STUDIES ON THE TRIMETHADIONE METABOLISM AS A TOOL FOR THE ASSESSMENT OF DRUG-METABOLIZING CAPACITY USING PLASMA AND URINE OF RATS PRETREATED WITH PHENOBARBITAL AND 3-METHYLCHOLANTHRENE

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To determine whether concentrations of trimethadione (TMO) and its metabolite 5,5-dimethyl-2,4-oxazolidinedione (DMO) in plasma as well as in urine of rats are useful indicator of drug-metabolizing capacity or not, the following experiments were carried out.

Plasma TMO and DMO concentrations were measured in phenobarbital (PB) or 3-methylcholanthrene (3-MC)-pretreated rats following the administration of TMO. In PB-pretreated rats, there was a good correlation between elimination rate constant ($K_e$) and plasma DMO/TMO ratio; $r = 0.991$ at 1 h, $r = 0.967$ at 2 h. However, there was no good correlation in 3-MC-pretreated rats ($r = 0.780$ at 1 h, $r = 0.720$ at 2 h). TMO and DMO excretion in PB and 3-MC pretreated rats following the administration of TMO were not significantly different in 24-h urine.

These experiments, together with the previous findings, indicate that concentrations of TMO and DMO in plasma, but not in urine, in PB-pretreated rats may be a useful indicator of drug-metabolizing capacity.

Keywords—trimethadione; 5,5-dimethyl-2,4-oxazolidinedione; enzyme induction; phenobarbital; 3-methylcholanthrene; drug-metabolizing capacity; pharmacokinetic parameter; urine; d-glucaric acid

In general, patients receiving long-term treatment with drugs such as anticonvulsants and sedatives, etc., show individual differences in capacity to metabolize these drugs. Epileptic patients given a standard dose of anticonvulsants on body-weight basis had extremely variable serum drug concentrations when measured after therapy was begun. It has shown that there are wide varieties in the response to the same dose of various drugs among patients. Consequently, individual's capacity for metabolizing drugs is varied and differed from each other. Thus, it is important to determine the individual's capacity for metabolizing drugs especially in the case of long-term treatment of patients with drugs.

Phenobarbital (PB) and 3-methylcholanthrene (3-MC) are commonly used as a liver enzyme-inducing agent. We have already reported plasma and saliva concentration ratio of 5,5-dimethyl-2,4-oxazolidinedione (DMO) to trimethadione (TMO) after administration of TMO might be a useful indicator of hepatic drug-metabolizing capacity of rats pretreated with some hepatotoxic chemicals.\textsuperscript{1-4} Namely, plasma and saliva concentration ratio of DMO to TMO was well reflecting the decreased hepatic drug-metabolizing capacity produced by the administration of these hepatotoxic chemicals, such as $\alpha$-naphthylisothiocyanate, carbon tetrachloride and $d$-galactosamine.

In this respect, the present investigation was designed to examine whether DMO to TMO ratio in plasma as well as in urine is reflecting hepatic drug-metabolizing capacity of rats treated with PB or 3-MC.

MATERIALS AND METHOD

Materials — TMO was purified from com-
commercial 66.7% powder (Mino-Aleviatin®: Dai-nippon Pharmaceutical Co., Ltd., Osaka). PB (So-
dium phenobarbital) was purchased from Sankyo Co., Ltd. (Tokyo); 3-MC, from Wako Pure
Chemicals Co. (Osaka); Aminopyrine, from Sanko Seiyaku Kogyo Co. (Tokyo); Olive oil,
from Tomozawa Yakuhin Co. (Tokyo).

**Animal Treatment** — Wistar male rats, weighing 160—220 g, were used in these experiments.
Rats were pretreated with PB (80 mg/kg/d, i.p.) for 3 d, 3-MC (20 mg/kg/d, i.p.) for 2 d and 10%
sodium bicarbonate (NaHCO₃, 0.2 ml/100 g body wt., p.o.) for 2 d 24 h prior to the oral
administration of TMO (100 mg/kg). The control animals were given an equal volume of saline and
olive oil. After overnight fasting, TMO (100 mg/kg, p.o.) was administered to rats treated with
drugs. In the experiments for studying possible
effect of different dose levels on TMO and
DMO excretion, TMO was administered at the
doses of 25, 50, 100, 200 and 300 mg/kg.

