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Note

Simultaneous determination of trimethadione and its metabolite in rat and human serum by high-performance liquid chromatography

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Recently we reported a sensitive method for the determination of low concentrations of trimethadione (TMO) and its metabolite, 5,5-dimethyl-2,4-oxazolidinedione (DMO), by using gas chromatography (GC) with flame-thermionic detection (FTD) [1]. In addition, a pharmacokinetic study using this sensitive method was carried out in carbon tetrachloride-treated rats [1]. However, GC methods for the determination of TMO and DMO in rat plasma normally are time-consuming.

In this present study, we report the development of a rapid and selective high-performance liquid chromatographic (HPLC) method for the simultaneous analysis of TMO and DMO in rat and human serum.

EXPERIMENTAL

Materials

TMO was purified from commercial powder containing 66.7% TMO (Mino-Aleviatin®, Dainippon, Osaka, Japan). DMO was purchased from Tokyo Kasei (Tokyo, Japan), acetonitrile from Wako (Tokyo, Japan), α-methyl-α-propyl-succinimide from Aldrich (Milwaukee, WI, U.S.A.) and PIC®-B_5 (low UV) from Waters Assoc. (Milford, MA, U.S.A.). All other chemicals were reagent grade.

Extraction procedure

To 50 μl of serum (or standard) in a 2.5-ml tube were added 50 μl of methyl alcohol with α-methyl-α-propylsuccinimide as internal standard. The tube was

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shaken for 2 min and centrifuged at 1500 *g* for 5 min. A 10- or 20-μl aliquot was injected into the chromatograph.

High-performance liquid chromatography

A high-performance liquid chromatograph (Model 633A, Hitachi, Tokyo, Japan) with a variable-wavelength ultraviolet (UV) spectrophotometer (Hitachi) and recorder (Model 056, Hitachi) was employed. The column was a reversed-phase type (Shodex® ODSpak F-411A, 4.6 mm × 150 mm, 5 μm). The detector was set at 200 nm and the mobile phase was water (85 ml), acetonitrile (15 ml) and PIC®-B_5 (3.5 ml). All analyses were performed at room temperature.

Standards

Standard solutions of TMO and DMO (1 mg/ml) were prepared in water and stored at 4°C. These solutions were then diluted as necessary to prepare the appropriate serum standards for each drug and assay run. The internal standard, α-methyl-α-propylsuccinimide, was prepared in methyl alcohol (100 μg/ml) and stored at 4°C. Peak height ratios of TMO and DMO to α-methyl-α-propylsuccinimide were determined.

Recovery and reproducibility

Drug standards were added to drug-free serum in amounts equivalent to 0.01—200 μg/ml. Recovery of drug from serum after protein precipitation was determined at concentrations of 0.1, 0.5, 1.0, 10.0, 50.0, 100.0 and 200.0 μg/ml serum by comparing the peak heights with those obtained for aqueous solutions containing the known concentrations of TMO and DMO. Reproducibility was determined for the same concentration range by quadruplicate analysis of samples at each concentration.

Gas chromatography

Analysis was carried out with a Shimadzu GC-7A equipped with a flame-thermionic detector (Kyoto, Japan). The glass column (50 cm × 2.6 mm I.D.) was packed with 5% PEG 6000 on 80—100 mesh Chromosorb W HP (Chromato Supply, Tokyo, Japan). The column oven temperature was raised from 100°C to 190°C at a rate of 16°C/min and held at 190°C for 5 min. The injection port and detector were at 210°C. The carrier gas was helium at a flow-rate of 50 ml/min.

Animal studies

After overnight fasting, male Wistar rats (Japan Laboratory Animals, Tokyo, Japan), weighing 195—230 g, received 100 mg/kg TMO in 2 ml of water by gavage. Blood samples were obtained from the jugular vein at 0.5, 1.0, 2.0, 4.0, 8.0, 12.0, 24.0, 48.0 and 72 h after administration.

