Disposition Kinetics of Propranolol Isomers in the Perfused Rat Liver

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ABSTRACT

The aim of this study was to define the determinants of the linear hepatic disposition kinetics of propranolol optical isomers using a perfused rat liver. Monensin was used to abolish the lysosomal proton gradient to allow an estimation of propranolol ion trapping by hepatic acidic vesicles. In vitro studies were used for independent estimates of microsomal binding and intrinsic clearance. Hepatic extraction and mean transit time were determined from outflow-concentration profiles using a nonparametric method. Kinetic parameters were derived from a physiologically based pharmacokinetic model. Modeling showed an approximate 34-fold decrease in ion trapping following monensin treatment. The observed model-derived ion trapping was similar to estimated theoretical values. No differences in ion-trapping values was found between \( R^+ \)- and \( S^- \)-propranolol. Hepatic propranolol extraction was sensitive to changes in liver perfusate flow, permeability-surface area product, and intrinsic clearance. Ion trapping, microsomal and nonspecific binding, and distribution of unbound propranolol accounted for 47.4, 47.1, and 5.5% of the sequestration of propranolol in the liver, respectively. It is concluded that the physiologically more active \( S^- \)-propranolol differs from the \( R^+ \)-isomer in higher permeability-surface area product, intrinsic clearance, and intracellular binding site values.

Propranolol is a widely used nonselective \( \beta \)-blocking agent that is highly extracted in the liver (Shand, 1976) and exhibits a nonlinear first pass metabolism in the rat (Suzuki et al., 1981) due to saturation of hepatic tissue binding (Anderson et al., 1978; Miyauchi et al., 1993) and Michaelis-Menten metabolic enzyme or sequestration clearance (Keding and Steinnes, 1984; Smallwood et al., 1988) as well as metabolic stereoselectivity. It has been described as a “problematic” drug in terms of its first pass metabolism not being properly described (Lalka et al., 1993).

Because the stereo-selective elimination of propranolol enantiomers remains poorly understood (Marier et al., 1998) and because propranolol is marketed as a racemate consisting of the two enantiomers, we examined the contribution of the stereochemistry of propranolol on its hepatic disposition kinetics by investigating the individual disposition kinetics of both \( R^+ \)- and \( S^- \)-propranolol and the racemate after bolus injection in the single-pass perfused rat liver. In addition, we measured the microsomal protein binding and metabolism of individual propranolol isomers in an in vitro study. These in vitro data were then used to validate the hepatic disposition kinetic model derived from the in situ impulse-response studies using perfusate concentrations.

This study follows our recently reported structure-hepatic disposition relationships of several cationic drugs in the normal and the diseased rat liver (Hung et al., 2001, 2002), and here we also determined pharmacokinetic parameters such as hepatocellular influx, efflux, binding, and elimination for these optical isomers. Kinetic parameters were derived from a two-phase physiologically based organ pharmacokinetic model (Weiss and Roberts, 1996; Hung et al., 2001). Of particular interest was fully defining the determinants of propranolol isomer hepatic extraction and mean transit time at nonsaturable propranolol concentrations, including the relative contribution of ion trapping and microsomal binding to the uptake of propranolol isomers.

Materials and Methods

Chemicals. Monensin sodium (2-[5-ethyltetrahydro-5-[tetrahydro-3-methyl-5-[tetrahydro-6-hydroxy-6-[hydroxymethyl]-3,5-dimethyl-2H-pyran-2-y1]-2-furyl]-9-hydroxy-β-methoxy-
buffer/RBC, and controls were perfused for 10 min with buffer/RBC.

perfusion was performed using an intravenous 16-gauge catheter,

where the permeation rate constant, $k_{cv}$, characterizing the intracellular binding sites) for model cationic

The unbound fraction of individual propranolol optical isomer in each

buffer solution was estimated using an ultra-filtration method. A

known concentration of the individual propranolol optical isomer

stock solution was added to 500 μl of each buffer solution to make

final concentration of 0.05 μM and placed in a centrifugal filter
device (Microcon YM-30, 30,000 molecular weight cut-off; Millipore
Corporation) and then centrifuged at 3000g for 10 min. The ultra-
filtre (in triplicate) was assayed by HPLC. The unbound fraction

