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The effect of probenecid on paracetamol metabolism and pharmacokinetics

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Summary. The influence of probenecid on the pharmacokinetics of paracetamol was investigated in a group of healthy volunteers.

Pretreatment with probenecid caused a significant decrease in paracetamol clearance (6.23 to 3.42 ml·min^{-1}·kg^{-1}). The urinary excretion of paracetamol sulphate (243 to 193 mg); and paracetamol glucuronide (348 to 74.5 mg) were significantly reduced, whereas that of paracetamol was unchanged.

Probenecid was shown to be an uncompetitive inhibitor of paracetamol glucuronidation in vitro, using rat liver microsomes.

Key words: Paracetamol, Probenecid; drug metabolism, drug interaction

The analgesic drug, paracetamol, is metabolised by the liver primarily to paracetamol glucuronide (55%) and paracetamol sulphate (33%) [1]. The renal elimination of paracetamol and its two major metabolites has been extensively investigated in the dog. Paracetamol is shown to undergo glomerular filtration followed by reabsorption in the renal tubules by passive diffusion. The glucuronide and the sulphate metabolites undergo glomerular filtration and at low plasma concentrations both compounds are secreted by active transport processes [2].

Probenecid is an organic acidic drug, which is excreted into urine by active tubular secretion via the anion pump. Concurrent administration of probenecid has been shown to reduce the renal elimination of a number of agents [3–7]. In addition, probenecid is shown to reduce the formation of glucuronide conjugates of drugs, such as naproxen [8], clofibrate [9] and more recently zidovudine [10], as a direct result of inhibition of the glucuronidation pathway. Probenecid is itself eliminated in part by glucuronidation [11]. It is therefore possible that probenecid may alter paracetamol pharmacokinetics and pharmacological activity by inhibiting its glucuronidation. This study investigated the possible effect of probenecid on paracetamol pharmacokinetics in a group of healthy volunteers. The mechanism of the interaction was also examined in vitro, using rat liver microsomes.

Methods

Ten healthy volunteers (5 females) aged 20–29 y and weighing 56.5–83.5 kg took part in a randomised crossover study which was approved by the Local Ethical Committee. Following an overnight fast, each subject received an oral dose of paracetamol (3 × 500 mg b.p. formulation) on two different occasions, once 1 h after taking probenecid (2 × 500 mg Benemid tablets). There was a minimum of a 14 days washout period between the two treatments. Serial blood samples were collected at 0 (pre-paracetamol dose), 15, 25, 35, 50, 65, 95, 120, 180, 240, 300 and 360 min post-dose. A 6 h urine sample was also collected. Concentrations of paracetamol in plasma and of paracetamol sulphate and paracetamol glucuronide in urine were measured by high performance liquid chromatography (HPLC) [12]. The coefficient of variation for the assay was less than 5%.

In vitro microsomal study

Rat liver microsomal fractions were prepared by an established method [13]. Incubations were performed for 30 min at 37°C in a total volume of 250 µl, containing phosphate buffer pH 7.4 (final concentration 200 mM), microsomes (2 mg protein·ml^{-1}), uridine diphosphoglucuronic acid (UDPGA, 3 mM), Triton X-100 (0.0025% w/v), probenecid (5 mM) and varying concentrations of paracetamol (0.25–5.0 mM). The reaction was terminated by the addition of 3 N perchloric acid (125 µl) and following centrifugation at 1250 g for 15 min, the supernatant was stored at −20°C prior to analysis by HPLC.

Pharmacokinetic analysis

The area under the plasma paracetamol concentration versus time curve (AUC) was calculated by the trapezoidal rule and extrapolated to infinity. Paracetamol terminal half-life ($t_{1/2\beta}$) was calculated by least squares regression analysis. Paracetamol apparent clearance (CL_{app}) was calculated by CL_{app} = dose / AUC. Paracetamol apparent volume of distribution (V) was calculated from the product of clearance (CL_{app}) and the terminal elimination rate constant (K_{el}).

The data were compared using the Student's paired *t*-test.

