinusoidal blood substrate concentration in the flow direction of the sinusoid, and the importance of the hepatic blood flow rate for the size of this fall. An abrupt fall in blood substrate concentration at the inlet of the sinusoid as assumed in the venous equilibration model is unlikely from a physiological point of view, and cannot explain the present findings.

The two models have previously been compared experimentally. The venous equilibration model predicts that if v is fixed by a steady infusion of a substrate into a recirculating system containing a perfused isolated liver, then changes in Q should leave C_o unchanged (equation 2); this was not fulfilled by experimental data of elimination of galactose [8] or propranolol in perfused rat livers [9], whereas the data were consistent with the sinusoidal perfusion model. A recent study [14], however, reaches the opposite conclusion for lidocaine and meperidine.

The physiological variation between sinusoids as to enzyme content and flow rates may be accounted for by an extended version of the sinusoidal model [15]. Including this variation, the calculated sinusoidal concentration will be a little higher than by neglecting it, and more so, the higher the flow rate. The present experimental set up and measurement accuracy does not allow any detailed evaluation of this since the data do not deviate systematically from the simple model, i.e. K_m being unchanged by flow changes. Thus at present the simple sinusoidal perfusion model seems to be the most suitable working model.

Dosage of drugs on the basis of the venous equilibration model may lead to erroneous predictions of blood concentrations obtained during conditions with variations in hepatic blood flow rate, because this model predicts blood concentrations of drugs given orally to remain unchanged during flow variations (cf. equation 2 with v equivalent to dose [16]). As an example it can be calculated (equation 1) that if the hepatic blood flow is increased by one third due to intake of food [17], the blood concentration of a drug given orally (with kinetic constants similar to the present values) will be elevated by a factor of about three, with consequent risks of overdosage.

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