HUMAN METABOLISM OF PARACETAMOL (ACETAMINOPHEN) AT DIFFERENT DOSE LEVELS

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SUMMARY

Urine (0-24 h) was collected from five subjects on separate occasions following the ingestion of paracetamol at five different dose levels (500, 750, 1000, 1250, 1500 mg) which spanned the normal therapeutic range. The major urinary metabolites were sulphuric and glucuronic acid conjugates which together accounted for around 50% of the administered dose. Unchanged paracetamol excretion was low (5-20%). This situation was similar over the entire dose range. These findings are discussed in relation to previous single dose studies reported in the literature.

KEY WORDS

paracetamol, glucuronidation, sulphation, conjugation, dose-dependent metabolism

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INTRODUCTION

Paracetamol (acetaminophen, N-acetyl-4-aminophenol) is a widely used non-opioid analgesic drug, similar in efficacy to aspirin but having no demonstrable anti-inflammatory activity and being less irritant to the stomach lining.

The compound is rapidly absorbed from the gastrointestinal tract and quickly moves through the plasma compartment (C_{max}~1 h; $t_{½}$~2h) to be eliminated in the urine /1/. The major routes of metabolism are via sulphuric and glucuronic acid conjugation and this metabolism is believed to be dose-dependent, as sulphate conjugates have been reported as being of proportionately greater importance at lower dose rates /2/. In cases of overdose, depletion of hepatic glutathione levels occurs through formation of toxic quinonemines which are not generally found at therapeutic dose levels. In healthy volunteers it has been shown that the ratio of sulphuric acid/glucuronic acid conjugates was reproducible within an individual for a given dose rate /3/. There seems, however, to be little information in the literature on the comparative metabolism of different dose-levels of paracetamol in the same individual. This study provides data on the metabolic conjugation of paracetamol in five healthy individuals at five dose levels over the normal therapeutic dose range of 500 to 1500 mg (usually 1 to 3 tablets).

MATERIALS AND METHODS

Human volunteers

Five subjects (2 male, 28 and 37 years old; 3 female, 25, 28 and 48 years old) volunteered to participate in this study. They were in good health and possessed normal hepatic and renal function. All were non-smokers and were taking no medication, having abstained from paracetamol ingestion for at least 14 days before the commencement of the study. No alcohol was permitted for 2 days prior to and during the investigation. Approval for the study was obtained from the local Ethics Committee.

After an overnight fast, paracetamol (500, 750, 1000, 1250 or 1500 mg) in gelatin capsules was taken orally with water (100 ml) at 08.00 a.m. The subjects were permitted normal access to food and drink from 09.30 a.m. The urine voided during the ensuing day was collected in 0-

8 h and 8-24 h samples, the total volumes noted and aliquots (20 ml) frozen (-20°C) until analysis. Repeat administration of paracetamol at a different dose level was not undertaken until at least 14 days had elapsed from the previous study.

Urine analysis

Paracetamol, both free and liberated from conjugates after enzyme hydrolyses, was quantified by high-pressure liquid chromatography (h.p.l.c.) employing a reverse-phase C18 column (Technopack 10, 5.0 μm particle size; 25 cm long, 0.39 cm i.d.; HPLC Technology Ltd., Macclesfield, UK). The mobile phase consisted of an aqueous component [0.02 M tetrabutylammonium hydroxide and 0.01 M tris(hydroxymethyl)aminomethane buffered to pH 5.0 with orthophosphoric acid] which was run against a linear gradient of acetonitrile from 15% (v/v) to 50% (v/v) over an eleven minute period with a flow rate of 2.0 ml/min. Detection was performed by ultraviolet spectrophotometry at 254 nm /4/.

Appropriate amounts of paracetamol were added to control urine to give final concentrations of 25-200 μg/ml. These standards and all urine samples were filtered before injection onto the column. The system employed had an intra-assay variation of 2.5% (N=5) and an interassay variation of 6.4% (N=5) across the above working range.

Glucuronic acid conjugates were hydrolysed by incubation of urine (1.0 ml) with glucuronidase (1000 units, bovine liver β-glucuronidase type B1; Sigma Biochemicals, Dorset, UK) in 0.05 M acetate buffer (1.2 ml, pH 5.0) at 37°C for 18 h. Sulphuric acid conjugates were hydrolysed employing sulphatase (200 units, *Helix pomatia* sulphatase type H-1; Sigma) with the addition of D-saccharic acid-1,4-lactone (10 mg) to inhibit any β-glucuronidase activity. The use of authentic samples of paracetamol sulphate and paracetamol glucuronide (gift from Sterling Winthrop Group, Fawdon/Guildford, UK) permitted the verification of these enzyme hydrolyses and established the lack of cross activity (e.g. sulphatase activity in β-glucuronidase) within these systems. All hydrolyses were complete within the 18 h time period, no additional paracetamol being released on further incubation. Aliquots (10 μl) of these incubations were then filtered and examined by h.p.l.c. as described above /5/.

