J. Pharm. Pharmac., 1977, 29, 235

COMMUNICATIONS

Paracetamol metabolism following overdosage: application of high performance liquid chromatography

D. Howie, P. I. Adriaenssens, L. F. Prescott*, *University Department of Therapeutics, The Royal Infirmary, Edinburgh, EH3 9YW, U.K.*

There has been renewed interest in the metabolism of paracetamol since Mitchell, Thorgeirsson & others (1974) described the mechanisms of hepatic necrosis induced by this drug. In particular, the significance of the cysteine and mercapturic acid conjugates has been established in relation to the formation of a hepatotoxic intermediate metabolite (Mitchell & others, 1974; Andrews, Bond & others, 1976; Davis, Simmons & others, 1976). Sulphydryl donors such as cysteamine and L-methionine protect against severe liver damage following overdosage (Prescott, Park & others, 1976), but the mechanisms involved are unknown.

The urinary excretion of paracetamol and its conjugates (as measured by high performance liquid chromatography, h.p.l.c.) was studied in 5 males and 5 females admitted to the Regional Poisoning Treatment Centre, Edinburgh, following severe paracetamol poisoning. Their ages ranged from 19–54 years (mean 30·4). The mean plasma paracetamol concentration 4 h after ingestion was 371 μg ml^{-1} and the mean total urinary recovery of paracetamol ranged from 11·8–24·2 g (mean 18·3 g). Serial blood samples were taken and urine collected for 3–5 days for estimation of paracetamol and its metabolites. Blood was also taken daily for 5 days for estimation of aspartate and alanine aminotransferases, bilirubin and prothrombin time ratio to obtain a composite 'liver damage score' as described previously (normal value $< 2·4$) (Prescott & others, 1976). Four patients were treated with L-methionine, 3 with cysteamine, 1 with L-cysteine and 2 with supportive therapy only. Treatment was begun in all within 10 h of ingestion (Prescott & others, 1976). Severe liver damage (aminotransferases > 1000 i.u. $litre^{-1}$) occurred in 3 patients given L-methionine and the two receiving supportive therapy, one of whom died in hepatic failure. None of the patients developed renal failure.

The pattern of urinary excretion of paracetamol metabolites did not appear to be influenced by the treatment given, but was related to the severity of liver damage (Table 1). The proportion of the total excreted as the sulphate conjugate was significantly less in the patients with severe liver damage than in those without, and was inversely related to the liver damage score ($r = 0·76$, $P = < 0·01$). In contrast, the proportion excreted as the cysteine conjugate was significantly higher in the patients with severe liver damage than in those without, and was directly related to the liver damage score ($r = 0·96$, $P = <0·0001$). A similar but less marked trend was observed with the mercapturic acid conjugate. In all patients there was a marked change in the proportional excretion of paracetamol conjugates with time. During the first 10–20 h after overdosage very little paracetamol was excreted as the sulphate (5–15%) and the glucuronide was the major metabolite (> 80%). After 50–60 h however, the proportions excreted as sulphate and glucuronide were similar and each accounted for about 40% of the total. The proportion excreted as cysteine and mercapturic acid conjugates tended to increase with time, and reached a peak at about 30 h in some patients with liver damage.

The urinary excretion of paracetamol metabolites was also studied in a healthy volunteer given single oral doses of 1·5 g of paracetamol with and without cysteamine (1·0 g infused intravenously over the first 6 h). In the control study 18·6% was excreted in 24 h as the sulphate, 76% as glucuronide, 3·1% as mercapturic acid, 1·0% as cysteine conjugates, and 3·3% as unchanged paracetamol. Cysteamine reduced the urinary excretion of mercapturic acid and cysteine conjugates. In the control study 2·0% of the dose was excreted as

Table 1. *Urinary excretion of paracetamol (P) and its sulphate (S), glucuronide (G), mercapturic acid (M) and cysteine (C) conjugates in relation to severity of liver damage following overdosage.*

Patients	4 h plasma P (μg ml^{-1})	Liver damage score	Urinary excretion (%) P	S	G	M	C
Liver damage (n = 5)	402 (140)	22·3 (8·2)	7·2 (1·5)	10·1 (4·0)	70·1 (5·2)	5·0 (0·9)	7·6 (2·0)
No liver damage (n = 5)	341 (80)	2·8** (0·8)	6·3 (0·8)	14·7* (1·7)	71·6 (3·4)	4·0 (1·8)	3 4** (1·0)

Values given are means with s.d. * $P = <0·05$, ** $P = <0·005$.

