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Short Communication

Improved method for the determination of trimethadione and its demethylated metabolite, dimethadione, in human serum by gas chromatography

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ABSTRACT

An improved gas chromatographic method, involving the use of a wide-bore capillary column, for the determination of trimethadione and its only demethylated metabolite, dimethadione, in human serum is described. The results indicate that both substances and the internal standard (maleinimide) were well separated with no tailing peak. The detection limit was 10 ng/ml for trimethadione and 50 ng/ml for dimethadione. This improved method is reliable in terms of sensitivity, selectivity and reproducibility for the simultaneous determination of both compounds in human serum.

INTRODUCTION

We have shown that trimethadione (TMO) is a useful indicator of the hepatic drug-oxidizing capacity in rats [1–4] and humans [5–7]. TMO is exclusively metabolized to dimethadione (DMO), its only metabolite, by a cytochrome P-450-dependent mono-oxygenase in hepatic microsomes.

Recently, we have reported a sensitive and selective method for the determination of serum TMO and DMO in rat and human serum by using gas chromatography (GC) with hydrogen flame ionization detection (FID) [1,9] or flame thermionic detection (FTD) [8,9]. However, these methods were not sensitive enough, and the separation between TMO and DMO was imcomplete [1,8,9]. In addition, the DMO peak exhibited occasional tailing.

This paper describes and improvement to the analytical reliability of the method, and a pharmacokinetic study in humans. The method is based on wide-bore capillary GC.

EXPERIMENTAL

Reagents and standards

TMO was obtained from Dainippon Seiyaku (Osaka, Japan). DMO and maleinimide (internal standard, I.S.) were purchased from Tokyo Kasei (Tokyo, Japan). All other chemicals were of analytical-reagent grade.

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Analytical procedure

To 100 μl of serum (or standard) in a 2.5-ml tube were added 200 μl of 5 *M* monobasic sodium phosphate, minimum amounts (*ca.* 50 mg) of sodium sulphate and magnesium sulphate and 100 μl of ethyl acetate containing 5 μg/ml I.S. After vortex-mixing for 1 min, the tubes were centrifuged at 1800 *g* for 5 min. A 2-μl aliquot of the organic phase was directly injected into the GC apparatus.

Accuracy and reproducibility

The reproducibility and the accuracy from spiking drug-free serum were calculated at concentrations of 0.01, 0.05, 0.1, 0.5, 1, 5 and 10 μg/ml in serum by comparing the peak-height ratio against the I.S. with those obtained for aqueous solutions containing known concentrations of TMO and DMO. For statistical analysis, a paired Student's *t*-test was used.

Chromatography

Analysis of serum TMO and DMO was carried out with a Shimadzu-9A instrument (Kyoto, Japan) with FTD. The column was a CBP 10-W12-100 (12 m × 0.53 mm I.D., film thickness 1 μm) from Shimadzu (Kyoto, Japan). The column oven temperature was held at 70°C for 2 min, then raised to 120°C at 10°C/min, and then raised to 220°C at 20°C/min. The injection port temperature was 300°C. Helium, hydrogen and air flow-rates were 30, 30 and 145 ml/min, respectively.

Human study

Five healthy male subjects (aged 25–46 years; body weight 63–70 kg), who had given their informed consent, participated in the study. All subjects ingested 4 mg/kg TMO in aqueous solution at 09:30 h after an overnight fast. Blood was sampled before drug administration and after 0.5, 1, 2, 4, 6, 8, 10, 12 and 24 h.

The half-life ($t_{1/2}$) of TMO was estimated by linear regression analysis. The apparent volume of distribution (V_d) was calculated by dividing the dose of TMO by the extrapolated value of TMO concentration at zero time. The apparent total body clearance (*Cl*) of TMO was calculated from the equation Cl = dose/AUC_0^∞, where AUC is the area under the concentration–time curve. Because the absolute bioavailability of orally administered TMO is not known, values of *Cl* and V_d are termed "apparent".

RESULTS AND DISCUSSION

Fig. 1 shows representative chromatograms obtained from human serum spiked with TMO and its only metabolite, DMO (5 μg/ml) (panel 2) and a human serum sample obtained 4 h after administration of TMO (panel 3). There were no interfering peaks in blank serum samples (Fig. 1, panel 1). The results indicate that TMO, DMO and the I.S. were better separated than in the previous method [3]. The retention times of TMO, the I.S. and DMO were 2.5, 3.1 and 6.3 min, respectively. The elution order of TMO and the I.S. was reversed when compared with the previous method [9]. This behaviour is due to the different polarity of the bulking agent.

The accuracy and reproducibility of the analysis of the method for TMO and DMO are indicated in Table I. The standard curve produced by using pooled human drug-free serum showed good linearity (TMO, r = 0.991; DMO, r = 0.989) at various concentrations of TMO (0.01–10 μg/ml) and DMO (0.05–10 μg/ml). The coefficient of variation (C.V.) was less than 10% (6.9–10%). These results were reproducible.

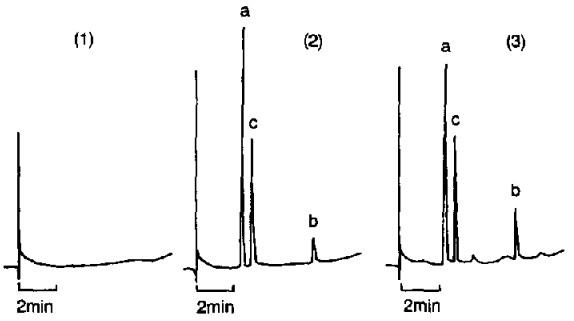

Fig. 1. Representative gas chromatograms of (1) blank human serum, (2) human serum spiked with trimethadione (TMO) and dimethadione (DMO) (5 μg/ml) and (3) a human serum sample obtained 4 h after oral administration of TMO (2 mg/kg). Peaks: a = TMO; b = DMO; c = internal standard (malcinimide).

TABLE I

ANALYTICAL ACCURACY AND REPRODUCIBILITY OF THE ANALYSIS OF TRIMETHADIONE AND DIMETHADIONE IN HUMAN SERUM

Each value represents the mean of three experiments. The concentration was calculated on the basis of the peak-height ratios against the I.S.

Concentration added (μg/ml)	TMO		DMO	
	Mean (μg/ml)	C.V. (%)	Mean (μg/ml)	C.V. (%)
0.05	0.051	9.8	0.049	8.9
0.1	0.099	10.0	0.11	9.8
0.5	0.52	8.6	0.51	8.9
1	0.99	7.9	1.06	8.1
5	5.05	8.1	4.99	7.7
10	9.89	7.2	10.08	6.9

Previously reported detection limits of TMO and DMO were 0.05 μg/ml [8,9]. With this method, the limits of quantitation after extraction were 0.01 μg/ml for TMO and 0.05 μg/ml for DMO. No interfering peaks appeared when the following drugs, which are usually co-administered with TMO to patients, were added to serum: acetazolamide, carbamazepine, pentobarbital, phenobarbital, phenytoin or primidone.

We carried out a pharmacokinetic study of TMO following the administration of the drug. The results were as follows: $t_{1/2} = 8.6 \pm 1.7$ h; $V_d = 67.5 \pm 3.4$ ml/kg; $Cl = 66.9 \pm 13.2$ ml/min.

The data suggest that this improved method is reliable in terms of sensitivity, selectivity and reproducibility for the simultaneous determination of TMO and DMO in human serum. The method can also be applied to plasma samples.

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