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PRELIMINARY COMMUNICATIONS

PREDICTION OF INTESTINAL FIRST-PASS EFFECT OF PHENACETIN IN THE RAT FROM ENZYME KINETIC DATA - CORRELATION WITH *IN VIVO* DATA USING MUCOSAL BLOOD FLOW

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Recent studies [1] have focused on the perfusion-limited model of clearance when investigating the hepatic clearance of a number of drugs. This model, originally proposed by Rowland et al. [2], described organ clearance in terms of organ blood flow (Q) and intrinsic clearance ($Cl_{organ,int}$), where metabolic intrinsic clearance is related to enzymatic parameters in that,

$$Cl_{organ,int} = \sum_{i=1}^{n} \frac{V_{max,i}}{K_{m,i}} \qquad (1)$$

where $V_{max,i}$ is the maximum rate of metabolism, mediated by the i-th enzyme (mass units/time units) and $K_{m,i}$ the Michaelis constant (concentration units). $K_{m,i}$ and $V_{max,i}$ can be determined *in vitro* from organ homogenates, microsomal or whole cell preparations. By substituting the experimental values in equation (1), one can calculate the extraction ratio by any organ using its blood flow,

$$E_{organ} = \frac{Cl_{organ,int}}{Q + Cl_{organ,int}} \qquad (2)$$

where Q denotes the blood flow to the eliminating organ. The extraction ratio, E_{organ}, can be determined *in vivo* as described by Cassidy et al. [3], by comparison of areas under blood concentration vs. time curves (AUCs) when the substrate is administered via different routes. For example, extraction by the small intestine can be determined by comparing AUCs after duodenal (oral) AUC_{po}, and intraportal, AUC_{pv}, administration of the drug [4]. Hence,

$$\frac{AUC_{po}}{AUC_{pv}} = 1-E_G \qquad (3)$$

where E_G denotes the intestinal extraction ratio, and thus the intestinal first-pass effect. In the present study we have determined intestinal phenacetin-O-deethylase activity *in vitro*, using isolated intestinal mucosal cells. Extraction ratios were calculated using the above described model (eq. 2) [4], substituting mucosal blood flow rather than portal vein blood flow [5, 6, 7], since from an anatomical point of view the one cell thick epithelial layer [8] (which contains the oxidative enzymes, and is therefore the eliminating organ) is "perfused" by mucosal blood flow. Calculated E_Gs are compared with extraction ratios obtained from AUCs found *in vivo* after intraportal and intraduodenal administration of phenacetin to control and 3-methylcholanthrene (3-MC) pretreated rats.

MATERIALS AND METHODS

Male Wistar rats (250 g, TNO, Zeist) received a 20 mg/kg oral dose of 3-MC in 1.0 ml corn oil for two consecutive days: control rats received the vehicle only. Twenty-four hr after the last administration cell-preparation was performed or *in vivo* experiments were started. Phenacetin (20 mg/kg; in propyleneglycol) was administered via the jugular vein, the portal vein via a cannula in the splenic vein or as a bolus injection into the duodenum. Portal administration involved infusion over 15 minutes to mimick the rate of entry in the liver after intraduodenal administration. All animals were kept under light ether anaesthesia throughout the experiments. Blood samples (600 µl) were taken from a cannula in the left carotid artery. Phenacetin and total paracetamol were determined in plasma after hydrolysis with β-glucuronidase/arylsulphatase (Sigma, type H-2) by HPLC and UV-detection. Details of the method will be given elsewhere [9].

