Stereoselective Disposition of Pranoprofen, a Nonsteroidal Antiinflammatory Drug, in Rabbits

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The stereoselective disposition of pranoprofen, a nonsteroidal antiinflammatory drug, was studied in rabbits. Plasma levels of S(+)-pranoprofen after oral and i.v. administration of the racemate pranoprofen were always higher than those of the R(-)-isomer. The elimination rate constant of the R(-)-isomer (2.74 h⁻¹), calculated using a 2-compartment model, was significantly larger than that of the S(+)-isomer (2.14 h⁻¹), while no significant difference was observed in the absorption rate constants between enantiomers. Pranoprofen was excreted in the urine exclusively in the form of pranoprofen glucuronide. The glucuronide of R(-)-isomer was excreted more rapidly than the S(+)-isomer. However, no metabolite of pranoprofen was detected in plasma, owing to its instability in liver and plasma. Moreover, the elimination of the S(+)- and R(-)-isomers of pranoprofen was more rapid when the enantiomers were separately administered than when administered as the racemate. The R(-)-isomer showed a significantly higher elimination rate than the S(+)-isomer, compared to what was observed upon the administration of the racemate. No inversion to an R(-)-form or S(+)-form to their corresponding antipode after administration of the isomers separately was detected. Pranoprofen, especially the R(-)-isomer, was significantly distributed in the kidney. An in vitro metabolism experiment of pranoprofen showed the predominant glucuronidation to be in the kidney rather than in the liver because of rapid hydrolysis of glucuronide in the liver. The glucuronidation of R(-)-isomer was 4-fold faster than S(+)-isomer in the kidney, although the hydrolysis from glucuronide to pranoprofen in the kidney was faster for R(-)-isomer than S(+)-isomer. It was concluded from the present limited data that conjugation of the R(-)-isomer depended predominantly upon its higher free plasma level and higher metabolic rate and it thus was rapidly excreted from the kidney. This describes the stereoselectivity in the elimination process of pranoprofen which might be responsible for a significant difference in plasma concentration between S(+)- and R(-)-pranoprofen.

Keywords pranoprofen; stereoselectivity; disposition; nonsteroidal antiinflammatory drug; HPLC.

Introduction

Many 2-arylpropionic acid derivatives, including ibuprofen and ketoprofen, are widely used as nonsteroidal antiinflammatory drugs (NSAIDs) in racemic form. However, for the last few decades, it has been demonstrated that the pharmacological activity and pharmacokinetic behavior are different for the optical isomers.¹,² Among these compounds, ibuprofen and fenoprofen are reported to undergo significant unidirectional conversion from an R(-)-isomer to the S(+)-isomer, the active form of the drug, in humans.³,⁴ Therefore, the investigation of the isomeric disposition of these drugs is of substantial significance. Studies on these subjects have recently received attention from pharmaceutical and pharmacological scientists.⁵⁻⁸

Pranoprofen, (2-5H-[1]benzopyran[2,3-b]pyridine-7-yl)propionic acid, is a derivative of propionic acid and is used as a racemic mixture of S(+)- and R(-)-pranoprofen.⁹ Although the pharmacological activity of the S(+)-isomer of pranoprofen is shown to be 3-fold higher than that of the R(-)-isomer on the inflammatory response of rat carrageenan edema,¹⁰ the stereoselective disposition of the drug has not yet been clarified.

So, the present work was undertaken with a view to study the stereoselective disposition of pranoprofen. Rabbits were used as experimental animals since pranoprofen, as shown in Fig. 1, exclusively in the form of pranoprofen glucuronide, is excreted in the urine of both rabbits and humans, and follows a less complicated metabolic pathway.¹¹ Moreover, it is easy to collect blood and urine samples. In order to elucidate the disposition of the pranoprofen optical isomers, the time-course of plasma concentration of the drug after oral and i.v. administration of RS(±)-pranoprofen was first determined through a chiral-stationary-phase HPLC column. Moreover, the pharmacokinetic characteristics after the administration of each isomer were examined and compared with the disposition of the corresponding isomer after the administration of the racemate pranoprofen.

