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The role of sulphate conjugation in the metabolism and disposition of oral and intravenous paracetamol in man

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1 The effects of paracetamol dose (5 and 20 mg/kg) and route of administration (intravenous and oral) on the urinary excretion of paracetamol and its glucuronide, sulphate, cysteine and mercapturic acid conjugates were studied in five healthy subjects.
2 The fractional urinary excretion of unchanged paracetamol and its conjugates was independent of the route of administration at both dose levels, suggesting that the gastrointestinal tract is not an important site for paracetamol metabolism.
3 The percentage of the dose excreted as the sulphate conjugate was significantly higher after 5 than after 20 mg/kg (37.7% and 33.3% respectively) and this is consistent with saturation of sulphate conjugation.
4 No significant effect of paracetamol dose upon the area under the plasma concentration-time curve (AUC), corrected for dose, was found for the sulphate or glucuronide conjugates.
5 The total plasma clearance of paracetamol and the renal clearance of the sulphate conjugate were significantly higher after the 5 than the 20 mg/kg dose (331 ± 42 ml/min and 295 ± 48 ml/min; 273 ± 74 ml/min and 205 ± 46 ml/min respectively).
6 The oral systemic availability of paracetamol was 80% and independent of dose.

Keywords paracetamol sulphate conjugation metabolism

Introduction

Sulphation is an important route of intestinal metabolism of some phenolic drugs (George, 1981). If this reaction occurs in both the intestine and the liver, the proportion of the dose converted to the sulphate conjugate should depend on whether the route of administration is oral or parenteral. For example, the sulphate conjugation of isoprenaline in man is extensive following oral but is minimal after parenteral administration (Conolly *et al.*, 1972; George *et al.*, 1974). Paracetamol undergoes extensive sulphate and glucuronide conjugation but it is not known whether its metabolism is similarly route-dependent.

To investigate the contribution of intestinal sulphate conjugation to paracetamol metabolism, we measured the urinary metabolites of oral and intravenous paracetamol in five healthy subjects given doses of 5 and 20 mg/kg on separate occasions. The sulphate conjugation of paracetamol is dose-dependent (Levy & Yamada, 1971) and to minimise the possibility of saturation of gastrointestinal sulphate conjugation, concentrations at the site(s) of metabolism were kept low by slow, constant-rate delivery of the drug over a period of 2 h.

Methods

Five healthy male subjects (age 29 to 39 years, mean 33.4 years; weight 66 to 83 kg, mean 72.4 kg) who were taking no other drugs participated in the study. Each fasted overnight and was given each dose of intravenous paracetamol (5

or 20 mg/kg) by constant infusion over 2 h using an IMED pump (IMED Corporation, California). Intravenous paracetamol (0.875 and 3.5 mg/ml) was prepared in phosphate buffer (pH 6.0) and sterilised by filtration. On subsequent occasions paracetamol was dissolved in 400 ml of 'Coca Cola' and ingested over 2 h; 50 ml aliquots were taken over successive 15 min periods. In this way, rates of administration were similar by both routes. The subjects remained seated for 2 h and then resumed normal activities. Food, drink and tobacco were allowed after 4 h. Each of the four studies was separated by at least one week.

Venous blood was taken at 0, 15, 30, 45, 60, 75 and 90 min and 2, 3, 4, 6 and 8 h. Urine was collected at 0, 0–1, 1–2, 2–3, 3–4, 4–6, 6–8, 8–10, 10–12 and 12–24 h. Plasma and urine were stored at −20°C until assayed for paracetamol and its glucuronide, sulphate, mercapturic acid and cysteine conjugates by modification of the high-pressure liquid chromatographic methods of Adriaenssens & Prescott (1978) and Knox & Jurand (1978). In order to measure low urinary concentrations of the sulphate conjugate in the presence of interfering compounds, its retention was increased by the use of cetyltrimethylammonium bromide as counter-ion; electrochemical detection (Model LC-4A, Bioanalytical Systems Inc.) was used to increase sensitivity and selectivity for unchanged paracetamol and the mercapturic acid and cysteine conjugates.

Total quantities of paracetamol and its conjugates excreted in the urine over 24 h were expressed as percentages of the total recovered material. Areas under the plasma concentration-time curves (AUC) were calculated by the trapezoidal rule with correction (for paracetamol only) for the area beyond the last data point (Gibaldi & Perrier, 1975). The total plasma paracetamol clearance was calculated as the intravenous dose/AUC (0 to infinity) and the systemic availability as the ratio of areas after oral and intravenous administration.