Table 1. Effect of probenecid on paracetamol pharmacokinetics and urinary excretion of paracetamol (P), paracetamol sulphate (PS) and paracetamol glucuronide (PG). The results are expressed as mean (SEM). * $P < 0.05$

	C_{max} ($\mu g \cdot ml^{-1}$)	t_{max} (min)	$AUC_{0-95\ min}$ ($\mu g \cdot ml^{-1} min$)	CL_{app} ($ml \cdot min^{-1} kg^{-1}$)	$t_{1/2\beta}$ (min)	V ($l \cdot kg^{-1}$)	0–6 h urinary excretion of		
							P (mg)	PG (mg)	PS (mg)
Without probenecid	18.2 (1.9)	61.0 (9.5)	1180 (190)	6.23 (0.51)	127.2 (9.1)	1.0 (0.05)	33.3 (4.2)	348.2 (33.4)	243.4 (44.8)
With probenecid	23.5* (1.1)	74.5* (11.8)	1350 (130)	3.42* (0.3)	206.0* (32.6)	0.8* (0.03)	37.9 (4.0)	74.5* (9.9)	193.4* (29.0)

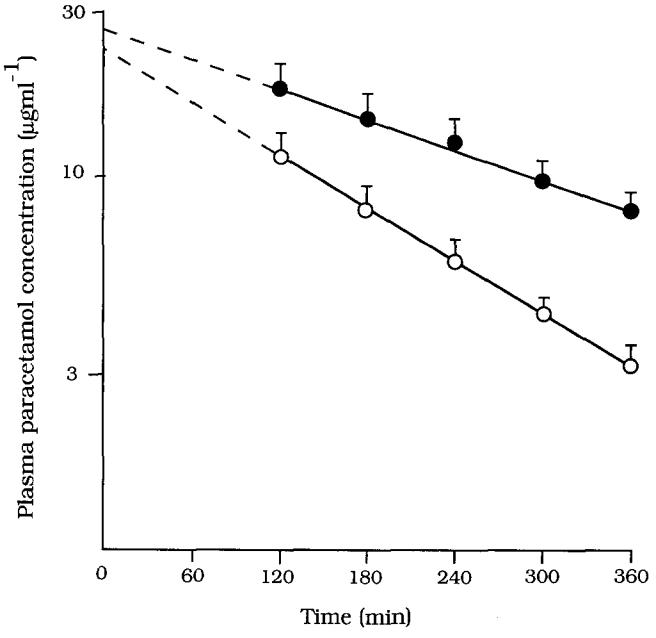

Fig. 1. Mean (SEM) plasma paracetamol concentration after a single oral dose of paracetamol with (●) and without (○) prior treatment with probenecid

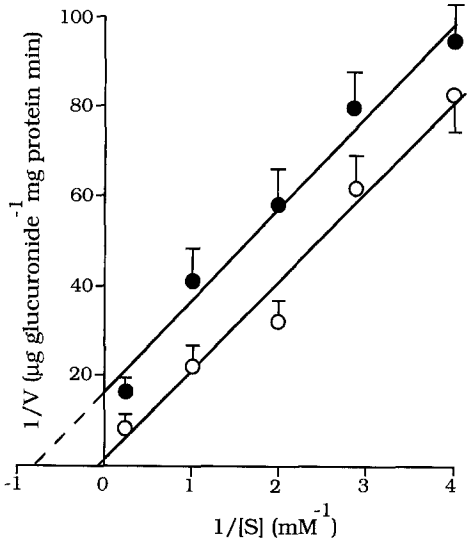

Fig. 2. Lineweaver-Burk plot of of UDPG-transferase activity towards paracetamol in the presence (●) and absence (○) of probenecid (5 mM). Each point represents the mean (SEM) obtained from 6 separate experiments

Results

Table 1 represents the effect of probenecid on paracetamol pharmacokinetics and the urinary excretion of paracetamol, paracetamol glucuronide and paracetamol sulphate. Probenecid pretreatment caused no significant change in the mean plasma paracetamol $AUC_{0-95\ min}$ or the mean time to peak paracetamol concentration (t_{max}). However, there was a significant increase in the mean peak plasma paracetamol concentrations (C_{max}). Prior administration of probenecid significantly decreased paracetamol clearance as well as the 0–6 h urinary excretion of paracetamol sulphate and paracetamol glucuronide, but not that of the unconjugated paracetamol (Table 1). Plasma paracetamol $t_{1/2\beta}$ was significantly increased whilst its V was significantly decreased following probenecid treatment (Fig. 1, Table 1).