Materials and Methods

Animals Male rabbits (Japanese white, 2.5–3.2 kg) were used in all the experiments. Rabbits were fasted for about 24 h before the experiments, but water was available ad libitum.

Chemicals Racemic pranoprofen and enantiomers of pranoprofen were kindly donated by Yoshihomi Pharmaceutical Industries (Fukuoka, Japan). The optical purities of S(+) and R(-)-isomers were 98.4 and 97.9%, respectively. Pranoprofen glucuronide was collected and purified from the urine of healthy volunteers after oral administration of 75 mg of racemic pranoprofen according to the method of Arima.¹² Uridine 5'-

Fig. 1. Structural Formulae of Pranoprofen and Its Conjugate with Glucuronic Acid

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diphasophogluconic acid trisodium salt was purchased from Nacalai Tesque (Kyoto, Japan). All other chemicals and solvents were of an analytical grade.

**In Vivo Experiments**

Dosing and Sample Collection: *In vivo* experiments were performed under non anesthetized. Racemic pranopron (5 mg/kg) and each enantiomer (2.5 mg/kg) were suspended in 50 ml of distilled water and administered orally to rabbits. In the case of intravenous bolus administration, **(R)**- and **(S)**-pranopron, dissolved in 0.15 M phosphate buffer (pH 7.4), were injected into the ear veins of same does used for oral administration. Blood and urine samples were then collected at appropriate time intervals. The urine sample from the bladder by using ureter cannulation was collected in a pH 3.0 citrate buffer to prevent the hydrolysis of glucuronide at a higher pH.

Sample Treatment and Assay: **(R)**- and **(S)**-pranopron in plasma and urine were determined by the HPLC method. Briefly, all blood samples collected from the ear vein were separated and the plasma (0.5 ml) was treated with 1 N NaOH for 30 min and 0.5 ml of 2 N HCl and extracted as before. In the case of urine, extraction was made by benzyne before and after hydrolysis of glucuronide under basic condition. Methyl **p**-aminobenzoate was used as the internal standard throughout the analysis.

**HPLC Conditions**

A Hitachi 655A-11 pump equipped with a fluorometric detector (Hitachi F-1000) and an HPLC system. The gradient elution was performed on a Chiracel OJ (25 cm × 4.6 cm i.d., Daicel Chemical Industries, Ltd., Tokyo, Japan) with a mobile phase composed of *n*-hexane: 2-propanol: acetic acid (70:40:1) at a flow rate of 1.0 ml/min. Detection was at excitation 250 nm and emission 330 nm.

Under these conditions, the capacity factors of **(R)**- and **(S)**- pranopron were 1.20 and 2.12, respectively. The separation factor (α) was 1.77 and the resolution factor (Res) was 2.51. Parameters were calculated as follows: capacity factor, k′ = (tR−t0)/t0; z = k′/k′S; and Res = 2(1−z)/z(wS + wR), where t0 = retention time of the unretained compound, tR = retention time of peak (with p = 1 for peak 1 and p = 2 for peak 2), and wS and wR = widths of peaks 1 and 2 in 2 min. The limit of reliable quantitation was set at 10 ng/ml and 50 ng/ml for each enantiomer in plasma and urine, respectively. The intraday and interday variations of these values were less than 5.0%.

**In Vivo Studies**

*In Situ* Intestinal Absorption Experiment: A male rabbit was anesthetized with urethane (1.2 g/kg, i.p.) and the small intestine was exposed by a midline abdominal incision. Two glass cannulae were inserted through small slits at the upper and lower jejunal portion. The intestine was flushed with saline solution maintained at 37°C. The length of the intestine used was 30 cm. The outflow and inflow glass cannulae were connected with rubber tubes to a flask containing 80 ml of racemic pranopron solution (10 mg/ml) and peroxidase reduced (5 mg/ml) in 0.15 M phosphate buffer solution (pH 7.4) and a perfusion pump. The drug solution was recirculated at the rate of 30 ml/min through the small intestine at 37°C. Aliquots were pipetted out at periodic intervals.