* Correspondence.

these metabolites in the first 6 h while the corresponding value during the infusion of cysteamine was only 1·0%.

Taken together, these observations are consistent with the hypothesis that the protective agents act by inhibiting the formation of the hepatotoxic metabolites of paracetamol. Had they acted by replacing hepatic glutathione (Strubelt, Siegers & Schütt, 1974), an increased excretion of mercapturic acid and cysteine conjugates would be expected in the patients without liver damage. No new metabolites of paracetamol were detected in the treated patients, and the excretion of the cysteine conjugate was directly related to the severity of liver damage irrespective of treatment. The initial very low proportional excretion of paracetamol sulphate is consistent with early saturation of sulphate conjugation (Levy & Yamada, 1971), although it is possible that available sulphur is diverted to vital glutathione synthesis in the face of impending hepatic necrosis. Generally similar findings were reported by Davis & others (1976) in untreated patients with different degrees of liver damage following paracetamol overdosage. However, they reported a much greater excretion of mercapturic acid and cysteine conjugates, ranging from 21% in healthy volunteers given high therapeutic doses to 39% in patients with severe liver damage. These differences may be related to methodology since our results are similar to those reported by Mitchell & others (1974).

The estimation of urinary cysteine and mercapturic acid conjugates of paracetamol has been a problem. Davis & others (1976) used two dimensional thin-layer chromatography and scanning densitometry which in our hands lacked reproducibility and had poor sensitivity. Other methods required the use of radiolabelled paracetamol (Mitchell & others, 1974) or analysis times of 21–45 h (Mrochek, Katz & others, 1974) and did not measure both the cysteine and mercapturic acid conjugates. Reported h.p.l.c. methods for the estimation of plasma paracetamol are complex, do not use an internal standard, and give no details of reproducibility (Riggin, Schmidt & Kissinger, 1975; Mrochek & others, 1974; Wong, Solomonraj & Thomas, 1976).

High performance liquid chromatographic methods have now been developed for (A) the simultaneous estimation of paracetamol and its sulphate, glucuronide, cysteine and mercapturic acid conjugates in urine and, (B) unchanged paracetamol in plasma.

H.p.l.c. system A assembly and running conditions
Orlita Model AE 10-4 pump, Cecil Model 212 ultraviolet detector (set at 250 nm, 10 μl flow cell), Honeywell Model 194 recorder, Hewlett-Packard Model 3370A integrator. Column: internally polished stainless steel tube, 170 × 4·9 mm. i.d., packed with octadecylsilane-bonded spherical silica, particle size 10 μm (Spherisorb 10-ODS, Phase Separations, Clwyd), with a septum injector. Mobile phase: 1% aqueous acetic acid–methanol–ethyl acetate (90:15:0·1) at 4·5 MNm^{-2} (650 lb in^2); flow rate 1·6 ml min^{-1}. Dilute urine sample up to 50-fold with distilled water if necessary. To 0·8 ml add internal standard solution (4-fluorophenol: 0·2 ml of a 20 mg ml^{-1} solution in water). Mix and inject 2–4 μl. For less concentrated urine samples (e.g. those collected several hours after a therapeutic dose of drug) reduce internal standard concentration to 4 mg ml^{-1}. Run appropriate aqueous paracetamol standards with each set of unknowns.

H.p.l.c. method B
Apparatus: as in A with a short column (90 × 4·5 mm i.d.) and the same reverse-phase packing. Mobile phase: water–acetic acid–ethyl acetate (98:1:1) at 2·75 MNm^{-2} (400 lb in^2); flow rate 3 ml min^{-1}. To 1 ml of plasma containing 25–500 μg ml^{-1} of paracetamol in a 10 ml glass tube add slowly 1·0 ml of 25% (w/w) aqueous trichloroacetic acid containing 5 mg ml^{-1} of 4-fluorophenol with agitation on a vortex mixer. Centrifuge off the precipitated protein and inject clear supernatant. For samples containing less than 25 μg ml^{-1} of paracetamol, add 100 μl of a solution containing 7 mg ml^{-1} of 4-fluorophenol in 75% (w/w) aqueous trichloroacetic acid to 1·0 ml of plasma and inject up to 25 μl of supernatant.

