STEREOSELECTIVE DISPOSITION OF PROPRANOLOL IN RABBITS
Role of Presystemic Organs and Dose

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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–∞ for (S)-(−)-propranolol was greater than the AUC0–∞ 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–∞ 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–∞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–∞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–∞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 pr-eysystemic metabolism in the epithelial cells of the intestine and/or in the liver (Krisha 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). Rabbits were selected as the animal model because the metabolism of propranolol in this species closely resembles that in humans.
in vitro studies were carried out with a 37.5
were separated by HPLC after derivatization with (R)-)
ol enantiomers were assayed in samples of 100
m from liver homogenates and every 30 min from the other tissue
homogenates. 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, 60, 90, 120, 180, 240, and 300 min after
administration. Preliminary studies were undertaken to assess the lowest po dose of pro-
pranolol 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)
drawn were before 5, 10, 20, 30, 60, 90, 120, 180, 240, 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 concen-
trations of propranolol enantiomers. The remaining plasma was centrifuged at
3500 rpm, in Centrivipul 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
assessing the ability of extrahepatic organs and liver to metabolize pro-
pranolol. 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, Rexton, Belgium).
The homogenates were centrifuged at 10,000g for 20 min, and 1 ml of the supernatant was
used to assess the ability 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; Sigma) or 0.5 ml of a solution with
7.5 µg/ml of the racemate (final concentration, 5.8 µM). The choice of propranolol concentrations was based on the fact that, after po administration, propranolol concentrations in the liver are
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)-(PEIC (Fluka, Switzer-
land), 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 carbonic buffer (pH 10.5), and 10 ml
of diethyl ether were added to 0.5- or 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-R4A integrator. The excitation and emission wave-
lengths of a Spectroflow 980 fluorescence detector were set at 220 and 340 nm,
respectively. The recoveries of (S)(–) and (R)(+) propranolol, at concen-
trations 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. (S)(–)/(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
AUC0–∞ values were estimated by 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

\[
CL_p = D_p / AUC_{0–∞}
\]

where Dp is the dose of the enantiomers and AUC0–∞ is the

AUC0–∞ value for the enantiomers given po (Gibaldi and Perrier, 1982).

All results are expressed as mean ± SE. Differences between the pharma-
koine kinetic 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 Vivo Studies. After the po administration of 40, 80, or 120
mg/kg racemic propranolol, peak plasma concentrations of both en-
antiomers 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
and (S)(–) propranolol were 33.3, 65.4, and 144.2 ng
kg/ml/mg, respectively. As the po administration of 80 and 120 mg/kg doses of (R)(+) propranolol were 30.5, 41.1, and 86.7 ng
kg/ml/mg, respectively.

Assuming that the whole dose of (R)-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, respect-
ively. 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 AUC0–∞ value for
(S)(–)-propranolol did not differ from that for (R)(+) propranolol.
However, after the po dose of 120 mg/kg, the AUC0–∞ for (S)(–)-
propranolol was greater (p < 0.05) than the AUC0–∞ for (R)(+)-

Abbreviation used is: (R)-PEIC, (R)-( + )-phenylethylisocyanate.

<ref>References</ref>
propranolol (table 1). The ratio of the AUC_{0→∞} for \((S)-(−)\)-propranolol to the AUC_{0→∞} for \((R)+(+)\)-propranolol increased \((p < 0.05)\) 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 racemate 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 AUC_{0→∞} 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 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 AUC_{0→∞} for \((S)-(−)\)-propranolol was smaller than that for the antipode \((p < 0.05)\). The ratio of the AUC_{0→∞} for \((S)-(−)\)-propranolol to the AUC_{0→∞} 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 \((i.e. \text{from 93.75 to 750 ng/ml})\) 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 \([0.39 ± 0.02 \text{ and } 0.40 ± 0.02 \text{ for } (R)+(+)\)-propranolol and \((S)-(−)\)-propranolol, respectively]. High concentrations of the racemic drug \((i.e. \text{1500 ng/ml})\) did not modify the unbound fractions of propranolol enantiomers.

**In Vitro Studies.** Intestinal mucosa and liver homogenates, but not lung and renal cortex supernatants, metabolized \((RS)\)-propranolol. After incubation of the racemate 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 racemate 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 racemate 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 racemic propranolol to conscious rabbits (\(N = 4 \text{ or } 5/dose\)). |
|-----------------|-----------------|-----------------|
|                | \((S)-(−)\)     | \((R)+(+)\)     | \((S)-(−)/\((R)+(+)\) Ratio |
| \(t_{1/2}\) (min) | Propranolol     | Propranolol     |                          |
| 40 mg/kg       | 69 ± 9          | 69 ± 5          | 1.00 ± 0.01              |
| 80 mg/kg       | 63 ± 6          | 62 ± 7          | 1.02 ± 0.02              |
| 120 mg/kg      | 58 ± 7          | 65 ± 8          | 0.89 ± 0.05              |
| \(C_{max}\) (ng/ml) | 13 ± 2          | 12 ± 2          | 1.04 ± 0.08              |
| 40 mg/kg       | 121 ± 28        | 70 ± 21         | 1.43 ± 0.23              |
| 80 mg/kg       | 356 ± 37        | 209 ± 29\(^b,c\) | 1.76 ± 0.21\(^c\)       |
| 120 mg/kg      | 17,308 ± 2,054  | 10,401 ± 952\(^b,c\) | 1.66 ± 0.19\(^c\)     |

Values are means ± SE.

