STEREOSELECTIVE DISPOSITION OF PROPRANOLOL IN RABBITS

Role of Presystemic Organs and Dose

JEAN-FRANÇOIS MARIER, VINCENT PICHETTE, AND PATRICK DU SOUICH

Department of Pharmacology, Faculty of Medicine, University of Montréal

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ABSTRACT:

The kinetics of propranolol enantiomers are stereoselective when high doses of the racemic drug are given po. To document whether the dose and/or the route of administration determines the stereoselective kinetics of propranolol enantiomers, conscious rabbits received 40, 80, or 120 mg/kg po or 0.5 or 10 mg/kg iv doses of racemic propranolol, and serial blood samples were obtained to assay propranolol enantiomers. At low po and iv doses, the kinetics of the propranolol enantiomers were identical. After the 120 mg/kg po dose, the kinetics of the enantiomers were stereoselective, i.e., the AUC0\textsubscript{t−∞} for (S)-(−)-propranolol was greater than the AUC0\textsubscript{t−∞} for (R)-(+) propranolol (p < 0.05). The iv injection of 10 mg/kg generated zero-order kinetics, and (S)-(−)-propranolol was eliminated faster than the antipode. Propranolol enantiomer plasma protein binding was not stereoselective. In vitro, after the incubation of 5.8 or 58 μM (RS)-propranolol with cells of the intestinal mucosa or the liver, (R)-(+) propranolol was more rapidly metabolized than (S)-(−)-propranolol at both concentrations in the intestine and at the higher concentration in the liver. Incubation of the individual enantiomers (2.9 and 29 μM) showed that in the intestine the intrinsic clearance of (R)-(+) propranolol was greater than that of (S)-(−)-propranolol but in the liver there was preferential saturation of (S)-(−)-propranolol clearance. In conclusion, at low po or iv doses the kinetics of (RS)-propranolol are not stereoselective because the liver overshadows the effect of the intestine, and at high po doses the kinetics of propranolol enantiomers are stereoselective because of hepatic saturation of (S)-(−)-propranolol clearance.

Propranolol is a nonspecific β-blocker used, as a racemic mixture of (S)-(−) and (R)-(+) propranolol, for the treatment of hypertension, angina, and cardiac arrhythmias (Ridell et al., 1987). The pharmacodynamic profiles of the propranolol enantiomers present some striking differences; for instance, (S)-(−)-propranolol is approximately 100 times more potent as a β-blocker than its antipode. On the other hand, both (S)-(−)- and (R)-(+) propranolol exert class II antiarrhythmic activity (Barrett and Cullum, 1968; Rahn, 1983; Stoschitzky and Lindner, 1990). The kinetics of propranolol enantiomers are also stereoselective (Olanoff et al., 1986; Lalonde et al., 1988; Stoschitzky et al., 1992; Egginger et al., 1994; Walle et al., 1983a) and, as a result, plasma concentrations of (S)-(−)-propranolol are higher than those of the antipode, i.e., the ratio of (S)-(−)-propranolol to (R)-(+) propranolol AUC0\textsubscript{t−∞} values is >1. The mechanism underlying the stereoselective elimination of propranolol enantiomers remains poorly understood. Stereoselective elimination appears to be influenced by the route of administration of propranolol. For instance, after the iv injection of 5 mg of racemic propranolol the kinetics of the propranolol enantiomers were almost identical, but when 40 mg were given po the ratio of (S)-(−)-propranolol to (R)-(+) propranolol AUC0\textsubscript{t−∞} values averaged 1.48 (Von Bahr et al., 1982). Olanoff et al. (1984) reported that, after the iv administration of a 0.1 mg/kg dose of a racemate of propranolol, the average ratio of (S)-(−)-propranolol to (R)-(+) propranolol AUC0\textsubscript{t−∞} values was 1.17. On the other hand, when racemic propranolol was administered po, in single doses ranging from 40 mg to 160 mg or in multiple doses to steady state, the average ratio of (S)-(−)-propranolol to (R)-(+) propranolol AUC0\textsubscript{t−∞} values ranged from 1.32 to 1.77 (Olanoff et al., 1986; Lalonde et al., 1988; Stoschitzky et al., 1992; Egginger et al., 1994). These data suggest that, in addition to route dependence, the stereoselective elimination of propranolol enantiomers appears to be modulated by the dose of racemic propranolol administered.

