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Influence of water deprivation on the disposition of paracetamol

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The effect of acute (96 h) water deprivation on the disposition of paracetamol (acetaminophen) has been examined in male Sprague-Dawley rats. Plasma and urinary concentrations of the drug and its two major metabolites, the glucuronide and sulphate, were determined by a sensitive and specific high performance liquid chromatographic assay. Following an intravenous dose of 100 mg kg^{-1} of paracetamol, no significant changes were found in the elimination rate constant (k), the mean residence time (MRT), total plasma clearance (Cl) and the apparent volume of distribution at steady-state (Vss). However, rats deprived of water for 96 h excreted a larger percentage of the administered dose as the glucuronide conjugate (15·3 vs 7·9%) and a smaller percentage as unchanged paracetamol (7·3 vs 20·7%) in the urine. In addition, there was a significant two-fold increase in the partial metabolic clearance to paracetamol glucuronide. Water deprivation also led to a significant reduction in the renal clearance of paracetamol accompanied by an increase in the renal clearance of the glucuronide.

It is known that humans do not always replace orally all the fluids lost in sweat and urine, a condition referred to as voluntary dehydration (Greenleaf & Sargent 1965). Most frequently, the system may be dehydrated as a result of (i) disease states such as polyurea, diarrhoea, oesophageal or pyloric obstructions, (ii) exposure to tropical climates and high or humid temperatures, (iii) accidental fluid loss as in the case of haemorrhage, and (iv) circumstantial water deprivation as in severe muscular exercise. Any type of water deprivation, either acute or chronic, whether developed or induced, subjects the system to a stress, leading to significant hormonal, enzymatic and physiological changes (Jones & Pickering 1969; Hatton 1971; Baetjer & Rubin 1976; Keil & Severs 1977).

Ahmad et al (1982) and Bakar & Niazi (1983) have reported that the pharmacokinetics of aspirin and chloramphenicol are altered in water-deprived rats. Specifically, it has been shown that the rate of microsomal oxidative reactions (phase I) using a model drug, antipyrine, can be altered substantially (Prasad et al 1985). However, there is a paucity of data on phase II enzymes. Of the major conjugation reactions in animals and man, glucuronide and sulphate formation are two of the most common routes of drug biotransformation for many drugs and are quantitatively the most important of the phase II conjugations.

The present study was undertaken to investigate the disposition kinetics of paracetamol (acetaminophen) in control (food and water freely available) and 96 h water-deprived (food freely available) rats. Paracetamol was chosen as a model drug for the study of phase II metabolic pathways because of its widespread use and clinical importance, low binding to plasma proteins and quantitative metabolism primarily to the glucuronide and sulphate conjugates without having to go through a phase I metabolism.

Methods

Male Sprague Dawley rats (Locke-Erickson Laboratories, Oak Park, IL), 225 to 300 g, were randomly assigned to a treatment (water-deprived) or control group and given free access to food. The treatment rats were deprived of water for 96 h before administration of the drug while the control group was allowed water. Body weights of all rats were recorded before and after the 96 h to assess the influence of water deprivation.

At the end of the 96 h the right jugular vein and carotid artery were catheterized with silastic and polyethylene tubing, respectively, under light ether anaesthesia. All animals were housed individually in plastic

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metabolism cages and allowed to recover overnight. Paracetamol (100 mg kg^{-1}) was administered intravenously as a 25 mg mL^{-1} solution in 40% propylene glycol-0·9% saline (v/v) into the jugular vein cannula. Blood (0·2 mL) was collected via the carotid artery cannula just before and at 5, 15, 30, 45, 60, 90, 120, 180, 240 and 300 min after drug administration. It was centrifuged immediately and the plasma separated and frozen at −20 °C for analysis. Urine was collected for 24 h following drug administration and the total volume recorded; an aliquot was frozen at −20 °C for later analysis.

The concentrations of paracetamol, its glucuronide and sulphate in plasma and urine, were determined by high performance liquid chromatography (Jung & Zafar 1985).

Individual plasma paracetamol concentrations were fitted to a first-order, single compartment open model using the nonlinear regression program, MULTI (Yamaoka et al 1981) to obtain estimates for the first-order elimination rate constant (k). Using non-compartmental methods (Gibaldi & Perrier 1982), the zero and first moments of the plasma concentration-time data were used to obtain values for MRT, Cl and Vss. All area terms were calculated by a linear trapezoidal rule with end correction where necessary. Renal clearances of paracetamol and its conjugates were determined from the total amount of the dose excreted as drug or its metabolites and the area under the plasma concentration-time curve of the respective compounds. Because over 80% of an administered dose of the metabolites has been found in the urine unchanged (Galinsky & Levy 1981), partial clearances of the drug to the conjugated metabolites were determined as a product of total body clearance and the fraction of the administered dose excreted as the metabolites.

Levels of statistical significance were assessed using Student's *t*-test between two means for unpaired data. Significant differences were judged as $P < 0.05$. All results are expressed as mean ± s.e.

