Short Communication

In Vivo Studies on Chiral Inversion and Amino Acid Conjugation of 2-[4-(3-Methyl-2-thienyl)phenyl]propionic Acid in Rats and Dogs

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ABSTRACT:

The relationship between chiral inversion and stereoselective amino acid conjugation of a new nonsteroidal anti-inflammatory agent, R,S-2-[4-(3-methyl-2-thienyl)phenyl]propionic acid (R,S-MTPPA), was investigated in rats and dogs. Only the S-enantiomer was found in plasma after oral administration of S-MTPPA to both species. In contrast, the R- and S-enantiomers were both detected after the dosing of R-MTPPA. In rats, the area under the curve of S-MTPPA in plasma was only 9% of that of R-MTPPA when R-MTPPA was dosed, whereas in dogs it was 2.5 times larger than that of the R-enantiomer. After administration of R-MTPPA, both enantiomers appeared in the urine. In rats, a small amount of S-enantiomer was found in the urine, whereas in the case of dogs the amount of the S-enantiomer was larger than that of the R-enantiomer. It appears that R-MTPPA is chirally inverted to S-MTPPA in both rats and dogs, although the extent of chiral inversion is greater in dogs than in rats. In dogs, the taurine conjugate was detected in plasma, urine and feces as a major metabolite after oral administration of either R- or S-MTPPA. In this case, only S-MTPPA-taurine was detected in the urine after the administration of S-MTPPA, and it was also the main component after administration of R-MTPPA.

Materials and Methods

Materials. The enantiomers of R,S-MTPPA and their taurine and glycine conjugates were prepared at the Maruho Kyoto Research Laboratory (Kyoto, Japan) as described in a previous article (Konishi et al., 1998a). A common intermediate of amino acid conjugation of 2-arylpropionic acid derivatives (Hutt and Caldwell, 1990; Asami et al., 1995) in this paper, we report the relationship of chiral inversion and taurine conjugation of MTPPA after administration of R- or S-MTPPA to rats and dogs.

R,S-2-[4-(3-Methyl-2-thienyl)phenyl]propionic acid (R,S-MTPPA), a 2-arylpropionic acid derivative, is a new, orally effective, nonsteroidal anti-inflammatory agent. The S-isomer is pharmacologically active, whereas the R-isomer is inactive in vitro. The biological activities of S-MTPPA (code: M-5011) in animal models have been reported (Murakami et al., 1996; Kataoka et al., 1997; Tobetto et al., 1997; Kido et al., 1998). In our previous article, the disposition of S-MTPPA was studied after oral administration to rats, dogs, and monkeys using the 1H-labeled drug. It was confirmed that the drug was metabolized mainly by oxidation of the thiophenyl moiety in these animals and by glucuronidation of the carboxyl group in rats and monkeys. In contrast, a major urinary and fecal metabolite in dogs was identified as the taurine conjugate of R,S-MTPPA (R,S-MTPPA-CoA) by means of isolation followed by mass spectrometry and 1H NMR analyses (Konishi et al., 1998a).

It is well known that enantiomers of 2-arylpropionic acid derivatives undergo chiral inversion from R- to S-isomer (Yamaguchi and Nakamura, 1987; Caldwell et al., 1988; Baillie et al., 1989; Shirley et al., 1995). This chiral inversion is general, although variations exist among species and drugs (Lee et al., 1985; Mayer et al., 1988; Muller et al., 1990). The R- and S-enantiomers of 2-arylpropionic acid derivatives are known to have different pharmaceutical activities. For example, the inflammatory activities of the S-enantiomers of ibuprofen, naproxen, carprofen, and fenoprofen are stronger than those of the R-enantiomers in vitro (Gaut et al., 1975; Adams et al., 1976; Buttinoni et al., 1983; Kean et al., 1989). Therefore, an understanding of the extent of chiral inversion of the drugs is needed for analysis of pharmaceutical activity and toxicity. The mechanism of chiral inversion of 2-arylpropionic acid derivatives is thought to involve the CoA thioester as an intermediate (Nakamura et al., 1981). The CoA thioester is also a common intermediate of amino acid conjugation of 2-arylpropionic acid derivatives (Hutt and Caldwell, 1990; Asami et al., 1995). In this paper, we report the relationship of chiral inversion and taurine conjugation of MTPPA after administration of R- or S-MTPPA to rats and dogs.