**Blood and Urine Sampling** — Bloods were
obtained from the jugular vein at 0.25, 0.5, 1, 2, 4,
6, 8, 12, 24 and 48 h after administration of TMO.
For collection of urine, rats were placed in individual
metabolism cages (Natsume Seisakusho
Co., Ltd., Tokyo) and supplied with water but no
food, and 24 and 48-h urine samples were collected.

**Determination of Urinary pH** — The samples
were subjected to determination of pH using a
glass electrodode pH meter (Blood Micro System
Type BMS, Radiometer, Denmark) within 0.5 h
after collection.

**Preparation of the Liver Sample** — After tak-
ing the blood sample from jugular vein, the livers
were perfused with cold saline, removed and ho-
 mogenized with 4 volumes of 0.25 M sucrose (pH
7.4). The microsomes separated by the Ca²⁺-
bound method of Kamath and Rubin⁶ were
resuspended in the 0.05 M Na⁺/K⁺ phosphate
buffer containing 1 mM EDTA (pH 7.6).

**Enzyme Assay** — The content of cytochrome
P-450 was determined by the method of Omura
and Sato.⁶ The activities of aminopyrine and
TMO N-demethylase were determined by

![Fig. 1. Time Course of Plasma TMO Concentration in the Rat pretreated with Phenobarbital and 3-
Methylcholanthrene.](image)

Each value is the mean of 5 male rats. Each
group consisted of 5 male rats. Rats were pretreated
with phenobarbital (80 mg/kg, i.p., daily for 3 d)
and 3-methylcholanthrene (20 mg/kg, i.p., daily for
2 d) prior to TMO (100 mg/kg, p.o.) administra-
tion.

○ : saline, ● : phenobarbital, △ : olive oil, ▲ : 3-
methylcholanthrene.
constant ($K_{el}$) were calculated from linear part of the curve obtained by means of 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 ($Cr/K_{el}$), where $Cr$ is the last value of TMO concentration and $K_{el}$ was calculated from the equation.

$$K_{el} = 0.963/T_{1/2}$$

Metabolic clearance was calculated according to the following equation.

$$\text{Clearance} = 0.963 \cdot V_d/T_{1/2}$$

RESULTS

1. Time Course and Pharmacokinetics of Plasma

**TABLE I. Pharmacokinetic Parameters of Trimethadione in the Rat pretreated with Phenobarbital and 3-Methylcholanthrene**

<table>
<thead>
<tr>
<th>Pretreatment</th>
<th>$T_{1/2}$ (h)</th>
<th>$V_d$ (l)</th>
<th>$Cl$ (l/h)</th>
<th>AUC (µg/ml/h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saline</td>
<td>1.79±0.16</td>
<td>0.081±0.004</td>
<td>0.0316±0.0012</td>
<td>378±13.8</td>
</tr>
<tr>
<td>Phenobarbital</td>
<td>0.64±0.09*</td>
<td>0.047±0.012*</td>
<td>0.0531±0.0024*</td>
<td>156±10.4*</td>
</tr>
<tr>
<td>Olive oil</td>
<td>1.72±0.47</td>
<td>0.079±0.017</td>
<td>0.0335±0.0024</td>
<td>555±49.3</td>
</tr>
<tr>
<td>3-Methylcholanthrene</td>
<td>1.79±0.24</td>
<td>0.069±0.003</td>
<td>0.0290±0.0015</td>
<td>343±38.1</td>
</tr>
</tbody>
</table>

*Values are means ± S.E., n = 5. $T_{1/2}$: The Half-life, $V_d$: The Apparent Volume of Distribution, Cl: The Metabolic Clearance, AUC: The Area Under the Curve. Rats were pretreated with phenobarbital (80 mg/kg, i.p., daily for 3 d) and 3-methylcholanthrene (20 mg/kg, i.p., daily for 2 d) prior to TMO (100 mg/kg, p.o.) administration. * $p < 0.01.$