The apparent route- and dose-dependent stereoselective kinetics of propranolol enantiomers could be associated with stereoselective presystemic metabolism in the epithelial cells of the intestine and/or in the liver (Krishna and Klotz, 1994; du Souich et al., 1995). This hypothesis is based on the fact that propranolol is subjected to important first-pass metabolism that takes place in these two organs (du Souich et al., 1995). The present study aimed to document in vivo and in vitro whether the intestinal mucosa and/or the liver is responsible for the stereoselective kinetics of propranolol enantiomers. The effects of increasing doses of propranolol on the stereoselective kinetics of its enantiomers were also assessed.

Materials and Methods

In Vivo Studies. Pharmacokinetic studies were performed with male New Zealand rabbits weighing approximately 2.7 kg (Ferme Cunicole, Mirabel, Québec, Canada). Rabbids were selected as the animal model because the metabolism of propranolol in this species closely resembles that in humans.
(Walpole et al., 1990). The rabbits were acclimatized in their cages for at least 1 week before any experimental procedure was undertaken. The central artery and the lateral vein of an ear were cannulated with a Butterfly-25 cannula (Venisystems; Abbott Ireland, Sligo, Ireland), which contained 0.02% of heparin dissolved in a 0.9% NaCl solution to maintain patency.

Preliminary studies were undertaken to assess the lowest po dose of propranolol generating first-order kinetics in rabbits and also to determine higher doses generating zero-order kinetics while yielding plasma concentrations in the range of those seen in humans, i.e. <500 ng/ml (Genco and Green, 1986). The experiments were performed with three groups of rabbits (N = 4 or 5/group), which received by gavage 40, 80, or 120 mg/kg doses of racemic propranolol (Sigma Chemical Co., St. Louis, MO); blood samples (2–4 ml) were drawn before and 5, 10, 20, 30, 60, 90, 120, 180, 240, and 300 min after po administration. Two other groups of rabbits (N = 5/group) received iv doses of 0.5 or 10 mg/kg racemic propranolol (dissolved in saline) through the venous catheter; blood samples (2–4 ml) were drawn before and 5, 10, 20, 30, 40, 50, 60, 70, 80, and 90 min after injection. The plasma was stored at −20°C until propranolol was assayed.

**Binding of Propranolol Enantiomers to Plasma Proteins.** To rule out stereoselective differences in plasma protein binding, the binding of (S)− and (R)+ propranolol to plasma proteins was assessed by ultrafiltration. Nine samples (15 ml) of pooled plasma from rabbits were spiked with 11.25, 5.625, or 1.40 μg of the individual enantiomers (final plasma concentrations of the enantiomers, 750, 375, and 94 ng/ml, respectively) or with 22.5, 11.25, or 2.8 μg of the racemate (final plasma concentrations of propranolol, 1500, 750, and 188 ng/ml, respectively). The samples were incubated for 20 min in a shaking bath at 37°C, and 1 ml of plasma was used to determine the concentrations of propranolol enantiomers. The remaining plasma was centrifuged at 3500 rpm, in Centriplus system devices (Amicon; W.R. Grace & Co., Beverly, MA), for 45 min at 25°C. The concentrations of unbound enantiomers were assayed in 1 ml of the resulting ultrafiltrate.