For *in vitro* studies, mucosal cells of the small intestine were obtained by vibration in a solution containing EDTA [10, 11]. Cell viability was measured by lactate dehydrogenase (LDH) leakage from the cell cytoplasm into the medium as described by Grafström et al. [12]. Cell concentration was estimated by suspending an aliquot of cells in 0.16% trypan blue and 3 mM tetraphenyl borate (TPB) containing solution, and counting the number of cells in a Bürker chamber. Mucosal cells were incubated in Krebs-Ringer solution containing 25 mM $NaHCO_3$ and 12 mM glucose for 30 minutes at 37°C under air. The incubates (3.0 ml) contained cells from 0.25 g intestine and phenacetin concentrations ranging from 1 to 200 µM. Phenacetin-O-deethylation was determined as the amount of paracetamol formed, after hydrolysis with 6000 Fishman Units β-glucuronidase/arylsulphatase. After pre-extraction with dichloromethane at pH 12, paracetamol was extracted with diethylether at pH 5 and chromatographed on a Lichrosorb 10 RP-18 column by reversed phase HPLC and electrochemical detection. Details will be described later [Borm et al., to be published].

RESULTS AND DISCUSSION

The results of *in vitro* phenacetin deethylation studies with isolated intestinal mucosal cells of control and 3-MC pretreated rats are presented in Fig. 1. The kinetic constants obtained from these experiments are summarized in Table 1. K_m- and V_{max}-values are significantly

Table 1. Phenacetin-O-deethylation in rat intestinal mucosal cells of control and 3-MC pretreated rats.

pretreatment	K_m* (µM)	V_{max}* (nmol/min·g int.)	Cl_{int}† ml·min^{-1}·kg body weight^{-1}
oil, controls	56.7 ± 7.0	0.254 ± 0.05	0.143 ± 0.04
3-MC	37.6 ± 3.8	2.704 ± 0.200	2.30 ± 0.45

*Values represent the mean of at least 4 animals, determined in two different batches as described in the text.

†When calculating intrinsic metabolic clearance 32 g small intestine·kg^{-1} rat was assumed.

different from controls (Student t-test, two tailed $P < 0.05$) after 3-MC pretreatment. Pretreatment with 3-MC did not interfere with cell yield (6.10^7 cells/g intestine) or cell viability (80-90% viable cells in all batches). After 3-MC pretreatment a higher affinity (lower K_m) and greater maximal capacity (higher V_{max}) for phenacetin-O-deethylation result in a 16-fold elevation of Cl_{int} as compared with control rats. Extraction ratios were calculated using Eq. (2), and the relationship between extraction ratio and mucosal blood flow [13-15] using the intrinsic clearances of Table 1, is presented in Fig. 2.

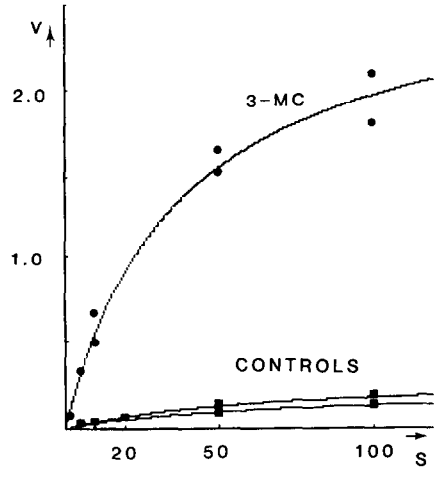


Fig. 1.
Plot of phenacetin metabolism in isolated intestinal mucosal cells of control (■) and 3-MC pretreated (●) rats. V is expressed as nmoles paracetamol/min·g intestine and substrate concentration as μM. Each point represents the mean of duplicates on the same batch of cells. Two different batches were tested. V_{max} and K_m values were calculated by means of a nonlinear (Duggleby) fitting procedure with bisquare weighing and proportional standard deviation.