Abbreviations used are: MTPPA, 2-[4-(3-methyl-2-thienyl)phenyl]propionic acid; MTPPA-TAU, 2-[4-(3-methyl-2-thienyl)phenyl]propionic acid taurine conjugate; MTPPA-CoA, 2-[4-(3-methyl-2-thienyl)phenyl]propionic acid CoA thioester; HPLC, high-performance liquid chromatography; AUC, area under the curve.

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Centrations of unchanged enantiomer after administration of R-enantiomer which corresponds to 2.5 times that of the S-enantiomer. About 27% of unchanged R,S-MTPPA was 88.5 mg/ml. In this case, AUC of S-MTPPA was 397.4 g/ml. In contrast, after administration of S-MTPPA, the S-enantiomer was detected in a small amount in an amount corresponding to 0.6% of the dose. The glycine conjugate was excreted after administration of S-MTPPA, whereas no R-MTPPA was detected in urine after dosing of S-MTPPA. When R-MTPPA was administered to dogs, the S-enantiomer (0.6%) was excreted in urine, and the amount was 0.7% of the dose of the respective excretion of unchanged S-enantiomer after administration of S-MTPPA was five times that of unchanged R-enantiomer after administration of R-MTPPA. The excretion of the S-enantiomer reached a peak, which corresponds to 2.5 times that of the R-enantiomer.

**Results and Discussion**

**Plasma Concentration of R- and S-MTPPA in Rats and Dogs.** Only the S-enantiomer was detected in plasma after oral administration of S-MTPPA to male rats. The area under the curve (AUC) of S-MTPPA was 88.5 μg·h/ml. In contrast, after administration of R-MTPPA, the S-enantiomer was detected in a small amount in addition to the unchanged R-enantiomer. The AUC of S-MTPPA after dosing of R-MTPPA was 5.1 μg·h/ml, which corresponds to 9% of that of unchanged R-MPTTA (56.0 μg·h/ml). When the plasma concentrations of unchanged enantiomer after administration of R- and S-MTPPA were compared, the $C_{\text{max}}$ values were almost the same at about 27 μg/ml. However, the $T_{1/2}$ values were different, being 1.3 and 2.0 h, respectively (Fig. 1). The plasma concentrations of R- and S-enantiomers after oral administration of R- or S-MTPPA to male dogs are also shown in Fig. 1. Only the unchanged S-enantiomer was detected at each time interval after dosing of S-MTPPA. In contrast, a large amount of the S-enantiomer was detected in plasma after administration of R-MTPPA. The plasma concentration of the S-enantiomer at 8 h after dosing of R-MTPPA reached a peak, $C_{\text{max}}$, of 14.5 μg/ml. In this case, AUC of S-MTPPA was 397.4 μg·h/ml, which corresponds to 2.5 times that of the R-enantiomer.

**Urinary Excretion of R- and S-MTPPA in Rats and Dogs.** The S- and R-enantiomer were both excreted in urine until 24 h after oral administration of S-MTPPA to male rats. The urinary excretion of the S-enantiomer was 0.07% and 3.2% of the dose, respectively. After administration of R-MTPPA, R- and S-enantiomer was also detected in the urine, and the amount was 0.7% and 0.2% of the dose, respectively. The excretion of unchanged S-enantiomer after administration of S-MTPPA was five times that of unchanged R-enantiomer after administration of R-MTPPA. The excretion of the S-enantiomer was 0.7% of the dose, but there was no R-enantiomer in dog urine after administration of S-MTPPA. When R-MTPPA was administered to dogs, the S-enantiomer (0.6%) was excreted in urine, in a higher amount than the unchanged R-enantiomer (0.2%).

It was detected S-MTPPA in plasma and urine after oral administration of R-MTPPA to rats. Conversely, a small amount of the R-MTPPA was detected in urine after dosing of S-MTPPA. The result shows that the inversion of S- to R-MTPPA also occurs in rats to a small extent, besides the R- to S-inversion. Chiral inversion of R-MTPPA to the S-enantiomer seems to be faster in dogs than in rats. Tanaka et al. (1992) reported that both R- to S- and S- to R-inversion of 2-phenylpropionic acid takes place in dogs. In this study, MTPPA was confirmed to show R- to S-inversion in dogs.