**In Vitro Studies.** The in vitro studies aimed to support the in vivo results by demonstrating the ability of extrahepatic organs and liver to metabolize propranolol. Rabbits (N = 6) were sacrificed by cervical dislocation, and the first 30 cm of the intestine, the right lobe of the liver, the kidneys, and the lungs were removed. The epithelial cells of the small intestine were isolated by gentle scraping of the mucosa. The medulla was removed from the kidneys. The tissues and intestinal mucosa cells were carefully washed and diluted to 25% with 1.15% KCl/0.05 M phosphate buffer (pH 7.4) and were finally homogenized with a Polytron homogenizer (Brinkman, Rixdale, Sweden). The homogenates were centrifuged at 10,000g, and 1 ml of the supernatant was used to assess the centrifugation of these tissues to metabolize propranolol, after addition of 0.5 ml of an aqueous solution with 3.75 μg/ml of the individual enantiomers (final concentration, 2.9 μM; Sigma) or 0.5 ml of a solution with 7.5 μg/ml of the racemate (final concentration, 5.8 μM). The mixture was incubated at 37°C, and the reaction was started by addition of 1 ml of 0.05 M phosphate buffer containing NADP (1.3 μM; Sigma), glucose-6-phosphate (20 μM; Sigma), nicotinamide (100 μM; Sigma), and MgCl2 (50 μM). Identical in vitro studies were carried out with a 37.5 μg/ml of the individual enantiomers (final concentration, 29 μM) or 75 μg/ml of (RS)-propranolol (final concentration, 58 μM). The choice of propranolol concentrations was based on the fact that, after po administration, propranolol concentrations in the liver were 20–50-fold greater than those in plasma (Hayes and Cooper, 1971). Propranolol enantiomers were assayed in samples of 100 μl that were withdrawn every 1 min from liver homogenates and every 30 min from the other tissue homogenates.

**Assay of Propranolol Enantiomers.** (S)− and (R)+-Propranolol were separated by HPLC after derivatization with (R)-PEIC2 (Fluka, Switzerland), as described elsewhere (Spanh-Languth et al., 1991), with some modifications. Briefly, 100 μl of a solution containing the internal standard (50 μg/ml metoprolol in methanol), 1 ml of carbonate buffer (pH 10.5), and 10 ml of diethyl ether were added to 0.5–1.0 ml aliquots of plasma, in centrifuge tubes with Teflon-coated, sealed, screw-caps. After mixing for 20 min and centrifugation at 2000 rpm for 5 min, 9 ml of the organic phase were transferred to test tubes and evaporated under a nitrogen stream at ambient temperature. The residue was reconstituted in 200 μl of methanol with 50 μl of (R)-PEIC (0.1% in dichloromethane), and the mixture was incubated at 4°C for 20 min. After evaporation under a nitrogen stream, the residue was reconstituted in 200 μl of mobile phase and 20–80 μl were injected into the HPLC system.

The samples obtained from the in vitro studies were processed as follows. To precipitate the proteins, the 100-μl samples were mixed with 600 μl of methanol, containing the internal standard (50 μg/ml metoprolol; Sigma), and were maintained for 10 min at 4°C. After centrifugation at 2000 rpm for 5 min, 200 μl of the supernatant were transferred to another tube, and derivatization with (R)-PEIC was carried out. Proteins in the homogenates were quantified according to the technique of Lowry et al. (1951).

The separation of enantiomers was performed with a reverse-phase Inertsil ODS column (5 μm, 150 mm × 4.6 mm i.d.), using a Waters 501 HPLC pump equipped with a WISP 710B autosampler. The mobile phase consisted of methanol/water/acetic acid (75:25:0.1, v/v/v) and was delivered isocratically at a flow rate of 1.0 ml/min. Propranolol enantiomer peak areas were integrated with a Chromatopack C-RRA integrator. The excitation and emission wavelengths of a Spectroflow 980 fluorescence detector were set to 220 and 340 nm, respectively. The recoveries of (S)− and (R)+-propranolol, at concentrations of 4, 8, 16, 32, and 64 ng/ml, after extraction from plasma averaged 92.2 ± 0.4 and 92.5 ± 0.5%, respectively. Mean within-batch and interday coefficients of variability were 2.2 and 8.2%, respectively. St (+)/R (+) enantiomer peak height ratios were near unity at all times, indicating that the two enantiomers reacted with the chiral derivatizing agent at the same rates.

**Data and Statistical Analyses.** The (S)− and (R)+-propranolol AUC values were estimated using means of the trapezoidal method. Standard noncompartmental equations were used to calculate the systemic clearance, terminal t1/2, and predicted apparent volume of distribution at steady state.

