Urinary Excretion of Mefenamic Acid and Its Metabolites Including Their Esterglucuronides in Preterm Infants Undergoing Mefenamic Acid Therapy

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Urinary excretion of mefenamic acid (MA) and its two oxidative metabolites, M-I (3'-hydroxymethyl derivative) and M-II (3'-carboxyl derivative), and their glucuronides was investigated in preterm infants undergoing MA therapy. MA was given orally at a dose of 2 mg/kg and the dose was repeated every 24 h a maximum of three times. Urine was collected for up to 5 d after the last dose, and MA and the metabolites were determined by a newly developed HPLC. The cumulative amounts of MA and the metabolites excreted in the urine varied from 7 to 46% of the total dose administered, and were less than those reported in adults and children. Significant correlation was observed between the plasma half-life of MA and the cumulative amount of MA and the metabolites excreted in the urine. These results suggest that long plasma half-lives of MA observed in preterm infants are due mainly to low activity of drug metabolizing enzyme(s). In an infant who received the two regimes of MA therapy about 2 weeks apart, the plasma half-life of MA was shortened and the urinary excretion of the MA metabolites including their glucuronides was greatly increased during this period. It is suggested that the activities of both cytochrome P-450(s) and glucuronyltransferase(s) related to MA metabolism rapidly increased during the first month of the infant’s life.

Key words mefenamic acid; urinary excretion; preterm infant; drug metabolism; metabolite; glucuronide

Mefenamic acid (MA) has been used widely in Japan for pharmacologic closure of patent ductus arteriosus (PDA) in preterm infants.1 We recently reported the pharmacokinetics of MA in preterm infants with symptomatic PDA.2 The plasma concentrations of MA were quite varied from infant to infant and marked interindividual differences in plasma half-lives and apparent total body clearances of MA were observed.2 As shown in Fig. 1, MA is extensively metabolized in man to 3'-hydroxymethyl (metabolite-I; M-I) and 3'-carboxyl (metabolite-II; M-II) derivatives, and also to its esterglucuronide (MA-G).3–5 Two oxidative metabolites, M-I and M-II, are further converted to the esterglucuronides, M-I-G and M-II-G, respectively, and these metabolites are mainly excreted in urine.3–5

In the present study, we investigated the urinary excretion of MA and its metabolites in preterm infants undergoing MA therapy for pharmacologic closure of symptomatic PDA, using a newly developed HPLC method.

MATERIALS AND METHODS

Subjects and Urine Collection The subjects were 5 preterm infants with symptomatic PDA under care in the Newborn Intensive Care Unit in Hokkaido Children’s Hospital and Medical Center (Table 1). As noted previously,2 this study was discussed with the parents and consent obtained. MA was given by nasogastric tube at a dose of 2 mg/kg and the dose was repeated every 24 h a maximum of three times. Plasma concentrations and plasma half-lives of MA were determined by the method described previously,2,6 and most of them except one case can be found in our previous report.2 The plasma

![Fig. 1. Metabolic Pathways of MA in Man](image.png)

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half-lives of MA in these infants are listed in Table 1. Urine samples were collected at 24 h intervals for up to 5 d after the last dose. Urine volume was measured and an aliquot of each sample was stored at −20°C until analysis.

**Determination of MA and Its Metabolites in Urine**

MA was kindly supplied by Sankyo Co. (Tokyo, Japan) and M-I and M-II by Warner Lambert Co. (Tokyo). Tolfenamic acid (TA) and N-(3-chloro-2-hydroxyethylphenyl) anthranilic acid (a TA metabolite; TAM), were generously supplied by Dr. T. Kuninaka (Tobishi Pharmaceutical Co., Tokyo). Acetonitrile (HPLC grade, Cica-Merck) was obtained from Kanto Chemical (Tokyo). The other reagents were of analytical-reagent grade.

