THE EFFECT OF INDOMETHACIN ON HEPATIC DRUG-OXIDIZING CAPACITY IN THE RAT: TRIMETHADIONE AND ANTIPYRINE METABOLISM AS AN INDICATOR

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In this study, trimethadione (TMO) and antipyrine were chosen as model drugs to investigate the extent of hepatic drug-oxidizing capacity. It was also studied whether pretreatment of rats with indomethacin affected the formation of antipyrine metabolite. Pretreatment with indomethacin in a dose of 5 mg/kg/d for 3 d did not change the serum half-life (T1/2), the total body clearance (CL), and the apparent volume of distribution (Vd) of TMO and antipyrine. However, in the rat treated with 8.5 mg/kg/d for 3 d of indomethacin, these parameters were significantly decreased as compared to controls except to Vd values in antipyrine kinetics in vivo.

The contents of cytochrome P-450, and the activities of aminopyrine N-demethylase and aniline hydroxylase were not changed by 5 mg/kg/d for 3 d of indomethacin. However, in the rat treated with 8.5 mg/kg/d for 3 d of indomethacin, these enzyme activities were significantly decreased as compared to controls. The activities of heme oxygenase were significantly increased as compared to controls in the rat treated with 5 and 8.5 mg/kg/d for 3 d of indomethacin, in vitro.

The excretions of 4-hydroxyantipyrine and 3-hydroxymethyl antipyrine were not changed in the rat treated with 8.5 mg/kg for 3 d of indomethacin as compared to controls, whereas the excretion of norantipyrine was significantly decreased.

These results, together with the previous findings, indicate that indomethacin treatment inhibited N-demethylation pathway of TMO and antipyrine metabolism.

Keywords — trimethadione; antipyrine metabolite; indomethacin; rat; hepatic drug-oxidizing capacity

INTRODUCTION

Indomethacin is a widely used non-steroidal antiinflammatory drug. In the treatment of inflammatory diseases, repeated use of this drug is not unusual. Long-term administration of indomethacin to rats produces degenerative changes in the liver. However, information about its hepatotoxic potential and the effect on hepatic drug-metabolizing capacity is still few.2,8

We have already reported a useful method for estimation of hepatic drug-oxidizing capacity. Serum concentration ratios of dimethadione (DMO) to trimethadione (TMO) after oral administration of TMO are well correlated to the drug-oxidizing enzyme activities in the rats pretreated with some chemicals such as hepatotoxic agents and the inducers of the enzyme system.4-7 In a similar fashion antipyrine is widely used as the model drug to investigate factors which influences the activity of the hepatic drug-oxidizing capacity.8-10 Based on these facts, trimethadione (TMO)4-7 and antipyrine8-10 were chosen as model drugs to investigate the extent of hepatic drug-oxidizing capacity after indomethacin treatment. It was also studied whether pretreatment of rats with indo-
methacin affected the formation of antipyrine metabolites.

MATERIALS AND METHODS

Materials — TMO, antipyrine and indomethacin were kindly provided by Dainippon Pharmaceutical Co. Ltd. (Osaka, Japan), Hoei Pharmacy Co. Ltd. (Tokyo, Japan) and Horiuchi Itaro Co. Ltd. (Tokyo, Japan), respectively. Norantipyrine (NORA), 3-hydroxymethylandipyrine (HMA) and 4-hydroxyantipyrine (OHA) were synthesized as described by Koike et al., Yoshimura et al., and Knorr and Pschorr, respectively. Metabolites were identified by infrared (IR) spectrometry, nuclear magnetic resonance (NMR) spectrometry, and mass spectrometry. β-Glucuronidase-sulphatase (limpet-acetone powder type I, Sigma, St. Louis, MO, U.S.A.) was used for the hydrolysis of the conjugated metabolite in urine. All solvents and chemicals used were of analytical grade and were purchased from Wako Pure Chemical Ind. (Osaka, Japan).

Animals — Male Wistar rats (Japan Laboratory animals, Inc., Tokyo), weighing 220—250 g, were used throughout the study. They were allowed free access to a commercially available diet (Sankyo Laboservice, Tokyo, Japan). From 15 h before to the start of the experiments, only water was given to the rats.