The presence of probenecid in the incubation medium significantly decreased both the apparent K_m (19.9 (4.23) to 1.82 (0.36) mM; $p < 0.05$) and the maximum enzyme turnover velocity (V_{max}) (2.89 (0.70) to 0.24 ± 0.02 μg paracetamol glucuronide formed mg protein^{-1} min^{-1}; $p < 0.05$). However, there was no significant difference in the slopes of the lines drawn according to a Lineweaver-Burk plot and calculated by linear regression (Fig. 2). The inhibitor constant K_i was found to be 0.60 (0.14) mM.

Discussion

The data obtained from the human volunteer study suggest that prior administration of probenecid significantly decreased the urinary excretion of paracetamol sulphate and paracetamol glucuronide, but not that of the free unconjugated paracetamol. This is in agreement with two previous reported studies in man, postulating that elimination of paracetamol glucuronide and paracetamol sulphate in the kidney is mediated by an active transport process [14–15]. The results of this study are also in agreement with those found in dogs, with the exception of paracetamol glucuronide. In dogs it was shown that probenecid did not inhibit the net tubular secretion of the glucuronide conjugate, either due to the lack of an inhibitory effect of probenecid on the secretory mechanism of paracetamol glucuronide or that both the secretory and reabsorption mechanisms of paracetamol glucuronide are equally sensitive to probenecid [2].

The prior administration of probenecid also significantly decreased paracetamol clearance and increased paracetamol half-life. Like paracetamol, probenecid is metabo-

lised in part by conjugation with glucuronic acid [11]. However, there is an abundant reserve of glucuronic acid available in the liver which is not expected to diminish following administration of therapeutic doses of drugs. On the other hand, paracetamol sulphation is saturated at therapeutic doses of the drug [16]. However, probenecid does not undergo sulphation, and it is most likely that the decrease in the urinary recovery of paracetamol sulphate is a direct result of inhibition of active renal excretion by probenecid.

It is possible that the reduced paracetamol clearance is caused by probenecid inhibiting the activity of the hepatic enzyme, uridine diphosphate glucuronyl transferase (UDPG-transferase), responsible for metabolism of paracetamol to its glucuronide conjugate. Thus whilst probenecid may interfere with the elimination of paracetamol glucuronide by impairing the renal active transport, it is also likely that it inhibits paracetamol glucuronidation. Probenecid has been shown to be a potent inhibitor of UDPG-transferase activity (using 4-methylumbelliferone as substrate) in microsomal preparations in vitro [17]. Therefore, rat liver microsomal preparations were used to examine the possible inhibitory effects of probenecid on paracetamol glucuronidation *in vitro*. The presence of probenecid in the incubation medium significantly decreased both the apparent Km and Vmax values for paracetamol glucuronidation, and there was no significant difference in the slopes of the lines drawn according to the Lineweaver-Burk plots. These results suggest that probenecid acts as an uncompetitive inhibitor of paracetamol glucuronidation. This may either be due to probenecid combining with the enzyme substrate complex (glucuronyltransferase-paracetamol complex) to produce an inactive enzyme substrate inhibitor complex which in turn cannot undergo further reaction to yield paracetamol glucuronide, or an interaction with an allosteric site on the glucuronyltransferase.

The *in vitro* data indicate that the reduction in the urinary excretion of paracetamol glucuronide and the decrease in paracetamol clearance in the volunteers pretreated with probenecid is mainly attributable to the inhibitory effect of probenecid on paracetamol glucuronidation.

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