Symmetrical peaks are obtained for all compounds (Fig. 1) and calibration graphs of the peak area ratios of paracetamol to 4-fluorophenol plotted against paracetamol concentrations are linear and pass through the origins for both assays. The responses to paracetamol and its sulphate, glucuronide, cysteine and

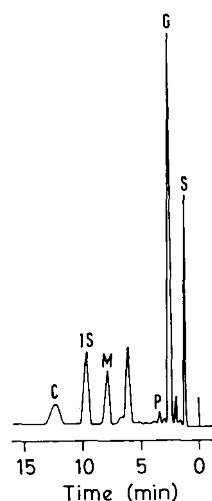

FIG. 1. High performance liquid chromatogram from a urine sample obtained 3–4 h after ingestion of 1·5 g of paracetamol in a healthy volunteer (2 μl injection). S = paracetamol sulphate, G = paracetamol glucuronide, P = paracetamol, M = paracetamol mercapturic acid, C = paracetamol cysteine, IS = internal standard (4-fluorophenol).

mercapturic acid conjugates in urine are linear over the ranges of 3–1600, 5–2600, 50–10 000, 2–800 and 2–1100 μg ml^{-1} respectively. The concentrations of conjugates are expressed as 'paracetamol equivalents' since their molar extinction coefficients are essentially the same as for paracetamol. Replicate analyses of urine samples containing different amounts of paracetamol and its conjugates yielded standard deviations of about 1, 2, 4 and 9% at concentrations of 1000, 200, 50 and 10 μg ml^{-1} respectively. Interference by other drugs has not been encountered in either assay, but interference by other glucuronide and sulphate conjugates in urine has not been excluded. Paracetamol and its conjugates in urine are stable at —20° for at least 9 months, but 4-fluorophenol solutions should be freshly made weekly.

The plasma paracetamol assay can be completed within 15 min. The retention times of the drug and internal standard were 2·1 and 3·7 min respectively, there were no interfering peaks with drug-free plasma and the limit of detection was about 0·1 μg ml^{-1}. The standard deviations of replicate analyses of samples containing 500–50 and 30–1 μg ml^{-1} were 3·7 and 5·8% respectively using peak height ratios. There was a highly significant correlation between the concentrations of paracetamol in 125 plasma samples assayed once by the above method and once by gas-liquid chromatography (Prescott, 1971) (Fig. 2).

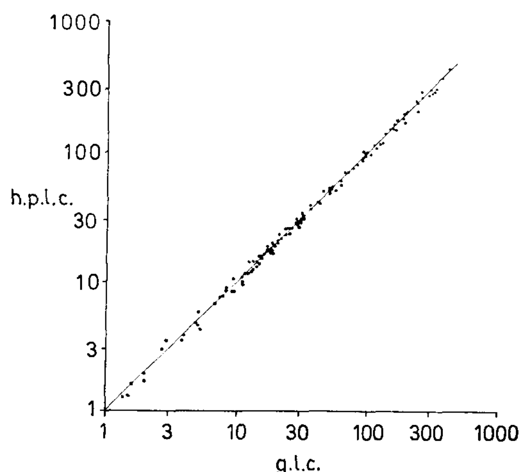

FIG. 2. Comparison of results of 125 plasma paracetamol estimations (μg ml^{-1}) by high performance liquid chromatography h.p.l.c. and gas-liquid chromatography g.l.c.

We gratefully acknowledge advice and guidance given by Dr J. Jurand and Professor J. H. Knox, Edinburgh University, Department of Chemistry, and we thank Dr R. Andrews, Sterling Winthrop, Newcastle-upon-Tyne, and Dr W. Madison, McNeil Laboratories, Fort Washington, Pa., U.S.A. for reference samples of metabolites. This work was supported by grants from the Scottish Home and Health Department and McNeil Laboratories. February 7, 1977

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