Mean results are shown with standard deviations; Student's t-test and analysis of variance were used in tests of statistical significance.

Results

For each dose, the plasma concentrations of unchanged paracetamol were similar after each route of administration, taking into account the first-pass loss and the difference in dose (Figure 1).

Urinary excretion

The percentage recovery of paracetamol and each of its conjugates was independent of the route of administration at both dose levels (Table 1). In particular, the production of sulphate conjugate was not significantly higher after oral administration. Indeed, when results for both doses were combined, less sulphate conjugate was recovered after the oral (35.0%) than after intravenous (36.1%) administration.

The dose had a significant effect upon the excretion of paracetamol sulphate and the mean recoveries after the 5 and 20 mg/kg doses were 37.7 ± 10.4% and 33.3 ± 8.9% respectively ($P < 0.001$). There were reciprocal changes in the glucuronide conjugation with higher recovery after the 20 than the 5 mg/kg dose (51.8 ± 11.9% and 47.1 ± 12.5% respectively; $P < 0.002$).

By analysis of variance, the 'subject' factor was highly significant ($P < 0.01$) for all the metabolites, reflecting large but very consistent differences between subjects in the proportion of metabolites excreted. In contrast, the excretion of unchanged paracetamol was surprisingly

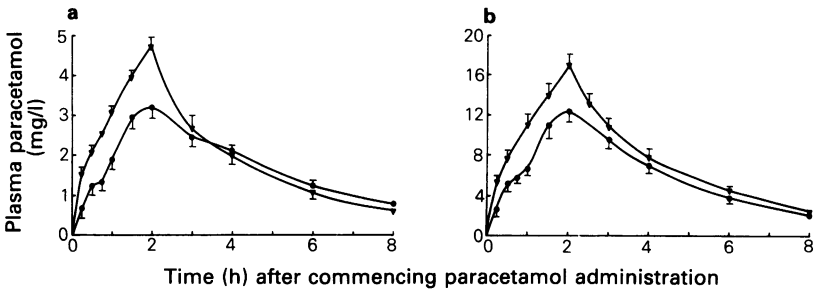

Figure 1 Mean plasma paracetamol concentrations (mg/l) in five subjects after oral (●) and intravenous (▲) administration of (a) 5 and (b) 20 mg/kg, (mean ± s.e. mean).

Table 1 Percentage urinary recoveries of paracetamol and metabolites after i.v. infusion and oral administration of 5 and 20 mg/kg over 2 h in five healthy subjects (mean ± s.d.)

	Dose (mg/kg)	*i.v. infusion*	*Oral solution*
Paracetamol	5	3.2 ± 0.4	5.5 ± 3.4
	20	4.3 ± 1.9	3.1 ± 0.4
Sulphate conjugate	5	38.4 ± 11.8	36.9 ± 11.4
	20	*33.7 ± 10.1	*33.0 ± 9.6
Glucuronide conjugate	5	47.6 ± 12.6	46.6 ± 15.2
	20	50.4 ± 13.5	*53.2 ± 12.8
Cysteine and mercapturic acid conjugates	5	10.8 ± 4.0	11.1 ± 6.3
	20	11.6 ± 8.7	10.7 ± 6.1

* Significant difference ($P < 0.025$) from 5 mg/kg. Differences between routes not significant ($P > 0.05$).

uniform with no significant differences between subjects. The ratio of sulphate to glucuronide conjugate excreted in 24 h was independent of the route of administration but was significantly higher after 5 than 20 mg/kg (0.90 ± 0.50 and 0.72 ± 0.32 respectively; $P < 0.02$).

Renal clearance

The renal clearance of the sulphate conjugate of paracetamol was significantly higher after 5 than after 20 mg/kg (273 ± 74 ml/min and 205 ± 46 ml/min, respectively; $P < 0.02$). There were no similar dose-dependent differences in the renal clearance of paracetamol or any of the other conjugates.

Areas under the plasma concentration-time curves

The AUC for paracetamol was significantly smaller after oral than intravenous administration because of 'first-pass' metabolism (Table 2) and the systemic availability of oral paracetamol was virtually identical after 5 and 20 mg/kg doses (79.6 ± 9.6% and 80.8 ± 7.8% respectively). When corrected for dose, the AUC for paracetamol was significantly higher after 20 than after 5 mg/kg for both routes of administration. Paracetamol plasma clearances after intravenous administration were 331 ± 42 ml/min and 295 ± 48 ml/min for the 5 and 20 mg/kg respectively and differed significantly ($P < 0.05$).