Treatments — Indomethacin was dissolved in an isomolar solution on sodium hydrogen carbonate to give a neutral pH, and injected i.p. once daily for 3 d (5 and 8.5 mg/kg) prior to TMO (100 mg/kg, p.o.) or antipyrine (50 mg/kg, i.p.) administration. Rats were pretreated with phenobarbital (PB, 80 mg/kg/d, i.p.) for 3 d or 3-methylcholanthrene (3-MC, 20 mg/kg/d, i.p.) for 2 d. The control and indomethacin-treated rats were fixed on a plate (CFK Lab., Tokyo, Japan) only at the time of blood sampling from the jugular vein after oral administration of TMO or intraperitoneal (i.p.) administration of antipyrine without anesthesia. The serum samples, prepared by centrifugation, were stored at −20 °C until used for the determination of TMO, DMO and antipyrine levels. For collection of urine after antipyrine (50 mg/kg, i.p.) administration, the control and indomethacin treated-rats were individually placed in metabolic cages (Natsume Seisakusho Co., Ltd, Tokyo, Japan) and were given only with water but no food, and 24 h urine samples were collected.

Preparation of the Liver Sample — After blood samples were taken from the jugular vein, the liver was perfused with cold 0.9% NaCl solution from the portal vein, removed and homogenized in 4 volumes of 1.15% KCl by using a glass homogenizer with a Teflon pestle. The homogenate was centrifuged at 9,000 × g for 20 min. The resulting supernatant was recentrifuged at 105,000 × g for 60 min. The microsomal pellet was resuspended in 0.05 M Na/K phosphate buffer (pH 7.6) containing 1 mM ethylenediaminetetraacetic acid (EDTA). The cytosol, prepared from normal rat liver homogenate, was saved and used as a source of biliverdin reductase for the determination of heme oxygenase activity.

Enzyme Assay — Contents of cytochrome P-450 were determined by the method of Omura and Sato. Activities of aminopyrine and TMO N-demethylase were determined according to the method of Cochin and Axelrod. The activity of aniline hydroxylase was determined by measuring the formation of p-aminophenol by the method of Imai et al. Heme oxygenase activity was assayed according to the method of Tenhunen et al.

Protein Concentration — Protein concentrations were determined by the method of Lowry et al. using bovine serum albumin as standard.

TMO and DMO Assay — TMO and DMO levels in serum were determined by a gas-liquid chromatographic method using paramethadione as internal standard, as reported previously.

Antipyrine and Its Metabolite Assay — Antipyrine in serum and urine, and OHA, NORA and HMA in the urine were determined by a high performance liquid chromatographic method. Phenacetin was used as internal standard.
A high-performance liquid chromatograph (HPLC-803D, Toyo Soda Manufacturing Co., Ltd., Tokyo, Japan) with a variable-wavelength ultraviolet (UV) spectrophotometer (UV-8 model II, Toyo Soda Manufacturing Co., Ltd., Tokyo, Japan) was employed. The column was of reversed-phase type (Lichrosorb RP-2, 4.6 × 150 mm, 5 µm). The detector was set at 244 nm, and 5% acetonitrile in 0.05 M phosphate buffer (pH 6.5) was used as mobile phase. Flow rate was set at 1.5 ml/min. All analyses were performed at room temperature.

Calculation — The half-life ($T_{1/2}$) and elimination rate constant ($k_e$) were calculated from linear part of the serum concentration–time curve of TMO and antipyrine, which was drawn on semilogarithmic scales obtained by means of linear regression analysis. The area under the serum concentration–time curve ($AUC$) of TMO was calculated according to the log-linear trapezoidal rule including extrapolation to infinity. The apparent volume of distribution ($V_d$) was calculated from the ratio extrapolated serum concentration at the time zero. The total body clearance (CL) of TMO and antipyrine were calculated according to the following equation:

$$CL = \frac{1n2 \cdot V_d}{T_{1/2}}$$

**Statistical Analysis** — The results were analyzed by the Cochran-Cox’s and Student’s $t$-test.

**RESULTS**

**Pharmacokinetic Study on TMO and ANtipyrine in Rats Pretreated with Indomethacin**

As shown in Tables I and II, $T_{1/2}$, CL and $V_d$ of TMO and antipyrine were not changed by pretreatment with 5 mg/kg/d for 3 d of indomethacin as compared to controls. However, in the rat treated with 8.5 mg/kg/d for 3 d of indometacin, these parameters were significantly decreased as compared to the control except for $V_d$ values in antipyrine kinetics.

**Hepatic Oxidizing Enzyme Activities in Rats Pre-

<table>
<thead>
<tr>
<th>TABLE I. Pharmacokinetic Parameters Following the Oral Administration of TMO to Rats Pretreated with Indomethacin</th>
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<tbody>
<tr>
<td>Treatment</td>
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<tr>
<td>--------------------------</td>
</tr>
<tr>
<td>Control</td>
</tr>
<tr>
<td>Indomethacin 5 mg/kg</td>
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<tr>
<td>Indomethacin 8.5 mg/kg</td>
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</tbody>
</table>

$^{a)} p < 0.01$. $^{b)} p < 0.05$. $T_{1/2}$= half-life; $V_d$= apparent volume of distribution; CL=total body clearance. Indomethacin (5 and 8.5 mg/kg) was injected i.p. daily for 3 d prior to TMO (100 mg/kg, p.o.) administration. Values are means ± S.E. (n=5).