Determination of Plasma Protein Binding: A protein binding experiment was carried out by means of an ultrafiltration method using a membrane filter device (MPS-1, Amicon Corporation, Danvers, MA, U.S.A.) using a membrane filter with a molecular weight cut-off of 3000. Racemic pranopron was added to rabbit serum made from freshly collected blood. The spike concentrations of the drug were 10, 50, 100 and 200 mg/ml as a racemate. The serum was centrifuged at 3000 g for 10 min at room temperature. Aliquots of the serum and ultrafiltrate were used to assay the concentration of pranopron and pranopron. Adsorption of pranopron onto the membrane was negligible.

Tissue Distribution: Different tissues were collected at 45 min after i.v. administration of racemic pranopron (5 mg/kg). Each tissue was then homogenized in 5 volumes of 0.067 M of phosphate buffer (pH 7.4). One ml of homogenate was then transferred to another test tube. The sum of pranopron and pranopron glucuronide concentration was determined after hydrolysis of glucuronide by the addition of 0.5 ml of 1 N HCl for 1 h. The sample was then acidified with 0.5 ml of 2 N HCl and extracted by benzene. The total concentration of pranopron enantiomers was determined by the HPLC method as described before.

Stability of Pranopron Glucuronide: Pranopron glucuronide (20 mg) in plasma, and in the 10000 g supernatant, cytosol and microsomes of kidney and liver was incubated at 37°C. The protein concentrations of each medium without plasma was 1 mg/ml. The concentrations of pranopron enantiomers produced after hydrolysis of pranopron glucuronide were determined at appropriate time intervals by the HPLC method as described before. The pranopron and its glucuronide were determined by lectin injection into the test solution into HPLC after deproteinization with acetonitile. The assay of pranopron glucuronide was performed by the non-chiral analytical method using a LiChrosorb RP-18 column (25 × 0.4 cm i.d., Chiron-Merck Industries, Tokyo, Japan) and a Hitachi 638-41 UV monitor (275 nm) with a mobile phase composed of acetonitile:0.14% phosphoric acid (9:11, v/v) at a flow rate of 0.5 ml/min. The capacity factors of pranopron and pranopron glucuronide were 1.38 and 2.42, respectively. The separation factor (α) was 1.76 and the resolution factor (Res) was 4.56.

**In Vivo Metabolic Experiments**

Rabbits were exsanguinated from the carotid artery, and the liver and kidney were removed immediately and were homogenized in a Potter-Elvehjem homogenizer with 0.1 M Tris-HCl buffer (pH 7.4). The homogenates were centrifuged at 10000 x g for 20 min and the resulting supernatant fluids were centrifuged at 100000 x g for 60 min to obtain microsomal pellets by using a Hitachi ultracentrifuge. The microsomal pellets obtained were resuspended in 0.1 M Tris-HCl buffer at pH 7.4. All procedures were done at 0–4°C. The incubation medium contained substrate (S**(+)**), (R**(-)**) or (S**(-)**)-pranopron: final concentration 0.25–2.0 mg/ml, 5 mM uridine 5′-diphosphogluconic acid, 5 mM MgCl2, microsome (2 mg as protein) and 0.1 M Tris-HCl buffer at pH 7.4 in a final volume of 2.0 ml. The incubation was carried out at 37°C for 30 min and terminated by adding 0.5 ml of 1 N HCl. The product, pranopron glucuronide, was estimated by the non-chiral analytical HPLC method as described before.

Taking bovine serum albumin as the standard, the protein concentration of the samples was measured by the method of Lowry et al. The K**m** and V**max** values for the formation of glucuronide by kidney microsomes were determined by extrapolation of the linear portion of the Lineweaver-Burk plots.

**Statistical Analysis**

The results were analyzed statistically with paired and unpaired Student’s t-tests. The p value of 0.05 or less was considered to be significant.