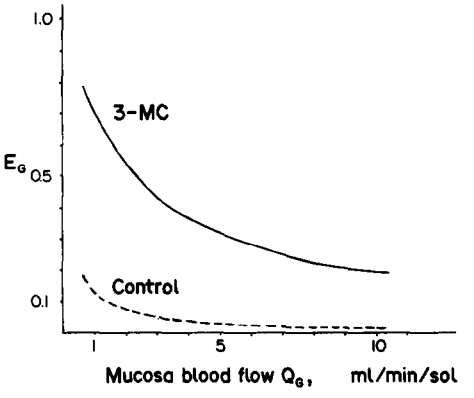
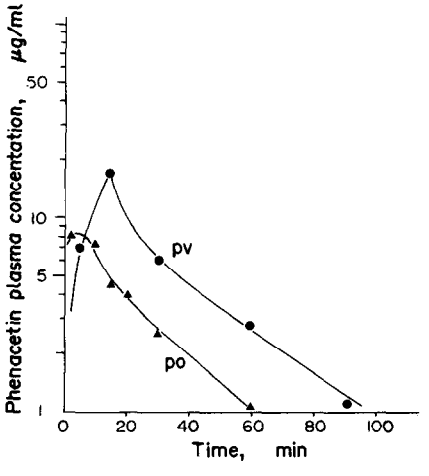


Fig. 2
Extraction ratio of the gut (E_G) as a function of mucosal blood flow (Q_G) by substituting Q_G and Cl_{int} (see Table 1) into eq. (2) for control and 3-MC pretreated rats.



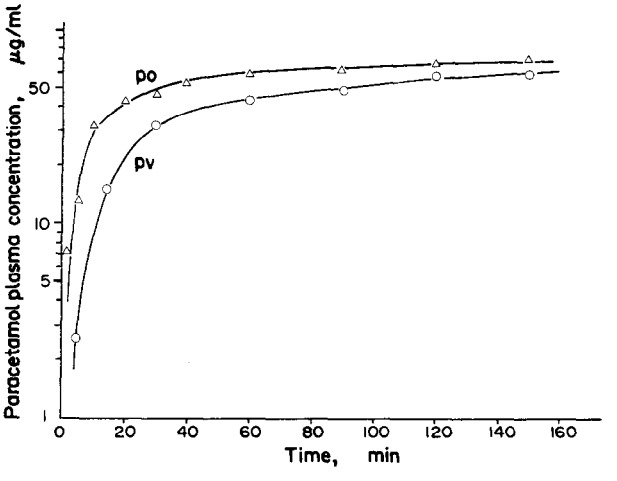


Fig. 3a (left) and 3b (right).
Plasma concentrations of phenacetin (Fig. 3a) and its metabolite paracetamol (free + hydrolyzed conjugates, Fig. 3b); phenacetin was administered intraduodenally (po, ▲, △) and via the portal vein (po, ●, ○) to rats pretreated with 3-MC (20 mg/kg, for 2 consecutive days). Each point represents the mean of at least 4 rats.

The *in vivo* disposition of phenacetin for two different administration routes in 3-MC pretreated rats is shown in Fig. 3a,b. The AUCs for phenacetin, for intraduodenal (po) and intraportal (pv) administration (Fig. 3a,b) are significantly different. This difference could not be explained by incomplete absorption as judged by metabolite concentration data (Fig. 3b). The results indicate that in 3-MC pretreated rats some 53% of the drug was metabolized by the gut wall. E_G, the extraction ratio by the gut, was calculated using Eq. (3) to be 0.53. In control rats no significant differences between AUC_{pv} and AUC_{po} were observed (data not shown). On the basis of *in vitro* data for 3-MC pretreated rats the calculated E_G varies between 0.31 and 0.53 when mucosal blood flow is varied between 5 and 2.5 $ml \cdot min^{-1} \cdot kg^{-1}$. These flow values have been estimated by several investigators [13-15]. On the other hand, E_G for controls varies between 0.03 and 0.07 under the same flow conditions.

In conclusion, the present study shows that there is a good correlation between the *in vivo* and *in vitro* rate of intestinal oxidative metabolism of phenacetin. This correlation was obtained using the above mentioned equations (Eq. 2), where mucosal blood flow is used instead of portal vein blood flow employed in previous physiological pharmacokinetic models [5, 6, 7]. We feel that using mucosal blood flow, rather than portal vein blood flow, is a better means of predicting gut wall first-pass metabolism.

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