**Amino Acid Conjugates of R,S-MTPPA.** Glycine and taurine conjugates were assayed in urine and feces after administration of R- or S-MTPPA to rats and dogs. The taurine conjugate of R,S-MTPPA was detected in dog urine and feces after administration of S-MTPPA in amounts corresponding to 10.9 and 13.5% of the dose, respectively (Table 1). The taurine conjugate was also detected in dog urine and feces after administration of R-MTPPA in similar amounts to those found after dosing of S-MTPPA. In the urine of only one rat, the taurine conjugate was excreted after administration of R-MTPPA, in an amount corresponding to 0.6% of the dose. The glycine conjugate was not detected in any of the samples from rats or dogs (data not shown).

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**Fig. 1. Plasma concentrations of S-MTPPA and R-MTPPA after oral administration of 10 mg/kg S-MTPPA (A and C) or R-MTPPA (B and D) to male rats (A and B) and male dogs (C and D).** Each value represents the mean ± S.D. of five rats or three dogs.
TABLE 1

Excretion of taurine conjugate of R,S-MTPPA in urine and feces for 48 h after oral administration of S-MTPPA or R-MTPPA at a dose of 10 mg/kg to male rats and dogs

<table>
<thead>
<tr>
<th>Species</th>
<th>Administered Compound</th>
<th>Excretion of Taurine Conjugate (as % of dose)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Urine</td>
</tr>
<tr>
<td>Dog</td>
<td>S-MTPPA</td>
<td>10.9 ± 1.9</td>
</tr>
<tr>
<td></td>
<td>R-MTPPA</td>
<td>13.5 ± 1.8</td>
</tr>
<tr>
<td>Rat</td>
<td>S-MTPPA</td>
<td>ND*</td>
</tr>
<tr>
<td></td>
<td>R-MTPPA</td>
<td>0.6±</td>
</tr>
</tbody>
</table>

Values are the mean ± S.D. of three animals.
* ND, not detected.
† Value for one animal.

The plasma concentration of R,S-MTPPA-TAU after administration of each enantiomer of MTPPA to male dogs is shown in Fig. 2. The plasma concentrations after administration of either R- or S-MTPPA peaked at 8 h after administration. The C_{max} of R,S-MTPPA-TAU after the administration of R-MTPPA was 5.41 μg/ml higher than that after the administration of S-MTPPA (0.97 μg/ml).

Stereoselectivity of taurine conjugation of R,S-MTPPA in dogs was investigated by chiral HPLC separation of the enantiomers of R,S-MTPPA-TAU. After administration of S-MTPPA, only the R-enantiomer of the taurine conjugate (S-MTPPA-TAU) was detected in dog urine for 48 h, in an amount corresponding to 10.3 ± 2.7% (mean ± S.D. of four experiments) of the dose. In the case of R-MTPPA administration, S-MTPPA-TAU was mainly excreted in an amount corresponding to 13.2 ± 2.0% of the dose, and the R-enantiomer of the conjugate was also detected in a small amount (0.5 ± 0.3%).

It was considered that R- and S-MTPPA are each transformed to the thioester, R- and S-MTPPA-CoA, which are then interconverted by epimerase as described for ibuprofen-CoA (Shieh and Chen, 1993). This may be followed by hydrolysis to R- or S-MTPPA or by conjugation with taurine to afford R- or S-MTPPA-TAU, respectively. It appears that the S-enantiomer does not convert to the S-enantiomer-CoA ester in rats, so presumably the S-enantiomer is not a suitable substrate for rat acyl-CoA synthetase (Fournel and Caldwell, 1986). However, in dogs, S-MTPPA is converted to S-MTPPA-CoA, as indicated by the appearance of S-MTPPA-TAU in the urine after administration of S-MTPPA. R-MTPPA-CoA was considered to be converted to R-MTPPA-TAU to a small extent and S-MTPPA was converted to S-MTPPA-TAU mainly via the S-MTPPA-CoA, because S-MTPPA-TAU was found mainly in the urine after administration of R- or S-MTPPA to dogs. Taurine N-acyl transferase may be selective for S-enantiomer-CoA, rather than R-enantiomer-CoA.

References