Results

Water deprivation for 96 h caused a significant decrease in mean body weight in male Sprague-Dawley rats from 258 ± 3 to 205 ± 11 g. The mean plasma paracetamol concentrations in control and water-deprived rats after an intravenous dose of 100 mg kg^{-1} are shown in Fig. 1. These appeared to decline exponentially with time following a single compartment open body model. However, since the disposition of the drug is concentration- as well as time-dependent, the estimates of total body clearance are time-averaged values (Galinsky & Levy 1981). The derived pharmacokinetic parameters based on non-compartmental analysis of the plasma concentration vs time data are presented in Table 1. There were no significant differences in the mean pharmacokinetic parameters (MRT, Vss, Cl, k) between the control and water-deprived rats but drug-treated rats excreted a larger percentage of the adminis-

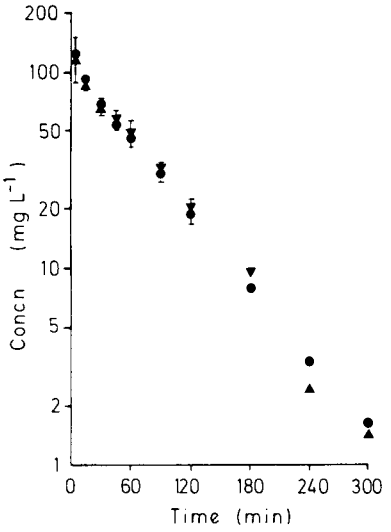

FIG. 1. Plasma paracetamol concentrations following intravenous administration of 100 mg kg^{-1} to control (●) and water-deprived (▲) rats. Each point is the mean ± s.e. for 8–9 animals.

Table 1. Pharmacokinetic parameters of paracetamol in water deprivation. Each value is the mean ± s.e.

Parameter	Control (n = 8)	Water-deprived (n = 9)
MRT (min)	73·4 ± 3·8	74·8 ± 2·6
Vss (L kg^{-1})	0·97 ± 0·03	1·04 ± 0·05
Cl (mL min kg^{-1})	13·8 ± 0·9	13·9 ± 0·7
k (min^{-1})	0·0154 ± 0·0006	0·0152 ± 0·0003

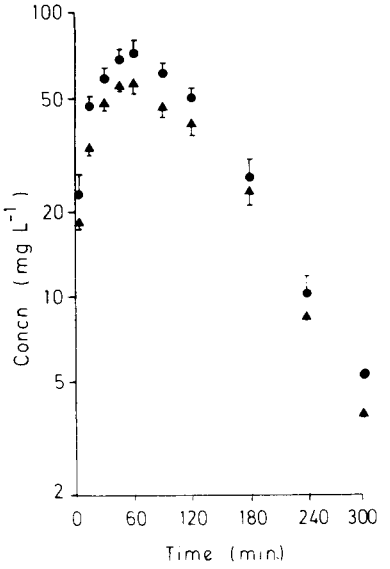

FIG. 2. Plasma paracetamol glucuronide concentrations (in terms of paracetamol) obtained in the experiments described in Fig. 1.

Table 2. Effect of 96 h water deprivation on the recovery of urinary excretion products of paracetamol in male rats. Results are percentage of administered dose and are expressed as mean ± s.e.

Urinary excretion product	Control (n = 8)	Water-deprived (n = 9)
Paracetamol	20·7 ± 3·4	7·3 ± 1·0*
Paracetamol glucuronide	7·9 ± 2·9	15·3 ± 1·2*
Paracetamol sulphate	60·4 ± 2·0	59·2 ± 1·7
Total	89·0 ± 2·8	81·8 ± 2·4

* $P < 0.05$.

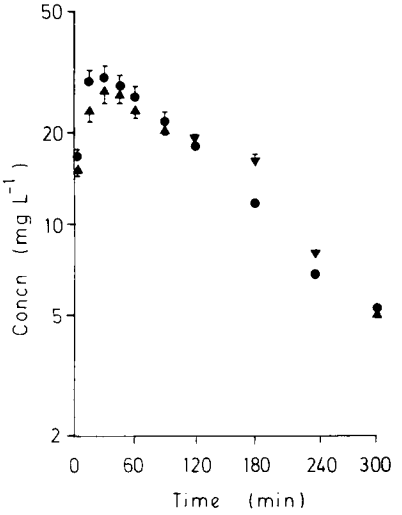

FIG. 3. Plasma paracetamol sulphate concentrations (expressed in terms of paracetamol) obtained in the experiments described in Fig. 1.

Table 3. Effect of 96 h water deprivation on the renal clearances of paracetamol, glucuronide and sulphate and on the time-averaged partial metabolic clearances of the drug to the glucuronide and sulphate conjugates after administration of 100 mg kg^{-1} of the drug to male rats. Results are in mL min^{-1} kg^{-1} and are expressed as mean ± s.e.

