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Note

Simple, rapid and sensitive reversed-phase high-performance liquid chromatographic method for the determination of mefenamic acid in plasma

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Mefenamic acid (MA), a potent prostaglandin synthetase inhibitor [1], has been widely used in Japan to produce pharmacological closure of symptomatic patent ductus arteriosus in premature infants [2]. Although the correlation between the plasma levels of MA and its therapeutic effects has not been fully established in man, the constriction effect of MA on foetal ductus arteriosus was reported to be dose-dependent in rats [3]. Therefore, measurement of plasma levels of MA may provide valuable information for MA treatment in premature infants with symptomatic patent ductus arteriosus and for preventing possible toxic effects [4].

For the determination of MA in plasma or serum, gas chromatographic [5,6] and high-performance liquid chromatographic (HPLC) [7,8] methods have been reported. These methods, however, require relatively large plasma samples and are not directly applicable to the determination of plasma levels of MA in infants. The method described in this paper was adapted for monitoring plasma MA in infants between sub-therapeutic and overdose levels using a small amount (50 μl) of plasma. The method requires minimum pretreatment of the plasma sample and has the advantage of being rapid and simple.

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EXPERIMENTAL

Chemicals

MA and tolfenamic acid (TA) were kindly supplied by Sankyo (Tokyo, Japan) and Tobishi Pharmaceutical (Tokyo, Japan), respectively. Acetonitrile (HPLC grade, Cica-Merck) was obtained from Kanto Chemical (Tokyo, Japan). The other reagents were of analytical-reagent grade.

Stock solutions of MA and TA were prepared at concentrations of 10.0 and 1.0 mg/l in acetonitrile. These solutions were stable at 4°C for at least two months.

Apparatus and conditions

A Model LC-3A liquid chromatograph equipped with a loop-type injector (Model SIL-1A) and a variable-wavelength ultraviolet absorbance detector (Model SPD-2A) (all from Shimadzu, Kyoto, Japan) was used. A microprocessor (Model C-R1A Chromatopac; Shimadzu) was used for peak-area integration and calculations. Analysis was performed on a 7-μm LiChrosorb RP-18 column (250 mm × 4 mm I.D.; Cica-Merck) from Kanto Chemical, operated at 45°C. The mobile phase was 6.5 m*M* phosphoric acid–acetonitrile (45:55, v/v). The flow-rate was kept at 2.0 ml/min and the column effluent was monitored at 280 nm.

Sample preparation

A 50-μl volume of plasma was pipetted into a 1.5-ml microcentrifuge tube and 250 μl of internal standard solution were added. After mixing on a vortex mixer for 30 s, the sample was centrifuged at about 8000 *g* for 2 min. A 250-μl aliquot of the supernatant was transferred into a 10-ml glass-stoppered centrifuge tube and was evaporated to dryness under reduced pressure. The residue was dissolved in 100 μl of the mobile phase and a 40-μl aliquot was injected into the HPLC system.

Quantitation

Quantitation was performed by the peak-area ratio method with TA as internal standard. Calibration graphs were obtained by analysing control plasma samples spiked with various amounts of stock MA solutions (0.2, 0.5, 1.0, 2.0, 5.0 and 10.0 mg/l). The within-run variation was determined by analysing two plasma samples containing 0.5 and 10.0 mg/l MA.

RESULTS AND DISCUSSION

Previous methods for the determination of plasma MA require a relatively large amount of plasma (more than 0.5 ml) and a time-consuming liquid–liquid extraction step [7,8]. As only small plasma samples can be taken from

infants, we developed a new HPLC procedure for the determination of MA in a small volume (50 μl) of plasma from which proteins were removed by acetonitrile precipitation. To improve the accuracy and precision of the method at low MA levels (less than 0.5 mg/l in plasma), the supernatant fraction after the precipitation of plasma proteins was evaporated to dryness, reconstituted with the mobile phase and then injected into the HPLC system.

Typical chromatograms of plasma samples are shown in Fig. 1. The total chromatographic time for analysis of MA was less than 10 min, with reproducible retention times. The retention times were 6.6 and 7.9 min for MA and TA, respectively. Under the chromatographic conditions adopted, flufenamic acid, which has been used previously as an internal standard [7,8], was coeluted with MA.

