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Note

Method for the determination of acemetacin, a non-steroidal anti-inflammatory drug, in plasma by high-performance liquid chromatography

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Acemetacin (Fig. 1), a non-steroidal anti-inflammatory drug (NSAID) with analgesic and anti-inflammatory properties, is the glycolic acid ester of indomethacin, produced in the laboratories of Troponwerke (F.R.G.) [1]. It is currently in clinical use on the continent of Europe as a treatment for chronic inflammatory diseases and is under clinical trials in the U.K. for the treatment of the major arthritic conditions.

In animal and clinical studies, the anti-inflammatory properties of acemetacin resemble indomethacin [2,3] although it is claimed that acemetacin is better

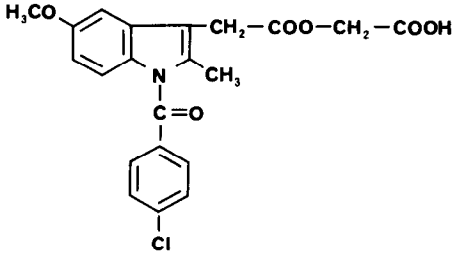

Fig. 1. Chemical structure of acemetacin, 1-(*p*-chlorobenzoyl)-5-methoxy-2-methylindole-3-acetoxy acetic acid.

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tolerated by the gastrointestinal tract than is indomethacin [4]. Metabolic studies of acemetacin suggest that the drug is rapidly absorbed orally and hydrolysed by the liver to produce indomethacin as its major metabolite. Another acemetacin metabolite is *p*-chlorobenzoic acid (PCBA). This is both a minor metabolite and a degradation product of acemetacin which may be formed during storage of samples (or standard solutions) above −20°C. Therefore in studying the metabolism of acemetacin account should be taken of its possible spontaneous hydrolysis. A recent paper [5] describing a high-performance liquid chromatographic (HPLC) assay for acemetacin (and indomethacin) does not enable PCBA to be measured.

During a study of the pharmacokinetics of acemetacin in man, we have reviewed and improved an assay for acemetacin and its metabolites in plasma by HPLC. The assay described will be of use in the further study of the pharmacokinetics and clinical pharmacology of this drug in man.

EXPERIMENTAL

Samples

Heparinized blood (10 ml) was collected by venepuncture at intervals over 24 h from a volunteer who had taken a single oral dose of acemetacin (120 mg).

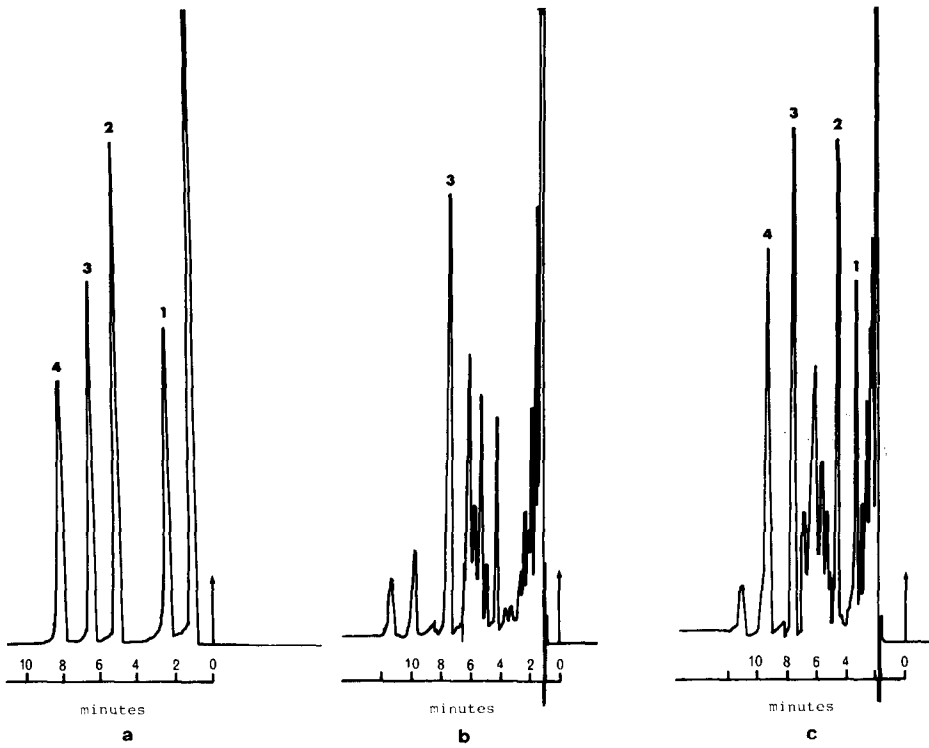

Fig. 2. Typical chromatograms of (a) unextracted mixture in mobile phase, (b) blank human plasma spiked with flurbiprofen extracted as described in the text and (c) plasma sample (extracted as described in the text) taken from a volunteer 6 h after an acute oral dose of acemetacin (120 mg). Peaks: 1 = PCBA; 2 = acemetacin; 3 = flurbiprofen; 4 = indomethacin.

Plasma was obtained immediately on collection of the blood by centrifugation at 4000 *g* for 10 min and stored at −20 °C prior to analysis.