**Results and Discussion**

**In Vivo Studies**

Figure 2 shows the time-course of the plasma concentrations of **(S)**- and **(R)**-pranopron after oral and i.v. administration of racemic pranopron to rabbits. With both administration routes, plasma levels of the **(S)**-isomer were higher than those of the **(R)**-isomer, particularly, the maximum plasma level (C**max**) of the **(S)**-isomer was approximately 1.7-fold higher than the **(R)**-isomer after oral administration. In addition, the area under the plasma concentration–time curve (AUC) of the **(S)**-isomer (7.0 mg·h/ml) was approximately 1.6-fold larger than that of the **(R)**-isomer (4.1 mg·h/ml). However, no significant difference in the peak concentration time (T**max**) was obtained for the enantiomers. The pharmacokinetic parameters were simultaneously calculated from the i.v. and p.o. data in Fig. 2 by the two-compartment model using a nonlinear least squares program (MULTI) and are listed in Table I. The elimination rate constant (k**e**) and the total body clearance (C**L**) of the **(R)**-isomer were significantly greater than those of the **(S)**-isomer, indicating that the elimination of the **(R)**-isomer from blood was significantly more rapid. However, there was no significant difference between the absorption rate constants of the two enantiomers. These data suggested that the different plasma levels observed for the enantiomers might be due to the difference in their elimination process. The different elimination processes might be produced by the interaction.
between enantiomers during the elimination process and/or the inversion from \( R(-) \)-isomer to \( S(+) \)-isomer. Therefore, the mean plasma levels of the isomers following the oral and i.v. administration of each enantiomer were compared to those obtained after the administration of racemic pranoprofen by the same routes. The results are shown in Fig. 3, and pharmacokinetic data are given in Table I. When one of the isomers was administered, the other isomer was detected to be equivalent to the impurity of the former isomer in either plasma or urine. The \( R(-) \)-enantiomer and the \( S(+) \)-enantiomer contained 2.1% of the \( S(+) \)-form and 1.6% of the \( R(-) \)-form, respectively. Therefore, unlike the case of 2-phenylpropionic acid and fenoprofen, the chiral inversion of pranoprofen enantiomers was not considered in rabbits. As shown in Table I, greater \( k_e \) and \( CL_{\text{tot}} \) and smaller \( AUC \) values were obtained for the enantiomers after administration of each enantiomer separately compared to those of racemate administration. These data indicate that each of the isomers was eliminated rapidly when the isomers were administered separately. For example, in case of the separate administration of the isomers, elimination rate constants of 2.23 and 5.96 h\(^{-1}\) were obtained for the \( S(+) \)- and \( R(-) \)-isomers, respectively. There was a significant difference in the elimination rate constant of the \( R(-) \)-isomer, while this difference in the case of the \( S(+) \)-isomer was not significant. The comparatively slow elimination of the isomers after racemate administration can be explained by the fact that the interaction of the \( S(+) \)- and \( R(-) \)-isomers in one or multiple processes of absorption, distribution, metabolism and excretion might result in suppression of the elimination of both isomers.
It has been reported that pranoprofen is conjugated with glucuronic acid and is exclusively excreted in the urine.\(^{11}\) So, we investigated whether urine excretion reflected the rapid elimination of \(R(-)-\)pranoprofen from the blood which was observed after i.v. administration of racemic pranoprofen. The results are shown in Fig. 4. Pranoprofen glucuronide was found to be excreted rapidly, and approximately 90% of both optical isomers were excreted in the urine 10 h past administration. At 30 min past administration, 43.9% of the \(R(-)-\)isomer and 19.9% of the \(S(+)-\)isomer were excreted, indicating that the \(R(-)-\)isomer was excreted more rapidly than the \(S(+)-\)isomer at the beginning of excretion. Moreover, pranoprofen, after oral administration, was excreted as glucuronides of 41.4% of the \(R(-)-\)isomer and 38.9% of the \(S(+)-\)isomer of the dose up to 6 h past administration, which was in good agreement with the bioavailability estimated from the \(AUC\) of plasma concentration. These results suggested that pranoprofen in the blood was rapidly transferred to the liver and kidney, where it was metabolized and rapidly excreted in the conjugated form.

