HIGH PERFORMANCE LIQUID CHROMATOGRAPHIC ESTIMATION OF PARACETAMOL METABOLITES IN PLASMA

There has been considerable interest in the metabolism of paracetamol following the elucidation of the mechanisms of hepatotoxicity after overdosage (Mitchell, Thorgeirsson, Potter, Jollow & Keiser, 1974). Only one method for the estimation of paracetamol metabolites in plasma has been published (Mrochek, Katz, Christie & Dinsmore, 1974) but this procedure takes at least 24 h and the mercapturic acid conjugate, a metabolite of major toxicological significance, was not identified. We describe here a simple, rapid method for the simultaneous assay of paracetamol and its sulphate, glucuronide, cysteine and mercapturic acid conjugates in plasma using high performance liquid chromatography.

An Orlita pump (Model AE 10-4) was used with a Waters model 440 U.V. absorbance detector (254 nm filter) and peak areas were measured with a Hewlett-Packard HP 3370A integrator. The columns were internally-polished stainless steel tubes 100 mm $\times$ 4.9 mm i.d. slurry packed with Hypersil-ODS (Shandon Southern Products) and fitted with a septum injector. The mobile phase was 0.1 M potassium dihydrogen

phosphate, 98% formic acid and isopropanol (100:0.1:1.7 v/v/v). Stock solutions of internal standard/protein precipitant were prepared containing 360 μ g/ml of N-propionyl-4-aminophenol in 6% aqueous perchloric acid and were stored deep frozen prior to use. Aliquots (1 ml) of plasma from patients with paracetamol overdosage were pipetted into glass tubes which were placed on a vortex mixer while 1.0 ml of internal standard/perchloric acid solution was added slowly. The tubes were centrifuged and up to 5 μ l of the clear supernatant injected directly onto the column. Samples containing lower concentrations of paracetamol and metabolites (e.g. after therapeutic doses) were assayed by the same procedure except that the internal standard/protein precipitant solution contained 300 μ g/ml N-propionyl-4-aminophenol in 30% aqueous perchloric acid and 100 μ l was added to 1.0 ml plasma.

With a solvent flow of 0.9 ml/min (650 p.s.i.) the glucuronide, sulphate and cysteine conjugates, paracetamol, internal standard and mercapturic acid conjugate eluted in 2.5, 4.8, 5.3, 6.5, 14.3 and 16.5 min respectively. The peaks were sharp and well resolved with no interference from endogenous compounds (Figure 1) and samples could be injected every 18 min. The limit of measurement with a 5 μ l injection was less than 1 μ g/ml for all compounds. Plots of the peak area ratios of paracetamol and the sulphate and glucuronide conjugates to internal standard against concentration were linear and passed through the origin. These three compounds normally account for practically all the paracetamol-related material in plasma. However, in patients developing severe liver damage after overdosage, easily measurable concentrations of the cysteine and mercapturic acid conjugates may be present (Figure 1). The extinction coefficients of the glucuronide and sulphate conjugates relative to paracetamol and the recoveries from plasma relative to water differ somewhat, but using the appropriate correction factors (0.84 for glucuronide, and 1.03 for sulphate at 254 nm) it is possible to calibrate the assay with plasma standards containing paracetamol only. Insufficient authentic mercapturic acid and cysteine conjugate was available for the preparation of plasma standards and the linear relationship previously demonstrated for the corresponding assay in urine (Howie, Adriaenssens & Prescott, 1977) was assumed to hold for plasma. The standard deviation of replicate assays of paracetamol and its glucuronide and sulphate conjugates was less than 3% (mean 1.0%) for each compound with concentrations ranging from 1–250 μ g/ml (paracetamol equivalent). Day-to-day reproducibility was 3–4%. Despite the direct injection

Figure 1 Chromatograms obtained from drug-free plasma (above) and plasma obtained 60 h after severe paracetamol overdosage (below). The peaks are: paracetamol glucuronide (G), sulphate (S), cysteine conjugate (C), free drug (P), internal standard (N) and mercapturic acid conjugate (M).

of deproteinized plasma, the columns have lasted for many months with daily use.

Figure 1 shows chromatograms of drug-free plasma and a sample obtained 60 h after paracetamol overdosage in a patient who developed severe liver damage.

The method has been used together with a previously described high performance liquid chromatographic assay for paracetamol metabolites in urine (Howie *et al.*, 1977) in studies of paracetamol

metabolism following therapeutic doses as well as overdosage of paracetamol.

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DOUBLE-BLIND TRIAL OF INDORAMIN IN DIGITAL ARTERY DISEASE

Trophic changes in the fingers resulting from digital artery disease secondary to scleroderma or other connective tissue disorders are difficult to treat (Rowell, 1972), and no wholly satisfactory form of therapy has been suggested. Vasoconstrictor tone in the periphery is controlled by α -adrenoceptor sympathetic innervation, and blockade of this control leads to peripheral vasodilation. Indoramin (3-[2-(4-benzamidopiperid-1-yl)ethyl]indole hydrochloride) has been shown to possess potent α -adrenoceptor blocking activity in all species so far studied (Alps, Hill, Johnson & Wilson, 1970; Alps, Hill, Johnson & Wilson, 1972; Collis & Alps, 1973) including man (Royds & Lockhart, 1972; Royds & Lockhart, 1974), and intravenous indoramin has been shown to be effective in increasing cutaneous blood flow to affected digits of patients with Raynaud's disease (Fares & Milliken, 1974). There is also a suggestion from other studies that indoramin may provide subjective improvement in both primary and secondary Raynaud's phenomenon (Alps *et al.*, 1972; Fares & Milliken, 1974). This preliminary study was undertaken to assess the effect of oral indoramin on digital blood flow, and on the symptoms of Raynaud's phenomenon associated with digital vascular insufficiency resulting from scleroderma and other connective tissue diseases.

Sixteen patients suffering from digital artery disease

in association with trophic changes in the digits were selected for study, eleven of whom were suffering from scleroderma with sclerodactyly as its most prominent manifestation and were receiving no other therapy during the trial. The primary disorder in the other patients, all but two of whom were female, was either rheumatoid arthritis, systemic lupus erythematosus or polymyositis, and these patients continued to receive their maintenance dose of prednisolone and/or azathioprine throughout the study. Apart from four subjects who continued to receive therapy for hypertension and two subjects who were receiving anti-inflammatory propionic acid derivatives for arthritic pain, all other agents were discontinued before the trial commenced and the dose of existing therapy which was not changed had been stabilized for at least 6 months before the period of assessment.

Patients were randomly allocated to two groups and the study was conducted in double-blind manner. Group A received indoramin 90 mg daily in three divided doses for 3 weeks followed by placebo for 3 weeks. Group B received placebo for the first 3 week period followed by 3 weeks receiving 90 mg indoramin in divided doses. Each patient entered a daily record of the number of attacks of Raynaud's phenomenon, and an overall estimate of the discomfort suffered in the day on a five point scale. Additional comments related to unusual physical activity, marked climatic changes