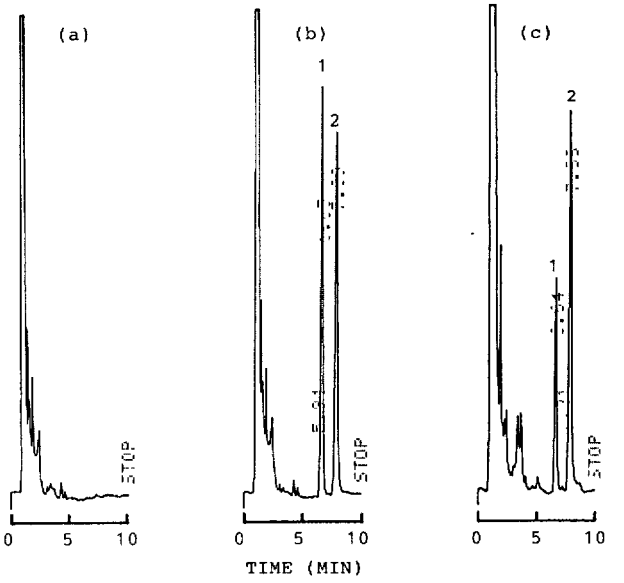

Fig. 1. Chromatograms of (a) blank plasma, (b) blank plasma spiked with mefenamic acid (5 mg/l) and (c) plasma obtained from a premature infant undergoing mefenamic acid therapy (mefenamic acid = 2.4 mg/l). Peaks: 1 = mefenamic acid; 2 = tolfenamic acid (internal standard).

TABLE I

WITHIN-RUN VARIABILITY OF THE METHOD

Added (mg/l)	Found (mean ± S.D.; $n = 10$) (mg/l)	Coefficient of variation (%)
0.50	0.48 ± 0.017	3.5
10.0	9.95 ± 0.210	2.1

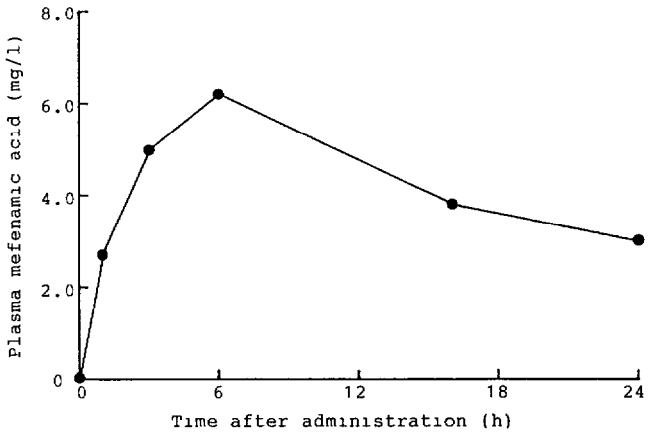

Fig. 2. Plasma concentration–time profile of mefenamic acid following a single oral administration of 2 mg/kg of the drug (Pontal syrup) to a premature infant with symptomatic patent ductus arteriosus.

The linearity of the method was studied for six different concentrations of MA in spiked plasma samples. The regression lines were linear ($y=0.185x+0.0079$, $r>0.9995$) over the concentration range examined (0.2–10.0 mg/l) and nearly passed through the origin.

The within-run precision was established for two concentrations of MA in spiked plasma samples. As shown in Table I, the recoveries of the drug were nearly 100% and the coefficients of variation were less than 4%. The detection limit of the drug, calculated for a signal-to-noise ratio of 5, was about 0.1 mg/l in plasma.

Ten drug-free plasma samples were analysed for possible interferences from endogenous constituents. As shown in Fig. 1a, no background interference was observed. So far, plasma samples from more than 25 premature infants undergoing MA therapy have been analysed and no interferences have been encountered.

Fig. 2 shows a plasma concentration–time profile of MA following a single oral administration of 2 mg/kg of the drug (Pontal® syrup; Sankyo, Tokyo, Japan) to a premature infant with symptomatic patent ductus arteriosus. In this patient, the peak plasma concentration (6.2 mg/l) was achieved about 6 h after administration and the plasma MA was easily detectable after 24 h. The plasma half-life of MA appeared to be more than 15 h, which is approximately five times greater than those reported in adults [9].

In conclusion, the proposed method, requiring only a small volume of plasma and with rapid sample preparation, is very suitable for therapeutic drug monitoring of MA in premature infants with symptomatic patent ductus arteriosus

and in patients undergoing conventional MA therapy [10] and is also applicable to pharmacokinetic studies of the drug.

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