Assuming that the absorption was complete, the apparent po clearance (CLp) values for (S)− and (R)+-propranolol were calculated using the equation

\[
CL_p = D_p / AUC_{\text{po}}
\]

where Dp is the po dose of the enantiomers and AUCpo is the AUCpo value for the enantiomers given po (Gibaldi and Perrier, 1982).

All results are expressed as mean ± SE. Differences between the pharmacokinetic parameters for the enantiomers in in vivo experiments were assessed using one-way analysis of variance for parallel groups, and the differences were determined using Dunnett’s table. Differences between the rate constants of elimination for the enantiomers from the racemate and the enantiomers incubated individually were assessed using paired or nonpaired t tests. The significance criterion was established at p < 0.05.

**Results**

**In Vitro Studies.** After the po administration of 40, 80, or 120 mg/kg racemic propranolol, peak plasma concentrations of both enantiomers were observed at about 20 min (fig. 1) and then declined with similar terminal t1/2 values. Compared with the 40 mg/kg dose, the po administration of 80 and 120 mg/kg doses of the racemate generated (S)− and (R)+-propranolol plasma concentrations higher than predicted. Consequently, the AUCpo values normalized to the 40, 80, and 120 mg/kg doses for (S)−-propranolol were 33.3, 65.4, and 144.2 ng min/ml/kg, and for (R)+-propranolol were 30.5, 41.1, and 86.7 ng min/kg/ml/kg, respectively.

Assuming that the whole dose of (RS)-propranolol was absorbed, the po clearance of (S)−-propranolol decreased from 17,444 ± 3,301 ml/min/kg for the 40 mg/kg dose to 9,874 ± 2,191 and 3,639 ± 489 ml/min/kg (p < 0.05) for the 80 and 120 mg/kg doses, respectively. The po clearance of (R)+-propranolol decreased from 20,859 ± 4,845 ml/min/kg for the 40 mg/kg dose to 12,077 ± 2,073 and 5,770 ± 53 ml/min/kg for the 80 and 120 mg/kg doses (p < 0.05), respectively.

After the po doses of 40 and 80 mg/kg, the AUCpo value for (S)−-propranolol did not differ from that for (R)+-propranolol. However, after the po dose of 120 mg/kg, the AUCpo value for (R)+-propranolol was greater (p < 0.05) than the AUCpo value for (S)−-propranolol.

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2 Abbreviation used is: (R)-PEIC, (R)-(-)-phenylethylisocyanate.
propranolol (table 1). The ratio of the AUC0→∞ for (S)-(−)-propranolol to the AUC0→∞ for (R)-(−)-propranolol increased ($p \leq 0.05$) as the dose was increased from 40 to 120 mg/kg (table 1).

After the iv injection of a 0.5 mg/kg dose of the racemate, plasma concentrations of (S)-(−)-propranolol and (R)-(−)-propranolol (fig. 2) were in the same range as the concentrations of propranolol enantiomers observed when the race was administered po at a dose of 40 mg/kg. The iv dose of 10 mg/kg generated plasma concentrations of (S)-(−)- and (R)-(−)-propranolol (fig. 2) higher than those observed after the po administration of a 120 mg/kg dose of the racemate. After the iv dose of 10 mg/kg, the AUC0→∞ values, corrected by the dose, for (S)-(−)- and (R)-(−)-propranolol were significantly greater than those observed after the low iv dose. Consequently, after the high dose, the systemic clearance values for the enantiomers were lower ($p < 0.05$) than those estimated after the iv injection of 0.5 mg/kg (table 2), suggesting that the kinetics of (S)-(−)- and (R)-(−)-propranolol were zero-order. At the dose of 10 mg/kg, the predicted volume of distribution at steady state for (R)-(−)-propranolol was smaller ($p < 0.05$) than that estimated after the 0.5 mg/kg dose (table 2).