In spite of the excretion of pranoprofen in the form of its glucuronide, no glucuronide of pranoprofen was detected in plasma. These apparently conflicting results can be explained on the basis of the high instability of pranoprofen glucuronide in plasma and liver (see Table IV), as has occurred with other NSAIDs such as zomepirac glucuronide.\(^{17}\)

**TABLE II. In Vitro Binding of \(S(+)-\) and \(R(-)-\) Pranoprofen to Rabbit Serum by Ultrafiltration**

<table>
<thead>
<tr>
<th>Racemate concn. (μg/ml)</th>
<th>% bound</th>
</tr>
</thead>
<tbody>
<tr>
<td>(S(+)-) Pranoprofen</td>
<td>(R(-)-) Pranoprofen</td>
</tr>
<tr>
<td>10</td>
<td>99.6±0.04</td>
</tr>
<tr>
<td>50</td>
<td>99.3±0.05</td>
</tr>
<tr>
<td>100</td>
<td>99.1±0.07</td>
</tr>
<tr>
<td>200</td>
<td>97.8±0.02</td>
</tr>
</tbody>
</table>

Values represent the mean±S.E. \(a\) \(p<0.01\) versus \(S(+)-\) pranoprofen. \(b\) \(p<0.001\) versus \(S(+)-\) pranoprofen.

**TABLE III. Tissue Distribution of \(S(+)-\) and \(R(-)-\) Pranoprofen at 45 min after Intravenous Bolus Administration of 5 mg/kg of Racemic Drug to Rabbits \((n=3)\)**

<table>
<thead>
<tr>
<th>Tissue</th>
<th>(S(+)-) Pranoprofen</th>
<th>(R(-)-) Pranoprofen</th>
</tr>
</thead>
<tbody>
<tr>
<td>Liver</td>
<td>1.15±0.14 (\pm 0.01)</td>
<td>0.81±0.09(^{a}) (\pm 0.01)</td>
</tr>
<tr>
<td>Kidney</td>
<td>16.24±2.51 (\pm 0.17)</td>
<td>30.24±4.25(^{a}) (\pm 0.60)</td>
</tr>
<tr>
<td>Heart</td>
<td>2.37±0.12 (\pm 0.01)</td>
<td>1.45±0.17(^{a}) (\pm 0.03)</td>
</tr>
<tr>
<td>Spleen</td>
<td>2.03±0.16 (\pm 0.06)</td>
<td>1.57±0.06(^{a}) (\pm 0.03)</td>
</tr>
<tr>
<td>Lung</td>
<td>2.09±0.25 (\pm 0.01)</td>
<td>1.53±0.25(^{a}) (\pm 0.03)</td>
</tr>
<tr>
<td>Muscle</td>
<td>0.60±0.02 (\pm 0.004)</td>
<td>0.57±0.01(^{a}) (\pm 0.009)</td>
</tr>
<tr>
<td>Plasma</td>
<td>8.11±0.57</td>
<td>4.86±0.37(^{a})</td>
</tr>
</tbody>
</table>

Values represent the mean±S.E. The values in parentheses were divided by plasma concentration. \(a\) \(p<0.05\) versus \(S(+)-\) pranoprofen. \(b\) \(p<0.01\) versus \(S(+)-\) pranoprofen. \(c\) \(\mu g/ml\).

**In Vitro Studies** In situ and in vitro experiments on the stereoselectivity of pranoprofen in absorption, metabolism and excretion were carried out to investigate the disposition of the pranoprofen optical isomers in detail.

Figure 5 shows the results of in situ perfused intestine experiments to examine the differences between the absorption properties of the \(R(-)-\) and \(S(+)-\)isomers. The absorption rate constant for the \(S(+)-\)isomer (0.61±0.05 h\(^{-1}\)) was also found to be slightly larger than that of the \(R(-)-\)isomer (0.55±0.05 h\(^{-1}\)). However, it is probably difficult to explain the difference in plasma concentrations between the two isomers on the basis of small differences in absorption rates. In fact, there was no significant difference between the absorption rate constants \(k_a\) of the isomers in vivo, as shown in Table I, though the absorption rate constants between in situ and in vivo were different because of different experimental conditions. Therefore, the difference in plasma levels can be explained on the basis of differences in the elimination process.

