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## Note

# Determination of trimethadione and its metabolite by gas chromatography with flame-thermionic detection and its application to pharmacokinetic studies of the drug in carbon tetrachloride-treated rats

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We have already reported a rapid and sensitive gas chromatographic method for the determination of plasma trimethadione (TMO) and its metabolite, 5,5-dimethyl-2,4-oxazolidinedione (DMO) [1]. We have shown that pharmacokinetic of TMO when administered in vivo is a good indicator for estimating drug-metabolizing enzyme activities in control, phenobarbital and hepatotoxins-treated rat liver [1—4]. Thus, it is expected that TMO could be suitable as a model drug for estimating drug-metabolizing enzyme activities in human. However, the detection limit of TMO and DMO of the previous gas chromatographic method seems to be not sufficient if this method is applied to humans, because the administered dose of TMO should be as low as possible because of its antiepileptic pharmacological effect. In this respect, we developed a more sensitive method for the determination of low concentrations of TMO and DMO by using gas chromatography equipped with a flame-thermionic detector (FTD). In addition, a pharmacokinetic study by using this new sensitive method was carried out in carbon tetrachloride-treated rats.

## EXPERIMENTAL

### *Materials*

TMO was purified from commercial 66.7% powder (Mino-Aleviatin®; Dainippon, Osaka, Japan). Paramethadione (PMO, internal standard) was synthesized from DMO by the method of Spielman [5]. DMO was purchased from Tokyo Kasei (Tokyo, Japan), olive oil from Sanko (Tokyo, Japan) and carbon tetrachloride from Wako (Osaka, Japan). All other chemicals were of reagent grade.

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*Extraction procedure*

To 50 $\mu$l plasma in a 2.5-ml tube were added 100 $\mu$l of 5 *M* monobasic sodium phosphate, a minimum quantity (about 50 mg) of sodium sulphate and magnesium sulphate, and 100 $\mu$l of ethyl acetate containing 5 $\mu$g of PMO as internal standard. The tube was shaken for 2 min and centrifuged at 1500 *g* for 8 min. A 2-$\mu$l volume of the organic phase was then analyzed by gas chromatography.

*Gas chromatography*

Analysis of plasma TMO and DMO was carried out with a Shimadzu GC-7A instrument equipped with an FTD. 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. Helium, hydrogen and air flow-rates were 50, 90 and 230 ml/min, respectively.

*Animal studies*

After overnight fasting, male Wistar rats (Japan Laboratory Animal, Tokyo, Japan), weighing 196—230 g, received intravenous administration of 10 mg/kg of TMO. Carbon tetrachloride was dissolved in olive oil and administered orally (0.5 ml/kg) 24 h prior to the intravenous administration of TMO. Blood samples (0.1—0.15 ml) were obtained from the jugular vein at 0.25, 0.5, 1, 2, 4, 8, 12, 24, 48 and 72 h after the intravenous administration of TMO.

*Pharmacokinetic studies*

Concentration—time curves of TMO and DMO were drawn on semilogarithmic scales. The half-life ($T_{½}$), metabolic rate constant ($K_m$) and elimination rate constant ($K_{el}$) were calculated from linear regression analysis. The apparent volume of distribution ($V_d$) was calculated from the ratio of the given dose to the plasma concentration extrapolated to the time zero. The area under the curve (AUC) was calculated by the trapezoidal rule, and area to infinite time was added by integration ($C_t/K_{el}$), where $C_t$ is the last value of 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 the gas chromatogram of TMO, DMO and the internal standard PMO. The results indicate that there was a good separation between TMO, PMO and DMO. The retention times for TMO, PMO and DMO were 1.7, 4.1 and 6.3 min, respectively. The calibration graphs showed the linear relationship between the peak height ratios of TMO or DMO to the internal

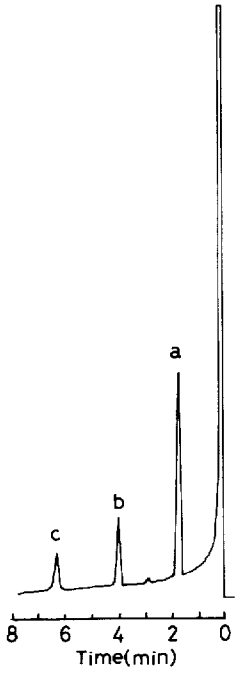


Fig. 1. Gas chromatogram of TMO (a) and DMO (c) in rat plasma. PMO (b) was used as internal standard. Retention times for a, b and c were 1.7, 4.1 and 6.3 min, respectively.

standard (PMO) in the concentration ranging from 0.1 μg/ml to 15 μg/ml ($r$ = 0.956). This method was capable of measuring at least 0.05 μg/ml of TMO and DMO, and was ca. 100-fold more sensitive than the method reported previously [1]. Extraction recovery of plasma TMO and DMO (0.1, 0.2, 0.5, 1, 5, 10 and 15 μg/ml) were 98.65 and 97.24 with standard deviations of ± 4.3 and ± 5.4% ($n$ = 4), respectively. No interfering peaks appeared when phenobarbital, phenytoin, pentobarbital, acetazolamide, carbamazepine and primidone, which are usually administered to the patients in combination with TMO, were added to plasma. From these results, it is reasonable to note that the method presented for the determination of TMO and DMO could be fully applicable to cases even if a lower dose of the drug was administered to animals or humans. Next, we carried out the pharmacokinetic study of TMO following the administration of a lower dose of the drug as compared to those reported previously [1—4]. The results of these experiments is shown in Table I. The plasma concentration of TMO in olive oil-treated rats reached its peak at 0.5—1 h, and plasma DMO reached its peak at around 9 h and then gradually decreased. The changed patterns of plasma TMO and DMO levels were almost similar to those reported previously [2]. In contrast, the peak levels of plasma concentration of TMO in carbon tetrachloride-treated rats were delayed to 5.7 h. Additionally, DMO production of the carbon tetrachloride-treated group occurred to a lesser extent than in the olive oil-group. Since $K_{el}$ values thus appeared to reflect $K_m$ values properly, estimation and analysis of $K_{el}$ values may enable us to predict the drug-metabolizing capacity in the given cases ($r$ = 0.988, Fig. 2). For the pur-