At the iv dose of 0.5 mg/kg, the dispositions of propranolol enantiomers were identical, i.e. no stereoselectivity was observed (table 2). After the iv dose of 10 mg/kg, the AUC0→∞ for (S)-(−)-propranolol was smaller than that for the antipode ($p < 0.05$). The ratio of the AUC0→∞ for (S)-(−)-propranolol to the AUC0→∞ for (R)-(−)-propranolol decreased from 1.00 ± 0.01 after the 0.5 mg/kg dose to 0.82 ± 0.02 ($p < 0.05$) after the 10 mg/kg dose, because at that dose level the systemic clearance of (R)-(−)-propranolol was smaller ($p < 0.05$) than that of (S)-(−)-propranolol (table 2).

**Binding of Propranolol Enantiomers to Plasma Proteins.** The fraction of unbound (R)-(−)-propranolol in plasma (0.33 ± 0.01) was not different from that of (S)-(−)-propranolol (0.34 ± 0.01), and the increase in the plasma concentrations of propranolol enantiomers (i.e. from 93.75 to 750 ng/ml) was not associated with elevations in their unbound fractions. When the enantiomers were added as a racemic mixture, their unbound fractions increased slightly, without stereoselective differences [0.39 ± 0.02 and 0.40 ± 0.02 for (R)-(−)-propranolol and (S)-(−)-propranolol, respectively]. High concentrations of the racemic drug (i.e. 1500 ng/ml) did not modify the unbound fractions of propranolol enantiomers.

**In Vitro Studies.** Intestinal mucosa and liver homogenates, metabolized (RS)-propranolol. After incubation of the race at 5.8 μM with epithelial cells of the intestine, propranolol enantiomer concentrations were diminished by 84%: the rate constant of elimination for (R)-(−)-propranolol was faster ($p < 0.05$) than that for (S)-(−)-propranolol (fig. 3A). This difference persisted when the race was incubated at 58 μM, although the decrease in propranolol enantiomer concentrations was 45% and the rate of elimination of the enantiomers was almost 10 times slower than at the concentration of 5.8 μM. When the enantiomers were incubated individually with the intestinal mucosa supernatant, the rate constant of elimination for (R)-(−)-propranolol was almost 3-fold faster ($p < 0.05$) than that for (S)-(−)-propranolol at both concentrations (fig. 3A). The rate constant of elimination for (R)-(−)-propranolol was greater when (R)-(−)-propranolol was incubated individually than when the race was used (fig. 3A).

**Table 1. Pharmacokinetic parameters for (S)-(−)- and (R)-(−)-propranolol after the po administration of 40, 80, and 120 mg/kg doses of racem propranolol to conscious rabbits (N = 4 or 5/dose).**

<table>
<thead>
<tr>
<th>Dose (mg/kg)</th>
<th>(S)-(−)- Propranolol</th>
<th>(R)-(−)- Propranolol</th>
<th>(S)-(−)/ (R)-(−) Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>AUC0→∞ (ng · min/ml)</td>
<td>AUC0→∞ (ng · min/ml)</td>
<td>AUC0→∞ (ng · min/ml)</td>
</tr>
<tr>
<td>40</td>
<td>69 ± 5</td>
<td>69 ± 5</td>
<td>1.00 ± 0.01</td>
</tr>
<tr>
<td>80</td>
<td>63 ± 6</td>
<td>62 ± 7</td>
<td>1.02 ± 0.02</td>
</tr>
<tr>
<td>120</td>
<td>58 ± 7</td>
<td>65 ± 8</td>
<td>0.89 ± 0.05</td>
</tr>
<tr>
<td>Cmax (μg/ml)</td>
<td>13 ± 2</td>
<td>12 ± 2</td>
<td>1.04 ± 0.08</td>
</tr>
<tr>
<td>80</td>
<td>121 ± 28</td>
<td>70 ± 21</td>
<td>1.43 ± 0.23</td>
</tr>
<tr>
<td>120</td>
<td>356 ± 37</td>
<td>209 ± 29</td>
<td>1.76 ± 0.21*</td>
</tr>
</tbody>
</table>

Values are means ± SE.

* $p < 0.05$, compared with 40 mg/kg.

* $p < 0.05$, compared with 80 mg/kg.

* $p < 0.05$, compared with (S)-(−)-propranolol.