Human studies

The subjects were normal healthy male volunteers, with a mean age of 30.4 years (22—51 years) and mean weight of 65.3 kg (54—76 kg). After overnight fasting, the subjects received an oral dose of 4 mg/kg TMO powder with

100 ml of water. Blood samples were obtained from an arm vein at 0.5, 1.0, 2.0, 6.0, 12.0, 48.0, 72.0, 96.0, 120.0, and 480.0 h after administration.

Pharmacokinetic studies

Concentration—time curves for TMO and DMO were drawn on semi-logarithmic scales. The half-life ($T_{½}$) and elimination rate constant (K_{el}) were calculated by linear regression analysis. The apparent volume of distribution (V_d) was calculated from the ratio of the administered dose to the plasma concentration extrapolated to time zero. The area under the curve (AUC) was calculated by the trapezoidal rule, and area to finite time was added by integration (C_t/K_{el}), where C_t is the last value of the TMO concentration. K_{el} was calculated from the equation $K_{el} = 0.693/T_{½}$.

Metabolic clearance (Cl) was calculated according to the equation $Cl = 0.693V_d/T_{½}$.

For statistical analysis a paired Student's t-test was used.

RESULTS AND DISCUSSION

Fig. 1. shows chromatograms of DMO, TMO and α-methyl-α-propyl-succinimide (internal standard). The results indicate that there was good separation among DMO, TMO and internal standard. The retention times for DMO, TMO and I.S. were 3.4, 5.5 and 12.7 min, respectively. Table I shows an extraction recovery of DMO and TMO between 1 μg/ml and 200 μg/ml from serum. The calibration graphs showed a linear relationship between the peak heights of TMO or DMO to the internal standard in the concentration range 1—200 μg/ml (TMO, $r = 0.995$; DMO, $r = 0.997$). No interfering peaks appeared

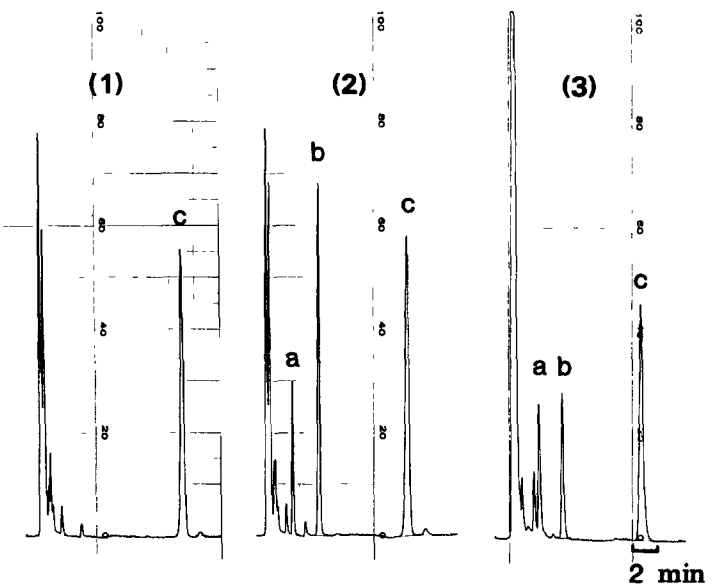

Fig. 1. Chromatograms of TMO and DMO after oral administration of TMO to rat (100 mg/kg) and human (4 mg/kg): (1), serum blank; (2) rat serum; (3) human serum. Peaks: a = DMO, b = TMO, c = internal standard (α-methyl-α-propylsuccinimide).