TABLE I

PHARMACOKINETIC PARAMETERS FOLLOWING THE INTRAVENOUS ADMINISTRATION OF TMO TO RATS PRETREATED WITH CARBON TETRACHLORIDE

Values are means ± S.E. ($n$ = 5). Rats were pretreated with carbon tetrachloride (0.5 ml/kg) 24 h prior to TMO (10 mg/kg, i.v.) administration.

| Treatment | $T_{½}$ (h) | $V_d$ (l) | $Cl$ (l/h) | $K_m$ (1/h) | AUC (μg/ml·h) |
|---|---|---|---|---|---|
| Olive oil | 1.61±0.04 | 0.220±0.007 | 0.095±0.003 | 0.463±0.027 | 18.03±0.92 |
| Carbon tetrachloride | 5.73±0.60* | 0.260±0.002** | 0.033±0.003* | 0.063±0.018* | 61.39±0.02* |

*$P < 0.01$.
**$P < 0.02$.

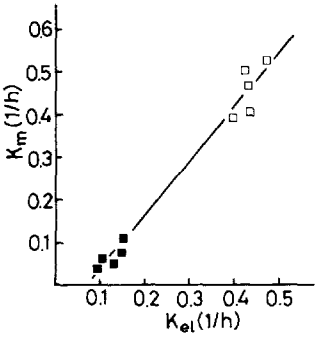


Fig. 2. Correlation between $K_m$ and $K_{el}$ of TMO in rat plasma pretreated with carbon tetrachloride. □, Olive oil; ■, carbon tetrachloride. Rats were pretreated with carbon tetrachloride (0.5 ml/kg, p.o.) 24 h prior to TMO (10 mg/kg, i.v.) administration. Each group consisted of 5 male rats. $r = 0.988$, $y = 1.3x - 0.1$, $n = 10$.

pose of shortening the time required for estimation of the drug-metabolizing capacity, we studied the possible correlation between $K_{el}$ values and ratios of plasma DMO to TMO at 1 and 2 h after intravenous administration of TMO. As shown in Fig. 3, there was a good correlation between the ratio of DMO to

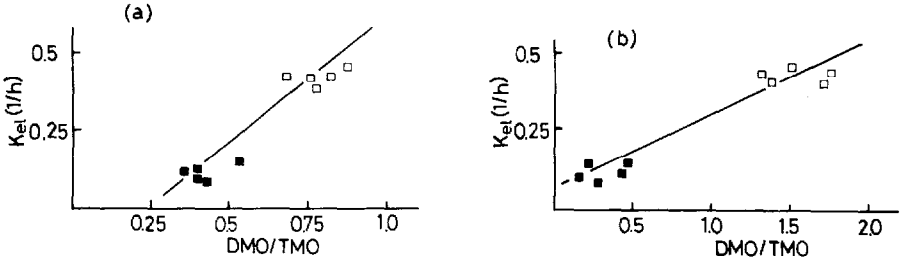


Fig. 3. Correlation between DMO/TMO ratio and $K_{el}$ in rat plasma 1 (a) and 2 h (b) after intravenous administration of TMO in rats pretreated with carbon tetrachloride. □, Olive oil; ■, carbon tetrachloride. Rats were pretreated with carbon tetrachloride (0.5 ml/kg, p.o.) 24 h prior to TMO (10 mg/kg, i.v.) administration. Each group consisted of 5 male rats. (a) $r = 0.955$, $y = 0.8x - 0.19$, $n = 10$; (b) $r = 0.958$, $y = 0.24x + 0.07$, $n = 10$.

TMO in plasma and $K_{el}$ values at 1 and 2 h after intravenous administration of TMO, respectively ($r$ = 0.955 at 1 h and $r$ = 0.958 at 2 h). The present investigation has revealed that the modified gas chromatographic method is sufficiently sensitive to determine plasma TMO and DMO in animals even when a lower dose of the drug was administered. Additionally, the results of the pharmacokinetics of TMO are well compatible with those reported previously [2].

REFERENCES

1 E. Tanaka, H. Kinoshita, T. Yoshida and Y. Kuroiwa, J. Pharm. Dyn., 4 (1981) 576.
2 E. Tanaka, H. Kinoshita, T. Yoshida and Y. Kuroiwa, J. Pharm. Dyn., 4 (1981) 961.
3 E. Tanaka, H. Kinoshita, T. Yoshida and Y. Kuroiwa, J. Pharm. Dyn., 5 (1982) 162.
4 E. Tanaka, H. Kinoshita, T. Yoshida and Y. Kuroiwa, Pharmacology, 25 (1982) 202.
5 M.A. Spielman, J. Amer. Chem. Soc., 66 (1944) 1244.