**Fig. 1. Mean plasma concentration-time curves for (S)-(−)-propranolol (●) and (R)-(−)-propranolol (○) after the po administration of 40, 80, and 120 mg/kg doses of racemic propranolol to conscious rabbits (N = 4 or 5/dose).**

**Fig. 2. Mean plasma concentration-time curves for (S)-(−)-propranolol (●) and (R)-(−)-propranolol (○) after the iv administration of 0.5 and 10 mg/kg doses of racemic propranolol to conscious rabbits (N = 5/dose).**
pranolol are route- and dose-dependent. 

suggest that the stereoselective kinetics of the enantiomers of pro-

enantiomers is stereoselective in both the intestine and the liver,

individual were significantly greater than those measured when the 

enantiomers were incubated individually at 

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R

)- (elimination for 

2

S

)- (propranolol was greater than that for 

1

R

1

)-propranolol. As observed with the intes-

tinal mucosa, the presence of (S)-( )-propranolol did not affect the 

rate constant of elimination for (S)-( )-propranolol; however, the 

presence of (S)-( )-propranolol reduced the rate constant of elimina-

tion for (R)(+)-propranolol.

Discussion

The present study demonstrates that, at low po (40 mg/kg) or iv (0.5 

mg/kg) doses of (RS)-propranolol, the kinetics of the enantiomers are 

very similar. When po doses of (RS)-propranolol are increased to 80 

and 120 mg/kg, there is a nonlinear increase in the AUC\text{0-\infty} values 

for both enantiomers. However, the increase in the AUC\text{0-\infty} value for 

(S)-( )-propranolol is greater than that for (R)(+)-propranolol, indi-

cating that propranolol enantiomers exhibit stereoselective kinetics 

after high po doses. Similar results have been reported for humans 

receiving an iv dose of 5 mg or a po dose of 40 mg, i.e. only after the 

po dose, the AUC for (S)-( )-propranolol was greater than that for 

(R)(+)-propranolol (Von Baehr et al., 1982). The iv dose of 10 mg/kg 

(RS)-propranolol generates a nonlinear increase in the AUC\text{0-\infty} values 

for both enantiomers but, in this case, the AUC\text{0-\infty} for (R)(+)- 

propranolol is greater than that for the antipode. In vitro, at 5.8 \mu M 

(RS)-propranolol, the rate of elimination of (R)(+)-propranolol is 

faster than that of (S)-( )-propranolol with epithelial cells of the 

intestine but not liver cells. At 58 \mu M, the elimination of propranolol 
enantiomers is stereoselective in both the intestine and the liver, 

although the rates of elimination are reduced. These results strongly 
suggest that the stereoselective kinetics of the enantiomers of pro-

pranolol are route- and dose-dependent.

After incubation of (RS)-propranolol at 5.8 \mu M with the liver 

10,000g supernatant, the concentrations of the enantiomers were 
decreased by 99%, with similar rate constants of elimination (fig. 3B). 
The rate constants of elimination for the enantiomers incubated indi-


cually were significantly greater than those measured when the 
racemate was used (fig. 3B), and no differences were observed be-
tween the two enantiomers. When the racemate was incubated at 58 
\mu M, the concentrations of the enantiomers were decreased by 96%, 
and the rate constants of elimination for the enantiomers were de-
creased to almost half of the values estimated at 5.8 \mu M. In addition, 

with the 58 \mu M concentration of the racemate, the rate constant of 

elimination for (R)-( )-propranolol was faster ( p < 0.05) than that 

for the antipode. When the enantiomers were incubated individually at 

29 \mu M, the rate constant of elimination for (R)(+)-propranolol 

was greater than that for (S)-( )-propranolol. As observed with the intes-

tinal mucosa, the presence of (R)(+)-propranolol did not affect the 

rate constant of elimination for (S)-( )-propranolol; however, the 

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suggest that the stereoselective kinetics of the enantiomers of pro-

pranolol are route- and dose-dependent.

Several factors, such as plasma protein binding and blood flow to 

the organs, may influence the systemic clearance of drugs. In the 
present study, (RS)-propranolol plasma protein binding was not ste-

reoselective, and the findings of stereoselective elimination of pro-

pranolol enantiomers in vitro confirm that plasma protein binding is 

not at the origin of the stereoselective elimination. This latter argu-

ment can also be applied to eliminate changes in blood flow as a cause 

for the stereoselective elimination of propranolol enantiomers.