The route of paracetamol administration had no important effect on the AUC values for the sulphate or glucuronide conjugates. However, the AUC for the sulphate (but not the glucuronide) conjugate was significantly higher after 20 than 5 mg/kg intravenously (Table 2).

Discussion

We were unable to demonstrate any effect of route of administration on the extent of paracetamol sulphate conjugation at either dose level.

Table 2 AUC (mg l^{-1} h) for plasma paracetamol (0 to infinity) and its sulphate and glucuronide conjugates (0 to 8 h) after i.v. infusion and oral administration of 5 and 20 mg/kg over 2 h in five healthy subjects. (Tabulated values of AUC for the 20 mg/kg dose are the observed values divided by 4.0) (mean ± s.d.)

	Dose (mg/kg)	*i.v. infusion*	*Oral solution*	*Difference*
Paracetamol	5	18.38 ± 1.65	14.67 ± 2.48	$P < 0.001$
	20	*20.62 ± 2.50	*16.67 ± 2.55	$P < 0.001$
Sulphate conjugate	5	5.32 ± 1.82	6.75 ± 2.42	NS
	20	*6.60 ± 2.58	6.65 ± 1.42	NS
Glucuronide conjugate	5	14.64 ± 4.97	17.67 ± 7.13	$P < 0.05$
	20	15.66 ± 4.84	16.95 ± 7.54	NS

* Significant difference from 5 mg/kg dose ($P < 0.05$).

This implies that no significant conjugation occurred in the gastrointestinal tract at these doses. It is possible that intestinal metabolism could play a more important role at very low doses, as found with isoprenaline (Conolly *et al.*, 1972), isoetharine (Williams *et al.*, 1974), rimiterol (Evans *et al.*, 1974) and terbutaline (Davies *et al.*, 1974).

Considerable inter-subject variation in the metabolism of paracetamol was observed but the fractional urinary recovery of each conjugate was remarkably constant within each subject.

There was dose-dependent sulphate conjugation of paracetamol, in keeping with previous reports of the limited capacity of this metabolic route after oral administration in man (Levy & Yamada, 1971). We have shown that the elimination of paracetamol as the sulphate conjugate is also dose-dependent after intravenous paracetamol administration.

The decreased formation of the sulphate conjugate at the higher dose of paracetamol was accompanied by a corresponding increase in the extent of glucuronide conjugation.

Neither dose nor route of administration had any significant effect on the formation of the cysteine and mercapturic acid conjugates.

After the 5 mg/kg oral dose of paracetamol, the extent of formation of the sulphate conjugate (as measured by the urinary recovery) and its renal clearance were both greater than after the 20 mg/kg dose. As a result, the dose-adjusted AUC values were not significantly different. However, after intravenous administration the AUC for the sulphate conjugate was significantly greater after the 20 mg/kg dose, despite the smaller proportion of sulphate conjugate formed. This was due to the much lower renal clearance of the sulphate conjugate at the higher plasma concentrations found after the 20 mg/kg dose. Thus, with the doses of paracetamol used in this study there is saturation of both the formation and the renal clearance of the sulphate conjugate. Clearly, the AUC is an unsatisfactory measure of fractional biotransformation when a metabolite is excreted by a saturable pathway.

A further observation was that the total plasma clearance of paracetamol was lower after 20 than 5 mg/kg doses. Although the difference was small (12%) and clearly not of therapeutic importance it should be taken into account in bioavailability studies. Our finding that 20% of an oral dose of paracetamol is lost by pre-systemic clearance is in agreement with previous reports (Perucca & Richens, 1979; Prescott, 1980). However, we were unable to confirm the dose-dependent systemic availability of paracetamol reported by Rawlins *et al.* (1977). In the latter study, the AUC values after oral doses of 500, 1000 and 2000 mg were compared with the AUC after a single intravenous dose of 1000 mg. In the present study the same dose was given by both routes. The systemic availability of the 500 mg oral dose reported by Rawlins *et al.* (1977) may have been spuriously low because the plasma paracetamol clearance was higher than after the 1000 mg intravenous dose.

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