Reagents and chemicals

Acemetacin was supplied by Troponwerke (F.R.G.). Indomethacin was purchased from Sigma (Poole, U.K.), PCBA from Aldrich (Gillingham, U.K.) and flurbiprofen was supplied by Boots (Nottingham, U.K.).

Organic solvents used were of HPLC grade and purchased from Fisons (Loughborough, U.K.).

Procedure

To 1 ml of plasma were added 1 ml of 0.15 *M* potassium dihydrogen phosphate buffer (pH 3.0), 0.1 ml of the NSAID flurbiprofen (1 mg/l) as internal standard and 5 ml of Analar-grade diethyl ether. The tubes were stoppered with PTFE-lined caps and agitated by mechanical rotation for 10 min. Following centrifugation at 4500 *g* for 5 min to separate the layers, the organic layer was transferred to a clean tapered glass tube. The ether layer was evaporated to dryness under nitrogen at 37 °C and the residue resuspended in 0.25 ml mobile phase by whirlimixing the tube for 30 s. The tubes were centrifuged for 1 min and the clear supernatant taken for HPLC analysis.

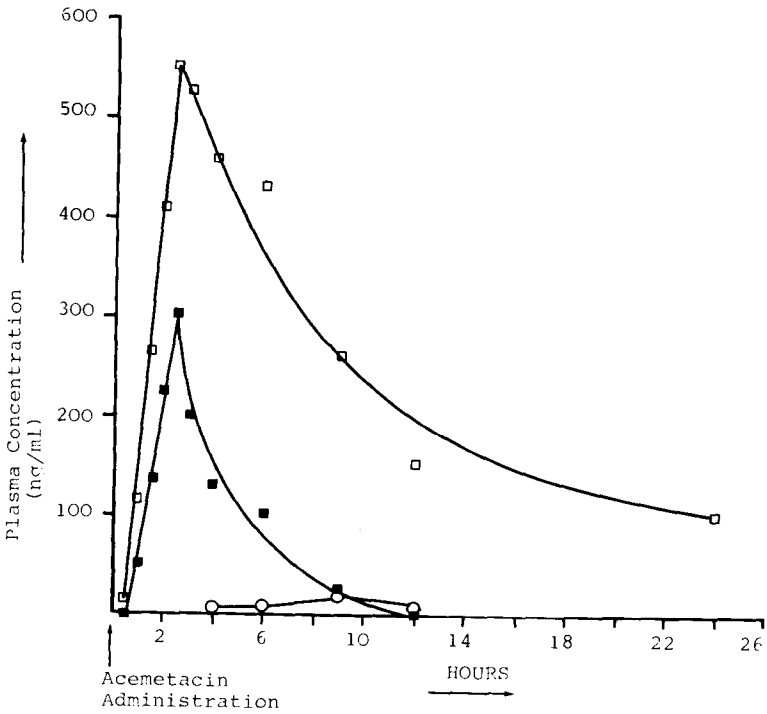

Fig. 3. Pharmacokinetic profiles of acemetacin and its metabolites indomethacin and PCBA in plasma following a single oral dose of acemetacin (120 mg) in a human volunteer. ■, Acemetacin; □, indomethacin; ○, PCBA.

High-performance liquid chromatography

Analysis was performed at 17°C using a Constametric III pump (LDC) combined with a Constamonitor variable UV detector (LDC) set at 245 nm and a Rheodyne 7125 injection valve fitted with a 0.1-ml loop. The analytical column used was a Hypersil ODS 5 μm, 15 cm×4.5 mm I.D. (Jones Chromatography). The mobile phase consisted of methanol–0.02 *M* potassium dihydrogen phosphate (57:43) adjusted to pH 4.5 with 88% phosphoric acid. The flow-rate employed was 1.25 ml/min giving a column pressure of 13.8 MPa. Under these conditions the following retention times were obtained: PCBA, 2.37 min; acemetacin, 5.58 min; flurbiprofen (internal standard), 7.37 min; indomethacin, 9.03 min.

Concentrations of acemetacin, indomethacin and PCBA were obtained using the method of peak-height ratios which were linear over the ranges 10–1000 ng/ml (acemetacin), 10–1500 ng/ml (indomethacin) and 20–1000 ng/ml (PCBA). The coefficients of variation at 1000 ng/ml were 4% for acemetacin and indomethacin, and 6% for PCBA. At 10 ng/ml, the coefficients of variation were 8% for acemetacin and 7% for indomethacin and at 20 ng/ml PCBA the value was 10%.

RESULTS AND DISCUSSION

Typical chromatographic traces of acemetacin and its metabolites both unextracted and extracted from plasma as described above are presented in Fig. 2. The plasma sample was taken from a volunteer 6 h after a single oral dose of acemetacin (120 mg). The pharmacokinetic profile of this drug in the volunteer is given graphically in Fig. 3. Peak plasma acemetacin and indomethacin concentrations were obtained 2.5 h after dosing. PCBA was not detected in plasma until 4 h after dosing. The areas under the concentration–time curves were 1.05 mg·h/l (acemetacin), 5.46 mg·h/l (indomethacin, 0–24 h). Plasma elimination half-life for acemetacin in this subject was 2.24 h and for indomethacin, 8.90 h.

This method affords a rapid and sensitive assay for the measurement of acemetacin and its metabolites in plasma.

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