The binding of pranoprofen to rabbit serum was determined by ultrafiltration to investigate the difference between the elimination rates of the enantiomers. The results are shown in Table II. At any concentration, \(S(+)-\) and \(R(-)-\)pranoprofen were strongly bound to serum protein and the binding percentage of the \(S(+)-\)isomer was significantly higher as compared to the \(R(-)-\)form. This result is in close agreement with the previous finding reported by us using human serum albumin,\(^{18}\) indicating that the \(R(-)-\)isomer had the advantage of
greater distribution than the $S(\cdot)$-isomer. So, we determined tissue concentrations of pranoprofen enantiomers including glucuronide at 45 min after i.v. administration of racemic pranoprofen. The concentrations of the sum of pranoprofen and glucuronide are shown in Table III.

Pranoprofen was mainly found to be distributed in the kidney at a remarkably high concentration. The greater distribution of the $R(\cdot)$-isomer, in spite of its lower protein binding than the $S(\cdot)$-isomer, was observed only in the kidney. On the other hand, the distribution in other tissues was greater for the $S(\cdot)$-isomer than the $R(\cdot)$-isomer. However, the normalized values obtained from dividing the tissue pranoprofen concentration by plasma concentration were always greater in the $R(\cdot)$-isomer than the $S(\cdot)$-isomer. The results are in good agreement with the data of the protein binding experiment. Although the ratio of conjugated to unchanged pranoprofen was not determined in this experiment, pranoprofen can be considered to be present in the kidney predominantly in the pranoprofen glucuronide form, as all pranoprofen was found to be excreted in the glucuronide conjugate form. In contrast, pranoprofen concentrations in the liver were significantly low. Very high distribution pranoprofen in the kidney might indicate that the kidney was the main tissue for metabolism as well as for excretion for this drug. The total amount of drug in the liver was estimated to be one-third to one-fifth that present in the kidney, suggesting that the liver might also take part in the metabolism of pranoprofen. In spite of the relatively large distribution of pranoprofen in the liver, the glucuronide was absent in the plasma after the administration of pranoprofen. So, the hydrolysis of pranoprofen glucuronide was examined in plasma, liver and kidney.

The hydrolysis rates of each enantiomer are listed in Table IV. The hydrolysis rate of $RS(\pm)$-pranoprofen conjugate was found to be increased in the following order, plasma $\geq$ liver homogenate $> \text{kidney homogenate}$. The glucuronide was mainly hydrolyzed in the microsome fraction of the liver and kidney. Furthermore, $R(\cdot)$-pranoprofen glucuronide was more rapidly hydrolyzed than $S(\cdot)$-pranoprofen glucuronide in all media. These data supported the finding that the glucuronide produced in the liver was rapidly hydrolyzed, resulting in the absence of pranoprofen glucuronide in plasma. However, the rapid hydrolysis of $R(\cdot)$-pranoprofen glucuronide was in conflict with its fast excretion in comparison with $S(\cdot)$-pranoprofen glucuronide.

The in vitro metabolism of pranoprofen was examined using liver and kidney microsomes. The activity of microsomes in the liver was markedly lower than in the kidney, as shown in Fig. 6. However, in general, the major organ for glucuronidation is the liver. This discrepancy can be explained on the basis of hydrolysis of pranoprofen glucuronide in a reaction medium, as shown in Table IV. Especially in the case of liver, not only was the formation of the pranoprofen glucuronide rapid, but also the hydrolysis of the produced pranoprofen glucuronide in the reaction medium was very fast. In contrast, because of the slow rate of hydrolysis in the kidney, it was considered that the pranoprofen glucuronide produced in the kidney was little hydrolyzed before its excretion in urine. These data suggested that the contribution of the liver to metabolizing pranoprofen was quite low and therefore the kidney was the major organ for metabolizing pranoprofen.

Steroselective glucuronidation of pranoprofen in the kidney was studied and the results are shown in Table V. The order of $V_{\text{max}}/K_{m}$ values for racemate and isomers of pranoprofen in the kidney was $R(\cdot) > RS(\cdot) > S(\cdot)$-pranoprofen. Although the $K_{m}$ value of the $R(\cdot)$-isomer was slightly higher than the $S(\cdot)$-isomer, the $V_{\text{max}}$ of the $R(\cdot)$-isomer was 6-fold higher than the $S(\cdot)$-isomer. The difference of $V_{\text{max}}/K_{m}$ values between the two enantiomers resulted from the difference of $V_{\text{max}}$ values in the kidney. Therefore, the $R(\cdot)$-isomer with lower protein binding than the $S(\cdot)$-isomer was immediately distributed in the kidney, and rapidly metabolized to the glucuronide and then excreted directly in the urine. These data indicated that the rapid elimination of $R(\cdot)$-pranoprofen can be explained by its rapid metabolism in the kidney, followed by direct excretion in the urine.