<table>
<thead>
<tr>
<th>TABLE II. Pharmacokinetic Parameters Following the Intraperitoneal Administration of Antipyrine to Rats Pretreated with Indomethacin</th>
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<tr>
<td>Treatment</td>
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</tr>
<tr>
<td>Control</td>
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<tr>
<td>Indomethacin 5 mg/kg</td>
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<td>Indomethacin 8.5 mg/kg</td>
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</tbody>
</table>

$^{a)} p < 0.01$. $^{b)} p < 0.05$. $T_{1/2}$= half-life; $V_d$= apparent volume of distribution; CL=total body clearance. Indomethacin (5 and 8.5 mg/kg) was injected i.p. daily for 3 d prior to antipyrine (50 mg/kg, i.p.) administration. Values are means ± S.E. (n=5).
### TABLE III. Effects of Indomethacin Pretreatment on Hepatic Microsomal Drug-Oxidizing Enzymes in Rats

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Content of cytochrome P-450 (^b)</th>
<th>Enzyme activity</th>
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</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Aminopyrine N-demethylase (^c)</td>
</tr>
<tr>
<td>Control</td>
<td>0.97 ± 0.03</td>
<td>10.00 ± 0.22</td>
</tr>
<tr>
<td>Indomethacin</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5 mg/kg</td>
<td>1.13 ± 0.10</td>
<td>10.59 ± 0.51</td>
</tr>
<tr>
<td>8.5 mg/kg</td>
<td>0.41 ± 0.04 (^a)</td>
<td>3.16 ± 0.49 (^a)</td>
</tr>
</tbody>
</table>

\(^a\) \(p < 0.01\). \(^b\) nmol/mg protein. \(^c\) nmol/mg protein/min. \(^d\) nmol/mg protein/h. Indomethacin (5 and 8.5 mg/kg) was injected i.p. daily for 3 d and the animals were sacrificed 24 h after the last injection. Values are means ± S.E. \((n=4)\).

**treated with Indomethacin**

As shown in Table III, the contents of cytochrome P-450, activities of aminopyrine N-demethylase and aniline hydroxylase were comparable between the controls and the rats treated with 5 mg/kg/d for 3 d of indomethacin. However, in the rat treated with 8.5 mg/kg/d for 3 d of indomethacin, these enzyme activities were significantly decreased as compared to the controls. The activity of heme oxygenase was significantly increased as compared to the controls in the rat treated with 5 and 8.5 mg/kg/d for 3 d of indomethacin.

*Serum DMO/TMO Ratios after TMO Administration in Rats Pretreated with Indomethacin*

As shown in Fig. 1, the serum DMO/TMO ratios at 2 and 4 h after TMO administration in the rat treated with 5 mg/kg/d for 3 d of indomethacin were not significantly changed as compared to the controls (2 h: 0.94 ± 0.10 vs. 0.84 ± 0.12, 4 h: 2.65 ± 0.21 vs. 2.17 ± 0.51). However, in the rat treated with 8.5 mg/kg/d for 3 d, those ratios were significantly decreased as compared to the controls (2 h: 0.94 ± 0.10 vs. 0.44 ± 0.04, 4 h: 2.65 ± 0.21 vs. 0.56 ± 0.22).

*Antipyrine Metabolite Profile in Urine of Rats Pretreated with Indomethacin, PB or 3-MC*

As shown in Table IV, the excretions of OHA and HMA were not changed in the rat treated with 8.5 mg/kg/d for 3 d of indomethacin as compared to the controls, whereas the excretion of NORA was significantly decreased. In the rat treated with PB and 3-MC, the excretion of HMA was significantly decreased, whereas the excretions of NORA and OHA were significant.

![Fig. 1. The Serum DMO/TMO Ratios at 2 and 4 h after TMO Administration in Rats Pretreated with Indomethacin](image-url)
ly increased in the rat treated with PB and 3-MC, respectively.