**TABLE II. Trimethadione and Aminopyrine N-demethylase Activity in Liver Homogenate**

<table>
<thead>
<tr>
<th>Pretreatment</th>
<th>P-450$^{a)}$</th>
<th>Trimethadione$^{b)}$ N-demethylase</th>
<th>Aminopyrine$^{b)}$ N-demethylase</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saline</td>
<td>0.56±0.025</td>
<td>1.54±0.681</td>
<td>6.58±1.260</td>
</tr>
<tr>
<td>Phenobarbital</td>
<td>1.60±0.102*</td>
<td>5.32±0.812*</td>
<td>29.79±2.812*</td>
</tr>
<tr>
<td>Olive oil</td>
<td>0.80±0.071</td>
<td>1.66±0.595</td>
<td>8.19±0.835</td>
</tr>
<tr>
<td>3-Methylcholanthrene</td>
<td>1.56±0.112$^{c)*}$</td>
<td>1.72±0.546</td>
<td>9.92±0.953</td>
</tr>
</tbody>
</table>

$a)$ nmol/mg protein, $b)$ nmol/mg protein/min, $c)$ P-448. *Values are means ± S.E., n = 4. Rats were pretreated with phenobarbital (80 mg/kg, i.p., daily for 3 d) and 3-methylcholanthrene (20 mg/kg, i.p., daily for 2 d) prior to TMO (100 mg/kg, p.o.) administration. * $p < 0.01.$
FIG. 2. Correlation between $K_m$ and $K_{el}$ in Rat Plasma pretreated with Phenobarbital (a) and 3-Methylcholanthrene (b).

Rats were pretreated with phenobarbital (80 mg/kg, i.p., daily for 3 d) and 3-methylcholanthrene (20 mg/kg, i.p., daily for 2 d) prior to TMO (100 mg/kg, p.o.) administration.

(a) ○ : saline, ● : phenobarbital, $r = 0.952$, $y = 0.44x + 0.109$, $n = 9$. (b) ○ : olive oil, ● : 3-methylcholanthrene, $r = 0.738$, $y = 0.46x + 0.209$, $n = 14$.

FIG. 3. Correlation between DMO/TMO Ratio and $K_{el}$ in Rat Plasma after 1 (a) and 2 h (b) Oral Administration of TMO in Rats pretreated with Phenobarbital.

Rats were pretreated with phenobarbital (80 mg/kg, i.p., daily for 3 d) prior to TMO (100 mg/kg, p.o.) administration.

○ : saline, ● : phenobarbital.

(a) $r = 0.991$, $y = 0.97x + 0.250$, $n = 10$. (b) $r = 0.967$, $y = 0.28x + 0.007$, $n = 8$. 
treatment on pharmacokinetic parameters of TMO in rats are shown in Table I. The pretreatment of rats with PB shortened half-life, decreased apparent $V_{dh}$, AUC and increased clearance rate, while the pretreatment with 3-MC did not cause any change in these parameters.

2. Effect of PB and 3-MC on Rat Hepatic Microsomal Drug-Metabolizing Enzymes

As shown in Table II, administration of PB resulted in the increase of cytochrome P-450 content (3-fold), and aminopyrine (4.5-fold) and TMO $N$-demethylase activities (3.5-fold), while administration of 3-MC resulted in the increase of cytochrome P-448 content and slight increase of aminopyrine $N$-demethylase activity, but TMO $N$-demethylase activity was not different from the corresponding controls.

3. Correlation between $K_m$ and $K_{cl}$ with PB and 3-MC Treated Rats

As shown in Fig. 2 (a), there were significant correlation between $K_{cl}$ and $K_m$ of TMO after oral administration of the drug to rats treated with PB ($r = 0.952, y = 0.44x + 0.109$), while no good correlation was seen in 3-MC treated rats ($r = 0.738, y = 0.46x + 0.207$), as shown in Fig. 2 (b).