Parameter	Control (n = 8)	Water-deprived (n = 9)
Renal clearance of paracetamol	2·8 ± 0·1	1·0 ± 0·1*
Renal clearance of the glucuronide	1·5 ± 0·5	4·6 ± 0·8*
Renal clearance of the sulphate	21·0 ± 2·4	24·6 ± 2·9
Partial clearance to the glucuronide	1·0 ± 0·4	2·1 ± 0·2*
Partial clearance to the sulphate	8·3 ± 0·6	8·1 ± 0·6

* $P < 0.05$.

tered dose as the glucuronide (15·3 ± 1·2% vs 7·9 ± 3·0%) and a smaller percentage as unchanged drug (7·3 ± 1·0% vs 20·7 ± 3·4%). However, no significant difference was found in the fraction of the dose recovered in the urine as paracetamol sulphate or total drug between the control and water-deprived animals (Table 2).

The mean time courses of paracetamol glucuronide and sulphate concentrations in plasma following intravenous injection of 100 mg kg^{-1} drug in both control and treated rats are shown in Figs 2 and 3, respectively. The lack of sufficient quantities of the glucuronide and sulphate conjugate prevented a detailed assessment of the disposition kinetics of both metabolites. The glucuronide concentrations in plasma were consistently lower in the water-deprived rats, suggesting either a decreased rate of formation of this metabolite and/or an increase in its apparent volume of distribution. The significant increase in the renal clearance of the glucuronide conjugate in the water-deprived rats may also have contributed to the lower plasma concentrations of the glucuronide (Table 3). Paracetamol sulphate concentrations, however, were not significantly different between the two groups of rats. The time averaged partial metabolic clearance of the drug to its glucuronide conjugate was significantly increased from 1·0 ± 0·4 to 2·1 ± 0·2 mL min^{-1} kg^{-1}, whereas there was no significant alteration in the partial metabolic clearance to the sulphate (8·3 ± 0·6 vs 8·1 ± 0·6 mL min^{-1} kg^{-1}) in the water-deprived rats.

Discussion

One factor that must be considered in the interpretation of the effect of acute water deprivation on paracetamol disposition is the capacity-limited formation of the glucuronide and sulphate conjugates and the depletion of endogenous sulphate which is used in the formation of paracetamol sulphate. In the present study, acute water deprivation did not significantly affect the primary pharmacokinetic parameters (MRT, Vss, Cl and k) of paracetamol in plasma. The drug is only slightly plasma protein-bound (about 10%) and its volume of distribution (1 L kg^{-1}) is slightly larger than the predicted volume of total body water. Since previous studies have shown that water deprivation led to decreases in total and pulmonary blood volumes (Aarseth & Klug 1972), plasma water (Libermann et al 1976) and total body water (Prasad et al 1985), the disposition of paracetamol should be sensitive to changes in total body water. However, unlike the decreases in Vss observed following intravenous administration of antipyrine (Prasad et al 1985), which is distributed in total body water, and gentamicin (Lecompte et al 1981), which is distributed in extracellular fluid, no significant alterations were seen in the Vss following paracetamol administration in water-deprived rats.

In addition to distribution, the elimination kinetics of paracetamol were not affected by 96 h water depriva-

tion. In contrast to the observed decrease in total body clearance of antipyrine (Prasad et al 1985), which is commonly used to assess hepatic oxidative phase I metabolic reactions, no alterations in Cl were seen for paracetamol, a model compound which is used for the study of hepatic phase II metabolism. The lack of effect of acute water deprivation on the clearance of the drug is a result of the simultaneous decrease in its renal clearance ($2{\cdot}8 \pm 0{\cdot}1$ to $1{\cdot}0 \pm 0{\cdot}1$ mL min^{-1} kg^{-1}) and the increase in the partial metabolic clearance to glucuronide ($1{\cdot}0 \pm 0{\cdot}4$ to $2{\cdot}1 \pm 0{\cdot}2$ mL min^{-1} kg^{-1}). As predicted by the pK_a value of 9·5, the renal clearance of paracetamol is not dependent on urine pH, but previous studies have shown a weak, but significant correlation with urine flow rates (Prescott & Wright 1973; Forrest et al 1982). Thus, the decreased renal clearance ($2{\cdot}8 \pm 0{\cdot}1$ to $1{\cdot}0 \pm 0{\cdot}1$ mL min^{-1} kg^{-1}) of paracetamol found in our study may be partially a result of the observed reduction in urine flow rate from about 20 to 5 mL/24 h in the water-deprived rats. In our study, although no significant differences were observed in the pharmacokinetic parameters following paracetamol administration, there was a significant alteration in the metabolic pathways as a result of water deprivation. A significant increase in the percentage of the administered dose excreted in the urine as the glucuronide conjugate was associated with a concomitant decrease in the fraction of the dose excreted as unchanged drug. However, no alteration was found in the metabolism of the drug to its sulphate conjugate. The finding that there is an increase in the fraction of the dose excreted as the glucuronide as well as the increased partial metabolic clearance to the glucuronide conjugate suggests that there is an increase in the amount and/or activity of UDP-glucuronyl transferase due to acute water deprivation.

In conclusion, the present study has demonstrated that the pharmacokinetics of paracetamol in plasma are not altered as a result of 96 h water deprivation. However, the increase in the conversion of the drug to its glucuronide conjugate may possibly be associated with an increase in the amount and/or activity of UDP-glucuronyl transferase in water-deprived rats. The clinical implications of the present findings are unknown.

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