A Model LC-9A liquid chromatograph equipped with a Model SPD-10A variable-wavelength ultraviolet absorbance detector (all from Shimadzu, Kyoto, Japan) was used. A micro-data processor (Model C-R6A Chromatopac, Shimadzu) was used for peak-area integration and calculations. Analysis was performed on a 7-μm LiChrosorb RP-18 column (250 × 4 mm i.d.; Cica-Merck) from Kanto Chemical, operated at 45°C. The mobile phases were acetonitrile–water–0.1 M phosphoric acid (55:42:3, v/v/v; mobile phase A) for analysis of MA, and acetonitrile–water–0.1 M phosphoric acid (33:64:3, v/v/v; mobile phase B) for analysis of M-I and M-II. The flow rate was kept at 2.0 ml/min and the column effluent was monitored at 280 nm.

For analysis of unconjugated MA, a 100 μl volume of urine was pipetted into a 1.5 ml microcentrifuge tube and 50 μl of the internal standard solution (TA, 5 μg/ml in acetonitrile) and 400 μl of acetonitrile were added. After mixing on a vortex mixer for 30 s, the sample was centrifuged at 8000 × g for 2 min. A 500 μl aliquot of the supernatant was transferred into a 10 ml glass-stoppered centrifuge tube and was evaporated to dryness under reduced pressure. The residue was dissolved in 120 μl of the mobile phase A and a 50 μl aliquot was injected into the HPLC system. For analysis of total MA (conjugated and unconjugated MA), a 100 μl volume of urine and 100 μl of 0.5 N sodium hydroxide was pipetted into a 1.5 ml microcentrifuge tube and allowed to stand for 30 min at room temperature. Then, 200 μl of acetic acid, 50 μl of the internal standard solution (TA, 5 μg/ml in acetonitrile) and 200 μl of acetonitrile were added to the alkali hydrolyzed urine. After mixing on a vortex mixer for 30 s, the sample was treated as described above.

For analysis of unconjugated M-I and M-II, a 200 μl volume of urine and 1 ml of 0.1 N hydrochloric acid were pipetted into a 10 ml glass-stoppered centrifuge tube and 6 ml of extracting solvent (n-hexane–2-propanol, 7:3, v/v) containing the internal standard (TAM, 0.2 μg/ml) was added. The sample was shaken for 10 min on a mechanical shaker and then centrifuged at about 1600 × g for 10 min. A 4 ml aliquot of the upper organic layer was transferred into a 10 ml glass-stoppered centrifuge tube and evaporated to dryness under reduced pressure. The residue was dissolved in 120 μl of the mobile phase B and a 50 μl aliquot was injected into the HPLC system. For analysis of total M-I and M-II (unconjugated and conjugated forms of M-I and M-II), a 200 μl volume of urine and 200 μl of 0.5 N sodium hydroxide were pipetted into a 10 ml glass-stoppered centrifuge tube and allowed to stand for 30 min at room temperature. Then, 800 μl of 0.25 N hydrochloric acid and 6 ml of extracting solvent (n-hexane–2-propanol, 7:3, v/v) containing the internal standard (TAM, 0.2 μg/ml) were added to the tube; the sample was shaken for 10 min on a mechanical shaker and treated as described above.

Quantitation was performed by the peak-area ratio method with an internal standard, TA for MA analysis and TAM for analysis of M-I and M-II. The concentration of each glucuronide was determined as the difference in values for total MA, M-I or M-II after alkali hydrolysis and free MA, M-I or M-II, respectively. The intra-assay coefficients of variation for MA and both the metabolites at a concentration of 0.2, 0.5 and 5.0 μg/ml were less than 8.2% both without and with alkali hydrolysis.

**RESULTS AND DISCUSSION**

It is known that the activities of drug metabolizing enzymes in preterm infants are generally extremely low at birth and gradually increase during the first year of life. The half-life of theophylline, for example, is about 30 h in preterm infants and is about 5 times longer than that in adults. The capacity for biotransformation of theophylline grows constantly due to the developing liver drug metabolizing enzyme system and attains adult values when the infant is approximately 6 to 12 months of age. We can also find many reports on the pharmacokinetics and metabolism of other drugs such as caffeine, indomethacin, phenobarbital, etc. in the neonates. However, little information is available on the metabolism of MA in preterm infants. We recently reported the pharmacokinetics of MA in preterm infants and found marked interindividual differences in half-lives and clearances of MA. To obtain further information on the metabolism of MA in preterm infants, we investigated here the excretion of MA and its metabolites in urine of the infants.