4. Correlation between $K_{cl}$ and Plasma DMO/TMO Ratio with PB and 3-MC Treated Rats

Figs. 3 and 4 show the results of determination of correlation between the ratio of DMO to TMO in plasma and $K_{cl}$ at 1 and 2 h after oral administration of TMO to rats treated with PB and 3-MC, respectively. A significant correlation was seen in PB treated rats ($r = 0.991, y = 0.97x + 0.250$ at 1 h, $r = 0.967, y = 0.28x + 0.007$ at 2 h), while there was no good correlation in 3-MC treated rats ($r = 0.780, y = 0.63x - 0.044$ at 1 h, $r = 0.720, y = 0.26x +$

![Graph](image-url)

**FIG. 4.** Correlation between DMO/TMO Ratio and $K_{cl}$ in Rat Plasma after 1 (a) and 2 h (b) Oral Administration of TMO in Rats pretreated with 3-Methylcholanthrene

Rats were pretreated with 3-methylcholanthrene (20 mg/kg, i.p., daily for 2 d) prior to TMO (100 mg/kg, p.o.) administration.

○ : olive oil, ● : 3-methylcholanthrene.

(a) $r = 0.780, y = 0.63x - 0.004, n = 13$. (b) $r = 0.720, y = 0.26x - 0.060, n = 12$. 
TABLE III. Effect of Fasting on D-Glucaric Acid Excretion in Rats

<table>
<thead>
<tr>
<th>D-Glucaric acid (n mol/mg creatine/24 h)</th>
<th>Before</th>
<th>Fasting (d)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>1</td>
</tr>
<tr>
<td>Mean</td>
<td>0.478</td>
<td>0.309</td>
</tr>
<tr>
<td>±S.E.</td>
<td>0.070</td>
<td>0.053</td>
</tr>
</tbody>
</table>

Each value is the mean of 6 male rats.

FIG. 5. Correlation between Urinary Excretion of TMO and Different Dose Levels of TMO
\[ r = 0.986, y = 1.7x + 1.15, n = 20. \]

FIG. 6. Correlation between Urinary Excretion of DMO and Different Dose Levels of TMO
\[ r = 0.978, y = 11.9x + 10.34, n = 20. \]

0.060 at 2 h).

5. Effects of Fasting on Urinary Excretion of GA in Rats

As shown in Table III, when rats were fasted 24 h and 48 h, urinary excretion of GA was significantly decreased to about 35% and 63% of the initial levels, respectively. In addition, treatment of rats with PB resulted in a significant increase of urinary excretion of GA (2-fold at 24 h, 4.7-fold at 48 h) as compared to controls. However, treatment of rats with 3-MC and 10% NaHCO₃ did not cause any change in urinary excretion of GA.

6. Urinary Excretion of TMO and DMO in PB, 3-MC and 10% NaHCO₃ Treated Rats

As shown in Table IV, TMO excretion in PB, 3-MC and 10% NaHCO₃ treated rats and DMO excretion in PB and 3-MC treated rats were not significantly different in 24-h urine, while DMO excretion in 10% NaHCO₃ treated rats were increased to about 1.5-fold as compared with controls. Urinary pH was increased in 10% NaHCO₃ treated rats (pH 8.22). Urinary excretion of TMO in controls, PB, 3-MC and 10% NaHCO₃ treated rats was about 1.4% of the administered dose, while urinary excretion of DMO in controls, PB and 3-MC treated rats was about 10.1% of the administered dose, but treatment of rats with 10% NaHCO₃ resulted in increase of the DMO excretion from 10.5 to 15.8% of the administered dose.

7. Urinary TMO, DMO and GA Excretion after the Administration of Different Dose Levels of TMO

As shown in Figs. 5, 6 and Table V, urinary TMO and DMO excretions in rats were proportional to the administered dose levels. Correlations between urinary excretion of TMO and DMO, and the administered different dose levels of TMO were good (TMO: \( r = 0.986, y = 1.7x + 1.15 \), DMO: \( r = 0.978, y = 11.9x + 10.34 \)). Urinary GA and pH were not changed significantly.