**DISCUSSION**

In this study, we have investigated the extent of hepatic drug-oxidizing capacity and studied a selective change in formation of antipyrine metabolite in the rats pretreated with indomethacin. According to Reinicke and Klinger, indomethacin (6 mg/kg, once daily for 3 d, i.p.) increases hexobarbital sleeping time in male Wistar rats. Burke et al. found in male Sprague-Dawley rats that treatment with 5 mg/kg (once daily for 3 d, i.p.) indomethacin did not significantly change the contents of cytochrome P-450, and the activities of aminopyrine N-demethylase, ethoxyresorcin O-deethylase and benzyloxyresorcin O-debenzylase. However, in higher dose (8.5 mg/kg, once daily for 3 d, i.p.), the contents of cytochrome P-450 and the activity of monooxygenase were significantly decreased as compared to controls.

In other studies, Vukoson et al. also reported that the contents of cytochrome P-450, and the activities of aniline hydroxylase, ethymorphine N-demethylase and Δ4-steroid hydroxynase were significantly lower in the male Sprague-Dawley rat treated with 5 mg/kg of indomethacin (twice daily for 2 or 3 d, i.p.).

In the recent study by Ogiso et al. it was also found in male Wistar rats that 4 mg/kg (8 times, every 16 h) of indomethacin significantly decreased the contents of cytochrome P-450, and the activities of aniline hydroxylase, biphenyl hydroxylase and aryl esterase as compared to controls. These findings were similar to our results indicating decreases in the activities of hepatic microsomal oxidizing enzymes (Tables I, II, III and Fig. 1).

Antipyrine metabolite profile is used as a model drug to assess the intensity of drug-induced changes in hepatic drug-oxidizing enzyme activities. Danhof et al. found that the formation of antipyrine metabolites (antipyrine, OHA, HMA and NORA) detected in 24 h urine of the rats treated with PB (100 mg/kg/d for 8 d, i.p.) was not significantly different from that of controls. In the rat treated with 3-MC (18 mg/kg/d for 3 d, i.p.), OHA was significantly increased from 13.4% to 25.6% of dose, whereas HMA was decreased from 26.8% to 8.5% of dose.

In other studies, Inaba et al. also found that after [N-14CH3] antipyrine dosing in male rats, the content of OHA in 24 h urine was markedly increased by both PB (100 mg/kg/d for 2 d and 50 mg/kg on the third day) and 3-MC (30 mg/kg/d for 2 d). However, the content of HMA was decreased by more than 50%.

In recent study, Teunissen et al. found that the percentages of antipyrine metabolites in urine of the rats pretreated with PB (100 mg/kg, i.p.) were not significantly changed except for NORA, which decreased from 17.5% to 13.3% of dose. On the basis of their results and some of the previously reported results, they concluded

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**TABLE IV. Excretion of Antipyrine and Its Metabolites Expressed as Percentage of Dose in 24 h Urine in Rats Pretreated with Indomethacin, Phenobarbital and 3-Methylcholanthrene**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Antipyrine</th>
<th>3-Hydroxymethylantipyrine</th>
<th>4-Hydroxyantipyrine</th>
<th>Norantipyrine</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>2.7 ± 0.20</td>
<td>24.6 ± 1.44</td>
<td>18.7 ± 1.16</td>
<td>2.1 ± 0.08</td>
<td>45.4 ± 1.66</td>
</tr>
<tr>
<td>Indomethacin</td>
<td>3.3 ± 0.51</td>
<td>20.6 ± 4.30</td>
<td>20.4 ± 1.78</td>
<td>1.5 ± 0.15a</td>
<td>42.5 ± 5.43</td>
</tr>
<tr>
<td>Phenobarbital</td>
<td>2.1 ± 0.34</td>
<td>11.4 ± 0.90a</td>
<td>43.6 ± 4.86a</td>
<td>1.8 ± 0.27</td>
<td>57.0 ± 4.44</td>
</tr>
<tr>
<td>3-Methylcholanthrene</td>
<td>2.9 ± 0.39</td>
<td>4.6 ± 1.05a</td>
<td>20.2 ± 2.88</td>
<td>4.6 ± 0.52a</td>
<td>29.4 ± 3.45</td>
</tr>
</tbody>
</table>

a) p < 0.01. b) p < 0.05. Indomethacin (5 mg/kg) was injected i.p. once daily for 3 d. Values are means ± S.E. (n = 5–10) and are expressed as % dose.
that the formation of antipyrine metabolites is entirely mediated by PB-induced cytochrome P-450. They further stated that the formation of OHA and NORA, however, is also mediated by 3-MC-induced cytochrome P-450, but HMA formation is probably not. The results of our study were slightly different from those of several previous studies. This variance among the in vivo results may suggest that some different types of cytochrome P-450 are involved in the three main pathways of antipyrine metabolism. These results indicate that indomethacin treatment inhibited N-demethylation pathway of TMO and antipyrine metabolism.

Acknowledgment

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