Table 2 shows the cumulative amounts of MA and its metabolites excreted in the urine obtained from the preterm infants undergoing MA therapy. The total amounts excreted in the urine varied from 7 to 46% of the total dose, and were less than those reported in adults and children. In infants 1, 3 and 5 (the first therapy), in whom the plasma half-life of MA was relatively long (more than 26 h), less than 10% of the total dose was recovered in the urine, while in infants 2, 4 and 5 (the
Table 2. Urinary Excretion of MA and Its Metabolites in Preterm Infants Undergoing MA Therapy

<table>
<thead>
<tr>
<th>Patient</th>
<th>Urine collection periods (d)</th>
<th>MA</th>
<th>M-I</th>
<th>M-II</th>
<th>MA-G</th>
<th>M-I-G</th>
<th>M-II-G</th>
<th>Total</th>
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<tr>
<td>1</td>
<td>5</td>
<td>0.2</td>
<td>0.5</td>
<td>0.5</td>
<td>2.4</td>
<td>0.4</td>
<td>7.5</td>
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<tr>
<td>2</td>
<td>5</td>
<td>1.3</td>
<td>5.0</td>
<td>3.9</td>
<td>8.1</td>
<td>18.2</td>
<td>37.5</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>6</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>4.7</td>
<td>1.9</td>
<td>6.6</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>6</td>
<td>0.3</td>
<td>0.3</td>
<td>0.1</td>
<td>2.2</td>
<td>0.9</td>
<td>4.0</td>
<td></td>
</tr>
<tr>
<td>5(1)</td>
<td>7</td>
<td>0.1</td>
<td>0.2</td>
<td>0.6</td>
<td>3.7</td>
<td>3.5</td>
<td>8.3</td>
<td></td>
</tr>
<tr>
<td>5(2)</td>
<td>7</td>
<td>0</td>
<td>0</td>
<td>0.9</td>
<td>13.7</td>
<td>1.0</td>
<td>25.8</td>
<td></td>
</tr>
</tbody>
</table>

a) Urine samples were collected for up to 5 d after the last dose.

Fig. 2. Relationship between Plasma Half-Life of MA and Cumulative Amount of MA and Its Metabolites Excreted in Urine

The regression equation calculated by the least-squares method is $y = -1.07x + 47.3$ ($a = 6, r = 0.8568, p < 0.05$), as shown by the solid line.

second therapy) in whom the half-life was relatively short (less than 18 h), 23 to 46% of the total dose was excreted. Excretion of the oxidized metabolites, M-I and M-II, and their esterglucuronides were markedly increased in the latter. As shown in Fig. 2, a significant correlation was observed between the plasma half-life of MA and the cumulative amount of MA and its metabolites excreted in the urine. These results suggest that long plasma half-lives of MA observed in preterm infants are primarily due to low activities of drug metabolizing enzymes.

In infant 5, the first dose of MA was given at 2 d after the birth, and the ductus was successfully closed after the third dose. Since the ductus reopened at 5 d after the last dose, the second MA therapy was started at 17 d after the birth. During this period (about 2 weeks), the plasma half-life of MA was shortened from 26.4 to 8.4 h (Table 1), and the urinary excretion of the MA metabolites including their glucuronides was greatly increased (Table 2). It is suggested that the activities of both cytochrome P-450(s) and glucuronyltransferase(s) related to MA metabolism rapidly increased during the first month of this infant’s life. Although further studies, such as bioavailability and biliary excretion studies of MA, are needed to obtain the full picture on MA disposition in preterm infants, these are quite difficult to perform in clinical trials. Our present data indicate, however, that a relatively rapid change in activities of drug metabolizing enzyme(s) may sometimes occur in neonates undergoing MA therapy.

Since the first report of Shimada et al. in 1984,11 the efficacy and usefulness of MA therapy in preterm infants with symptomatic PDA have been widely accepted by Japanese pediatricians.12 Although intravenous indomethacin therapy has recently been introduced in Japan for closure of PDA in preterm infants, more frequent and severe side effects than those observed in MA therapy were reported.13 For this reason, MA therapy is still being used in more than 50% of the neonatal intensive care units of children’s hospitals in Japan.14 We confirm, therefore, that not only our previous report2 but the data presented here provide useful information for effective and safe MA therapy in preterm infants with symptomatic PDA.

REFERENCES