\(^a\) \(p < 0.05\), compared with 40 mg/kg.
\(^b\) \(p < 0.05\), compared with 80 mg/kg.
\(^c\) \(p < 0.05\), compared with \((S)-(−)\)-propranolol.

**FIG. 1.** Mean plasma concentration-time curves for \((S)-(−)\)-propranolol (\(Δ\)) and \((R)+(+)\)-propranolol (\(●\)) after the po administration of 40, 80, and 120 mg/kg doses of racemic propranolol to conscious rabbits (\(N = 4 \text{ or } 5/dose\)). Vertical bars, SE.

**FIG. 2.** Mean plasma concentration-time curves for \((S)-(−)\)-propranolol (\(Δ\)) and \((R)+(+)\)-propranolol (\(●\)) after the iv administration of 0.5 and 10 mg/kg doses of racemic propranolol to conscious rabbits (\(N = 5/dose\)). Vertical bars, SE.

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**TABLE 1**

| Pharmacokinetic parameters for \((S)-(−)\) and \((R)+(+)\)-propranolol after the po administration of 40, 80, and 120 mg/kg doses of racemic propranolol to conscious rabbits (\(N = 4 \text{ or } 5/dose\)). |
|-----------------|-----------------|-----------------|
|                | \((S)-(−)\)     | \((R)+(+)\)     | \((S)-(−)/\((R)+(+)\) Ratio |
| \(t_{1/2}\) (min) | Propranolol     | Propranolol     |                          |
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Values are means ± SE.

\(^a\) \(p < 0.05\), compared with 40 mg/kg.
\(^b\) \(p < 0.05\), compared with 80 mg/kg.
\(^c\) \(p < 0.05\), compared with \((S)-(−)\)-propranolol.
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, 29 for the antipode. When the enantiomers were incubated individually at
\( p \rightarrow (\pm) -\) propranolol, the rate of elimination of (\( R \) ) propranolol is 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 presence of (\( S \) ) propranolol reduced the rate constant of elimination 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_{0→∞} values for both enantiomers. However, the increase in the AUC_{0→∞} value for (\( S \) ) propranolol is greater than that for (\( R \) ) propranolol, indicating 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 Bahr et al., 1982). The iv dose of 10 mg/kg (\( RS \) ) propranolol generates a nonlinear increase in the AUC_{0→∞} values for both enantiomers but, in this case, the AUC_{0→∞} 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 propranolol are route- and dose-dependent.

### Table 2

<table>
<thead>
<tr>
<th>Dosage (mg/kg)</th>
<th>AUC_{0→∞} (ng·min/ml)</th>
<th>CL_{a,b} (ml/min/kg)</th>
<th>( V_{d,r} ) (liter/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.5</td>
<td>1,067 ± 72</td>
<td>248 ± 16</td>
<td>14.6 ± 0.7</td>
</tr>
<tr>
<td>10.0</td>
<td>38,689 ± 981*</td>
<td>47,222 ± 1,358**</td>
<td>124 ± 4*</td>
</tr>
</tbody>
</table>

Values are means ± SE.

\( a \) p < 0.05, compared with 0.5 mg/kg.

\( b \) p < 0.05, compared with (\( S \) ) propranolol.

**Fig. 3.** Rate constants of elimination for (\( S \) ) propranolol and (\( R \) ) propranolol incubated in the 10,000g supernatant of homogenates of epithelial cells of the intestinal mucosa (A) and the liver (B) after incubation of 5.8 or 58 \( \mu \)M concentrations of racemic propranolol or 2.9 or 29 \( \mu \)M concentrations of the individual enantiomers.

Vertical bars, SE. * p < 0.05, compared with (\( S \) ) propranolol.

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 stereoselective, and the findings of stereoselective elimination of propranolol enantiomers in vitro confirm that plasma protein binding is not at the origin of the stereoselective elimination. This latter argument 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 saturation 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...
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 (RS)-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 (RS)-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 (RS)-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 (RS)-propranolol the intestine might generate stereoselective kinetic differences of the enantiomers, the stereoselectivity would not be apparent systemically, 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 high 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 (RS)-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 40 mg of (RS)-propranolol (Olannof 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 (RS)-propranolol are insufficient to saturate the metabolism of (S)(−)-propranolol in the liver.

Compared with the 0.5 mg/kg iv dose of (RS)-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 (RS)-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 (RS)-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 sulfo- and glucuronocoujugation (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 preferentially 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 (RS)-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 (RS)-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