DISCUSSION

To find out marker compound which could reflect hepatic drug-metabolizing capacity has received much attention recently because clini-
cians have become interested in examining drug metabolism as an indicator of hepatic function. Enzyme induction has been assessed in experimental animals and humans by measuring changes in the pharmacokinetics of market drugs, such as antipyrine, paracetamol and phenylbutazone, or changes in the urinary excretion of endogenous compounds such as d-glucaric acid and 6β-hydroxycorticosteroids. Plasma half-life of antipyrine was widely used as an indicator of drug metabolizing activity in experimental animals and in humans. However, some disadvantages of antipyrine are that the drug itself is a mild inducer of the drug-metabolizing enzymes in humans and the other is that its metabolism is regulated by different types of hepatic mono-oxygenase in experimental animals and probably also in humans.

It has been shown that TMO, anticonvulsant agent, is a neutral compound which is rapidly absorbed from the gastrointestinal tract and distributed into the total body fluids. TMO is extensively N-demethylated to DMO by hepatic microsomal enzyme system. TMO nor DMO is bound to plasma protein or any other macromolecules in biological materials. Considering these facts, it might be possible to estimate hepatic drug-metabolizing capacity of rats determining the concentration ratio of DMO to TMO in blood collected after the administration of TMO. In this context, we have shown that pharmacokinetic parameters of TMO metabolism in rats treated with hepatotoxic chemicals are well reflecting the hepatic drug-metabolizing capacities of the animals, especially the decreased drug-metabolizing capacities produced by the administration of the chemicals.

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**TABLE IV. Amounts of Trimethadione and Its Metabolite (DMO) Excreted in 24 H Urine in Rats pretreated with Phenobarbital, 3-Methylcholanthrene and 10% Sodium Bicarbonate**

<table>
<thead>
<tr>
<th>Pretreatment</th>
<th>TMO (μg)</th>
<th>% dose</th>
<th>DMO (μg)</th>
<th>% dose</th>
<th>Urinary pH</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>168 ± 16.2</td>
<td>1.30</td>
<td>1219 ± 133.8</td>
<td>10.5</td>
<td>7.50</td>
</tr>
<tr>
<td>Phenobarbital</td>
<td>143 ± 18.0</td>
<td>1.18</td>
<td>1338 ± 160.0</td>
<td>8.9</td>
<td>7.33</td>
</tr>
<tr>
<td>3-Methylcholanthrene</td>
<td>132 ± 32.7</td>
<td>1.42</td>
<td>1146 ± 112.9</td>
<td>12.3</td>
<td>7.42</td>
</tr>
<tr>
<td>10% NaHCO₃</td>
<td>152 ± 25.0</td>
<td>1.58</td>
<td>1687 ± 83.2</td>
<td>15.8</td>
<td>8.22</td>
</tr>
</tbody>
</table>

a) p < 0.02. Values are means ± S.E., n = 5. Rats were pretreated with phenobarbital (80 mg/kg, i.p., daily for 3 d), 3-methylcholanthrene (20 mg/kg, i.p., daily for 2 d) and 10% NaHCO₃ (20 mg/kg, p.o., daily for 2 d) prior to trimethadione (100 mg/kg, p.o.) administration, respectively.

**TABLE V. Amounts of d-Glucaric Acid, Trimethadione and Its Metabolite (DMO) excreted in 24 H Urine in Rats After Administration of Different Dose Levels of Trimethadione**

<table>
<thead>
<tr>
<th>Treatment (mg/kg)</th>
<th>TMO (μg)</th>
<th>% dose</th>
<th>DMO (μg)</th>
<th>% dose</th>
<th>d-Glucaric acid</th>
<th>Urinary pH</th>
</tr>
</thead>
<tbody>
<tr>
<td>25</td>
<td>44 ± 4.6</td>
<td>1.2</td>
<td>325 ± 33.4</td>
<td>9.5</td>
<td>0.521 ± 0.043</td>
<td>7.50</td>
</tr>
<tr>
<td>50</td>
<td>90 ± 4.4</td>
<td>1.2</td>
<td>623 ± 45.3</td>
<td>9.1</td>
<td>0.512 ± 0.054</td>
<td>7.23</td>
</tr>
<tr>
<td>100</td>
<td>168 ± 16.2</td>
<td>1.3</td>
<td>1219 ± 133.7</td>
<td>10.5</td>
<td>0.547 ± 0.107</td>
<td>7.51</td>
</tr>
<tr>
<td>200</td>
<td>336 ± 19.2</td>
<td>1.2</td>
<td>2268 ± 182.3</td>
<td>9.3</td>
<td>0.546 ± 0.033</td>
<td>7.18</td>
</tr>
<tr>
<td>300</td>
<td>510 ± 20.2</td>
<td>1.3</td>
<td>3557 ± 126.5</td>
<td>9.7</td>
<td>0.576 ± 0.201</td>
<td>7.38</td>
</tr>
</tbody>
</table>