Three mechanisms could explain the stereoselective metabolism of 

propranolol in the intestine and in the liver, i.e. differences in the 
intrinsic clearances of the enantiomers, competitive inhibition of 

(S)-( )-propranolol elimination by its antipode, or preferential satu-

ration of the metabolism of (S)-( )-propranolol over that of (R)(+)- 

propranolol. In intestinal epithelial cells, the rate of elimination of 

(S)-( )-propranolol was not influenced by the presence of (R)(+)- 

propranolol and (R)(+)-propranolol was always eliminated more 

quickly than (S)-( )-propranolol, suggesting that in the intestine the 
intrinsic clearance of (R)(+)-propranolol is greater than that of its

TABLE 2

Pharmacokinetic parameters for (S)(-)- and (R)(+)-propranolol after the iv 

administration of 0.5 and 10 mg/kg doses of racemic propranolol to conscious 

rabbits (N = 5/dose)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>(S)(-)-Propranolol</th>
<th>(R)(+)-Propranolol</th>
<th>(S)(-)/R(+) Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>t\text{1/2} (min)</td>
<td>0.5 mg/kg</td>
<td>48 ± 7</td>
<td>47 ± 6</td>
</tr>
<tr>
<td></td>
<td>10.0 mg/kg</td>
<td>59 ± 3</td>
<td>65 ± 3</td>
</tr>
<tr>
<td>AUC\text{0-\infty} (ng • min/ml)</td>
<td>0.5 mg/kg</td>
<td>1,067 ± 72</td>
<td>1,069 ± 100</td>
</tr>
<tr>
<td></td>
<td>10.0 mg/kg</td>
<td>38,689 ± 981</td>
<td>47,222 ± 1,358</td>
</tr>
<tr>
<td>CL\text{int} (ml/min/kg)</td>
<td>0.5 mg/kg</td>
<td>248 ± 16</td>
<td>248 ± 24</td>
</tr>
<tr>
<td></td>
<td>10.0 mg/kg</td>
<td>124 ± 4</td>
<td>106 ± 3</td>
</tr>
<tr>
<td>V\text{diss} (liters/kg)</td>
<td>0.5 mg/kg</td>
<td>14.6 ± 1.0</td>
<td>14.5 ± 1.0</td>
</tr>
<tr>
<td></td>
<td>10.0 mg/kg</td>
<td>12.4 ± 0.5</td>
<td>11.0 ± 0.5</td>
</tr>
</tbody>
</table>

Values are means ± SE.

\text{*} p < 0.05, compared with 0.5 mg/kg.

\text{**} p < 0.05, compared with (S)(-)-propranolol.
antipode. In the liver, with 5.8 μM racemate, the rates of elimination of the two enantiomers were similar. On the other hand, with 58 μM, the rate of elimination of (S)-(−)-propranolol was slower than that of (R)-(+) propranolol, suggesting that in the liver (R)-propranolol stereoselectivity is associated with greater metabolic saturation of (S)-(−)-propranolol, compared with (R)-(+) propranolol. Supporting this premise, it has been shown that an immediate-release po formulation of verapamil generates higher plasma concentrations of (S)-(−)-verapamil than those measured when the racemate is administered in a sustained-release formulation, indicating that the rate of absorption of (R)-verapamil determines differences in verapamil plasma concentrations great enough to produce a preferential saturation of (S)-(−)-verapamil elimination and therefore stereoselective kinetics (Karim and Piergies, 1995).

In the present study, the stereoselective clearance of (R)-propranolol is apparent only when high po doses of the racemate are administered. In vitro, in epithelial cells of the small intestine the kinetics of propranolol enantiomers are stereoselective at concentrations of the racemate lower than those required in the liver. However, the ability of the liver to biotransform the enantiomers of propranolol is 10 times greater than that of the small intestine (du Souich et al., 1995). Hence, even if at low po doses of (R)-propranolol the intestine might generate stereoselective kinetics of the enantiomers, the stereoselectivity would not be apparent systematically, because the liver, at that dose, does not contribute to the stereoselective elimination of propranolol enantiomers. To reveal stereoselective kinetics of propranolol enantiomers in vivo, high po doses and consequently hepatic concentrations are needed.