The rapid elimination of the $R(\cdot)$-isomer after the

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**Table IV. The Apparent First Order Degradation Half-Life of Pranoprofen Glucuronide at 37°C**

<table>
<thead>
<tr>
<th>Solvent</th>
<th>$S(\cdot)$-Pranoprofen</th>
<th>$R(\cdot)$-Pranoprofen</th>
<th>Half-life (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Liver</td>
<td>65.4</td>
<td>25.5</td>
<td></td>
</tr>
<tr>
<td>10000 x g supernatant</td>
<td>258.8</td>
<td>122.7</td>
<td></td>
</tr>
<tr>
<td>Cytosol</td>
<td>24.8</td>
<td>15.7</td>
<td></td>
</tr>
<tr>
<td>Kidney</td>
<td>233.7</td>
<td>183.1</td>
<td></td>
</tr>
<tr>
<td>10000 x g supernatant</td>
<td>394.0</td>
<td>242.6</td>
<td></td>
</tr>
<tr>
<td>Cytosol</td>
<td>141.0</td>
<td>126.5</td>
<td></td>
</tr>
<tr>
<td>Microsome</td>
<td>29.3</td>
<td>21.8</td>
<td></td>
</tr>
<tr>
<td>Plasma</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Concentration of plasma was 58.7 mg/ml and others were 1 mg/ml.

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**Table V. Apparent Kinetic Parameters of Pranoprofen Glucuronide Formation in Rabbit Kidney Microsomes**

<table>
<thead>
<tr>
<th>$S(\cdot)$</th>
<th>$RS(\cdot)$</th>
<th>$R(\cdot)$</th>
</tr>
</thead>
<tbody>
<tr>
<td>$V_{\text{max}}$</td>
<td>7.86</td>
<td>17.35</td>
</tr>
<tr>
<td>$K_{m}$</td>
<td>0.086</td>
<td>0.095</td>
</tr>
<tr>
<td>$V_{\text{max}}/K_{m}$</td>
<td>91.40</td>
<td>182.6</td>
</tr>
</tbody>
</table>

$V_{\text{max}}$ (nmol/min/mg protein), $K_{m}$ (nm).

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![Fig. 6. The Activity of Glucuronide Formation of $RS(\pm)$-Pranoprofen in the Liver and Kidney Microsomes](image_url)
administration of each isomer separately can be explained in the following way. Because of the high affinity and low $V_{\text{max}}$ value, the activity in forming pranoprofen glucuronide for the $S(+)$-isomer was lower than that of the $R(-)$-isomer. When the racemic pranoprofen was administered, the metabolic enzyme was predominantly and strongly occupied by the $S(+)$-isomer, preventing the $R(-)$-isomer from occupying the metabolic enzyme, and thus causing the reduction of the metabolic rate of the $R(-)$-isomer. However, when the $R(-)$-isomer was administered, the $R(-)$-isomer was able to occupy the metabolic enzyme freely, which caused enhanced metabolism of the isomer, and thus the elimination was fast. The metabolism of the $S(+)$-isomer was little affected upon the addition of the $R(-)$-isomer to the $S(+)$-isomer. This finding is supported by the previous results reported by Okazaki et al., where $R(+)$-ofloxacin was shown to competitively inhibit the $S(-)$-ofloxacin glucuronidation in vitro using rat liver microsomes.\(^{19}\)

In conclusion, the disposition of pranoprofen enantiomers in rabbits was found to be different, especially with respect to the elimination process. This can be explained on the basis of differences obtained in protein binding, metabolism, and excretion of these two enantiomers. The $R(-)$-isomer was predominantly conjugated depending upon its higher free plasma level and higher metabolic rate than the $S(+)$-form, and thus was rapidly excreted from the kidney in the form of pranoprofen glucuronide.

References