a) n mol/mg creatinine/24 h. Values are means ± S.E., n = 4.
Thus, TMO metabolism was also taken into consideration as an indicator of drug-metabolizing capacity in rats pretreated with microsomal enzyme inducers. Plasma TMO half-life in PB treated rats was extremely shortened in comparison with controls. On the other hand, TMO metabolism was not significantly changed in 3-MC treated rats. In pharmacokinetic parameters, pretreatment of rats with PB resulted in the shortened plasma half-life, the decrease of $V_d$ and AUC, and the increase of clearance rate. These results well coincided to the increase of cytochrome P-450 content, and of TMO and aminopyrine N-demethylase activity caused by PB pretreatment. On the other hand, pretreatment with 3-MC resulted in the increase of cytochrome P-448 content, while TMO and aminopyrine N-demethylase activities were not changed. The results suggest that TMO is mainly metabolized by the cytochrome P-450 system, but to a lesser extent by cytochrome P-448 system.

In all the rats treated with and without PB treatment, $K_m$ values were significantly correlated to $K_{el}$ values ($r = 0.952$). On the other hand, correlation between $K_{el}$ values and $K_m$ values in 3-MC treated rats ($r = 0.738$) was not good. The reasons for this deviation in 3-MC treated rats is now under investigation. Since $K_{el}$ values thus appeared to reflect $K_m$ values properly in PB treated rats, estimation and analysis of $K_{el}$ values may enable us to predict the drug-metabolizing capacity in the present case. In the next step, for the purpose of shortening the time required for estimation, we studied possible correlation between $K_{el}$ values and blood concentration ratios of DMO to TMO. In PB treated rats, 1 and 2 h values were well correlated in the respect ($r = 0.991$ at 1 h, $r = 0.967$ at 2 h). On the other hand, pretreatment with 3-MC did not show any good correlation ($r = 0.780$ at 1 h, $r = 0.720$ at 2 h). These experiments, together with the previous findings, provide an information that plasma concentration of TMO and DMO in PB treated rats may be useful as an indicator of drug-metabolizing capacity.

The significance of urinary $\text{GA}_{12,15,21}^{12,15,21}$ and $6\beta$-hydroxycortisol$^{26-28}$ as an indicator of microsomal enzyme induction has been emphasized in many recent publications. Thus, we also examined as to whether TMO and DMO excretion in plasma as well as in urine are useful indicator of microsomal enzyme induction or not. Despite the fact that TMO metabolism in PB treated rats was faster than controls, urinary excretion of DMO was unchanged as compared with controls. This reason could be explained by the fact that DMO is reabsorbed in the renal tubule by a process of passive diffusion, because the tubular epithelium is permeable to the undissociated form and impermeable to the ionic form.

In fact, urinary excretion of DMO (weak acidic compound, $pK_a$ 6.123) increased in 10% NaHCO$_3$ treated rats. In this context, Jensen$^{42}$ showed already that the elimination rate of DMO in man was $K_{el} = 6.9$, and the value increased to 13.9 when NaHCO$_3$ was given at a dose of 6 g/d. Waddell and Butler$^{49}$ also reported a similar tendency of the effect of NaHCO$_3$ administration on the change in $K_{el}$ and DMO excretion.

Since urinary excretion of DMO was dependent on urinary pH, it is not suitable at present that the TMO and DMO excretion in urine is used as an indicator of drug-metabolizing capacity. In this respect, further detailed studies would be required.

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