TABLE I

RECOVERY OF TMO AND DMO FROM SERUM

Amount added (μg/ml)	TMO		DMO	
	Amount found (μg/ml, mean ± S.E.)*	Recovery (%)	Amount found (μg/ml, mean ± S.E.)*	Recovery (%)
1	0.97 ± 0.01	97	0.96 ± 0.01	96
10	10.2 ± 0.33	102	10.1 ± 0.41	101
50	49.1 ± 0.28	98	48.7 ± 0.28	97
100	99.9 ± 2.18	99	98.3 ± 1.41	98
200	198.2 ± 3.16	99	197.1 ± 2.01	98

*$n = 4$

when phenobarbital, phenytoin, pentobarbital, acetazolamide, carbamazepine or primidone, which are usually administered to patients in combination with TMO, were added to serum. This method was capable of measuring at least 0.1 μg/ml TMO and 0.5 μg/ml DMO. From these results it is reasonable to note that the method presented for the determination of TMO and DMO would be useful even if a lower dose of the drug was administered to animals.

Rat or human serum samples were analysed for DMO and TMO by the method described here and by gas chromatography (GC). The results are shown in Fig. 2 which shows excellent agreement between the two procedures: TMO: $r = 0.998$, $Y = 1.01X - 0.288$; DMO: $r = 0.997$, $Y = 1.01X - 0.301$.

Table II shows the pharmacokinetic parameters following the oral administration of TMO to rats and humans. The serum concentration of TMO in rats reached its peak at 0.5—1 h, and serum DMO reached its peak at around 9 h and then gradually decreased, whereas the serum concentration of TMO in humans reached its peak at 0.5—1 h, and serum DMO reached its peak at around 60—72 h and then gradually decreased. The patterns of serum TMO

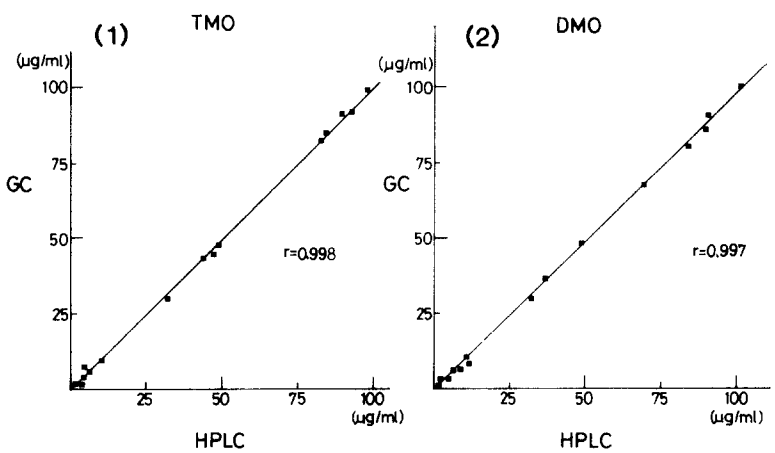

Fig. 2. Correlation between serum TMO (1) and DMO (2) concentrations assayed by HPLC and GC. TMO: $r = 0.998$, $Y = 1.01X - 0.29$; DMO: $r = 0.997$, $Y = 1.01X - 0.30$. $n = 15$.

TABLE II

PHARMACOKINETIC PARAMETERS* FOLLOWING THE ORAL ADMINISTRATION OF TMO TO RATS AND HUMANS

Each value indicates the mean ± S.E. Rats and humans received, respectively, 100 mg/kg and 4 mg/kg TMO orally.

	$T_{1/2}$ (h)	V_d (l/kg)	Cl (l/kg/h)	AUC (μg/ml/h)
Rat	2.66 ± 0.36	0.647 ± 0.013	0.039 ± 0.018	386.5 ± 5.5
Human	10.21 ± 0.69	0.648 ± 0.032	0.043 ± 0.003	100.1 ± 9.5

*$T_{1/2}$ = half-life; V_d = apparent volume of distribution; Cl = metabolic clearance; AUC = area under the curve.

and DMO levels in rats were quite similar to those reported previously by the GC method [2]. These data suggest that the new HPLC method is rapid, sensitive and selective for the simultaneous analysis of TMO and DMO in rat and human serum.

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