The present results show that the ratio of the AUC₀–∞ for (S)-(−)- propranolol to the AUC₀–∞ for (R)-(+) propranolol increased from 1.00 after the iv injection of 0.5 mg/kg to 1.14, 1.32, and 1.66 after the po administration of 40, 80, or 120 mg, respectively. These ratios, reflecting the amplitude of stereoselectivity, were estimated at concentrations included in the range of those observed clinically in humans (Genco and Green, 1986). In addition, the results of the present study are similar to those reported for humans; for instance, after iv doses of 5–10 mg of (R)-propranolol, the ratio of the AUC₀–∞ for (S)-(−)-propranolol to the AUC₀–∞ for (R)-(+) propranolol is smaller than that estimated after the po administration of 20 mg of (R)-propranolol (Olanoff et al., 1986; Lalonde et al., 1988; Stoschitzky et al., 1992; Egginger et al., 1994; Von Bahr et al., 1982; Silber et al., 1986). We are tempted to speculate that in humans, as in the present study, dose-dependent differences in stereoselectivity result from the route of administration, i.e. the iv doses of (R)-propranolol are insufficient to saturate the metabolism of (S)-(−)-propranolol in the liver.

Compared with the 0.5 mg/kg iv dose of (R)-propranolol, the 10 mg/kg dose reduced the apparent volume of distribution of (R)-(+) propranolol. Because binding of (R)-(+) propranolol to plasma proteins is concentration-independent up to 750 ng/ml, we must assume that, after the iv injection of (R)-propranolol into rabbits, at high plasma concentrations the binding of (R)-(+) propranolol to tissues is more rapidly saturated than that of the antipode.

The iv injection of 10 mg/kg (R)-propranolol significantly decreased the clearance of both enantiomers, implying that at this dose level the kinetics of propranolol enantiomers are zero-order. Surprisingly, the ratio of the (S)-(−)-propranolol AUC₀–∞ to the (R)-(+) propranolol AUC₀–∞ was 0.82. The differences in the propranolol enantiomer stereoselectivity produced by the different routes of administration may tentatively be explained by the heterogeneous distribution of isozymes in the liver. The rates of sulfation and glucuron conjugation (Pang and Terrell, 1980; Pang et al., 1994), deethylolation (Pang et al., 1988), and hydrolysis (Pang et al., 1991) in the periportal region differ from those observed in the perihepatic artery region of the liver. Therefore, it is conceivable that, depending upon the route of arrival (portal vein or hepatic artery), one enantiomer might be potentially metabolized over its antipode. Supporting such an hypothesis, conjugation is rather selective for (S)-(−)-propranolol (Silber et al., 1986; Walle et al., 1983b), and the formation of desisopropylpropranolol is (R)-(−)-enantioselective at low substrate concentrations and (S)-(−)-enantioselective at high concentrations (Marathe et al., 1994). Indeed, additional studies are required to explain why high iv doses reverse propranolol stereoselectivity.

In conclusion, the in vitro studies show that in the intestine the stereoselective kinetics of (R)-propranolol are secondary to differences in the intrinsic clearances of the enantiomers, whereas in the liver there is preferential saturation of the metabolism of (S)-(−)-propranolol. In vivo, low doses of (R)-propranolol administered iv or po do not generate stereoselective kinetics of the enantiomers of propranolol, possibly because the contribution of the liver overshadows the contribution of the intestine. On the other hand, without changing the protein binding, high po doses generate zero-order kinetics and more profoundly depress (S)-(−)-propranolol metabolism, producing stereoselective differences in the rates of elimination of the enantiomers. Finally, high iv doses, generating zero-order kinetics, induce small stereoselective differences in the elimination of the enantiomers of propranolol, but in this case (S)-(−)-propranolol is eliminated more rapidly than (R)-(+) propranolol.

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References