RESULTS AND DISCUSSION

When the 0-8 h versus the 0-24 h urine samples were compared it was evident that the first eight hours were the most important for conjugate excretion (Table 1). During this period, over 80% of the total conjugate output was voided at the lower dose levels (500, 750 mg) with this decreasing to approximately 60% following the higher doses of the drug (1000, 1250, 1500 mg). Excretion of free paracetamol appeared equally divided between the 0-8 h and 0-24 h urine samples. Overall, the 0-24 h urinary excretion of free paracetamol was low, accounting for between 5-20% of the dose, whereas the combined sulphuric and glucuronic acid conjugates represented just over 50% of the administered compound with glucuronic acid conjugation being roughly twice as important over the entire dose range investigated.

TABLE 1

Percentage of dose excreted as paracetamol and conjugates, the composition of conjugate metabolites and the sulphate/glucuronide ratio for the five oral paracetamol dose levels studied

Dose (mg)	Percentage administered dose		Percentage excreted conjugates		Sulphate / glucuronide ratio
	paracetamol	conjugates	sulphate	glucuronide	
500	2.4 ± 2.0	48.4 ± 19.2	27.0 ± 5.8	73.0 ± 5.8	0.4
	5.4 ± 3.1	57.9 ± 28.8	22.3 ± 5.3	77.7 ± 5.3	0.3
750	3.6 ± 2.4	43.0 ± 16.6	34.7 ± 15.0	65.3 ± 15.0	0.5
	5.1 ± 3.7	52.1 ± 18.0	32.4 ± 13.9	67.6 ± 13.9	0.5
1000	6.0 ± 4.9	29.7 ± 15.9	24.4 ± 19.7	75.6 ± 19.7	0.3
	13.6 ± 7.3	55.3 ± 21.5	26.7 ± 15.6	73.3 ± 15.6	0.4
1250	6.2 ± 4.8	28.7 ± 14.3	26.6 ± 18.2	73.4 ± 16.2	0.4
	15.1 ± 5.1	48.7 ± 20.4	28.1 ± 17.4	71.9 ± 15.9	0.4
1500	7.9 ± 4.5	30.3 ± 22.2	27.5 ± 17.8	72.5 ± 17.8	0.4
	19.8 ± 3.2	45.4 ± 27.0	33.7 ± 15.6	66.3 ± 15.6	0.5

Values given are the mean ± 1 s.d. for the five volunteers.
For each dose level, the top row of results relates to the 0-8 h urine collection and the bottom row to the total 0-24 h urine collection.

TABLE 2

Urinary paracetamol sulphate/glucuronide ratios calculated from human studies previously cited in the literature

Dose (g)	Volunteers number/sex		Urine collection period (h)	Sulphate/ glucuronide ratio	Literature reference
0.362[a]	5	M	0-24	0.79	/6/
0.650[b]	7	M	0-6	0.47	/7/
1.000	34	M&F	0-8	1.05	/3/
1.000[c]	10	M&F	0-12	0.54	/8/
1.000	8	M	0-24	0.42	/9/
1.000	8	F	0-24	0.47	/9/
1.000	3	M	0-24	0.65	/10/
1.448[a]	5	M	0-24	0.62	/6/
1.500	65	M	0-24	0.55	/11/
1.500	46	F	0-24	0.63	/11/
2.000	3	M	0-24	0.53	/10/
3.000	3	M	0-24	0.57	/10/
4.000	3	M	0-24	0.36	/10/

The mean paracetamol sulphate/glucuronide ratios have been recalculated from data given in the references cited. In some instances data have been extracted from graphical plots.

a taken over 3 hours
b same dose taken every 6 hours
c overnight urine collection

It appears that over the normal therapeutic dose range the metabolism remains constant within a given individual and conjugate formation remains constant. At lower, non-therapeutic doses the sulphation process may become more prominent, as has been suggested, and this may be relevant in paediatric medication.

In concordance with the present results, the literature shows that similar metabolic sulphate/glucuronide ratios were observed over a wide range of paracetamol doses (0.36 to 4.0 g), indicating the predominance of the glucuronidation pathway /6-11/ (Table 2). However, this may not be true for all individuals. A recent study examining the metabolism of a low dose (500 mg) of paracetamol in patients with degenerative neurological diseases and also healthy controls reported a mean sulphate/glucuronide ratio of 5.6 (n=50) suggesting that sulphation was the major conjugation pathway. On closer examination, however, it was evident that the individual ratios ranged from 0.6 to 58.0, nearly a hundred-fold difference, suggesting gross interindividual variation /12/. Similar observations had been previously made on healthy volunteers (n=34) ingesting paracetamol (1.0 g); whereas a mean sulphate/glucuronide ratio of 1.05 was quoted, a range between 0.22 and 2.86 (10-fold variation) was observed, with four individuals displaying a marked relative inability to form the sulphate conjugate /3/. A three-fold variation in glucuronide and sulphate formation has also been noticed during an ethnicity study (n=111) /11/.

Until a large population (several hundreds) is repeatedly examined over a wide paracetamol dose range, knowledge of the extent of these problems will remain elusive. In addition, single dose studies presumably do not allow prediction of the relative capacities of the sulphuric and glucuronic acid conjugation mechanisms following chronic therapeutic dosage of paracetamol. In particular, sulphation has a much lower capacity than glucuronidation, the whole body K_ms for paracetamol being approximately 1.0 g /3/, and is also profoundly influenced by the availability and potential depletion of sulphate within the body. As sulphate supply is relatively limited /13/ this may be of importance in individuals with low serum sulphate levels /14/.

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