$\alpha_{1,2,8}$-tetramethyl-1,6-dioxaspiro[4,5]decane-7-butyc sodium

salt, $S$-propranolol, $\alpha$-(1-$\alpha$-methylene)-$\alpha$-aminino-3-(1-naphtale-
nolyoxo)-2-propanol, $R$-propranolol, and $R,S$-propranolol all were obtained from Sigma-Aldrich (St. Louis, MO). Dimethylox-
aldazine-2,4-dione ([U-14C]DMO), [U-14C]sucrose, and [3H]water

were obtained from Sigma-Aldrich (St. Louis, MO). Dimethylox-
aldazine was treated with 37°C. Assess-

were placed in a temperature-controlled environment at 37°C. Assess-

ment of liver viability was by macroscopic appearance, measurement of

bile flow, oxygen consumption, and portal resistance pressure

(Cheung et al., 1996). Perfusion was performed using an intravenous 16-gauge catheter,

The collected samples were centrifuged, and 75-μl aliquots of the

supernatant containing [3H]water, [U-14C]sucrose, or [14C]DMO

were taken for scintillation counting (MINAXI beta TRI-CARB 4000

series liquid scintillation counter; PerkinElmer Life and Analytical

Science, Boston, MA). The remainder was vortexed and treated with

trichloroacetic acid/mobile phase (1:2) for HPLC analysis to deter-

mination of unbound fraction of individual propranolol optical isomer.

Analytical Procedure. The HPLC method employed in this work has

been described and validated previously (Hung et al., 2001).

Data Analysis. A two-phase physiologically based organ pharma-
cokinetic model was used to analyze propranolol isomer disposition

in the perfused liver. This model, which describes intersinuosoidal mixing also called vascular dispersion (Roberts et al., 1988), transfer across a permeability barrier, and the intracellular and elimination kinetics (Weiss and Roberts, 1996; Weiss et al., 1997), has been previously applied to the disposition of diclofenac (Weiss et al., 2000). In the present work, this model (as shown in Fig. 1 and eq. 1) has been developed to accommodate both ion trapping ($K_i$, character-

izing the vesicular ion-trapping sites) and intracellular binding ($K_{in}$, characterizing the intracellular binding sites) for model cationic

drugs. The underlying mathematics of the model and the estimation of relative contribution of ion trapping, microsomal binding, and distribution of unbound drug for hepatic sequestration of propranolol have been described in detail previously (Siebert et al., 2004).

$$\frac{d\phi}{dt} = \frac{(s + k_{in})(s + k_{out})}{\sigma^2 + k_{in} + k_{out} + k_{in} + k_{out} + (1 + K_i)}$$

where the permeation rate constant, $k_{in}$ = $f_{in}$PS/V_Ve, is the perme-

ation clearance per extracellular volume ($V_Ve$) PS is the permeability-
surface area product, and $K_{out}$ is the efflux rate constant. The equi-

librium amount ratio $K_{e} = K_{in}/K_{out}$ characterizes the slowly accessible

dpool for ion trapping, $K_{in}$ is defined as a rapidly equilibrating intra-
cellular binding sites (microsomal and nonspecific binding), and $K_{in}$ and $K_{out}$ represent the rate constant for transport from cytosol into

acidic vesicles (lysosomes and mitochondria) or from acidic vesicles

into cytosol, respectively. The elimination rate constant defined as $K_{e} = CL_{e}/V_{C}$ is the intrinsic elimination clearance normalized per

cellular volume $V_{C}$ (Hung et al., 2001). Data were fitted and calcu-

lated using Scisent (MicroMath Inc., Salt Lake City, UT).

Intracellular pH (pHv) was calculated from the concentration out-


$$pHv = log[p(10^{12} - 10^{-13})]$$

where $p$ is the distribution ratio of DMO described as

$$p = \frac{MTT_{DMO} - MTT_{sucrose}}{MTT_{water} - MTT_{sucrose}}$$
6.13 is the pH of DMO and 7.4 is the pH of pH, (extracellular pH) used for the estimation of pH.

**Sensitivity Analyses.** The predicted extraction ratio and mean transit time defined by the two-phase physiologically based pharmacokinetic model (\(E_{\text{pred}}\) and \(\text{MTT}_{\text{pred}}\)) are defined by eqs. 4 and 5:

\[
E_{\text{pred}} = 1 - pG_1 - (1 - p)G_2
\]

\[
\text{MTT}_{\text{pred}} = \frac{g_p}{pG_1 + (1 - p)G_2} \left[ \frac{G_1 \text{MTT}_1}{\sqrt{1 + 2CV^2 \cdot \text{MTT}_1 R_N}} \right] + \frac{G_2 (1 - p) \text{MTT}_2}{\sqrt{1 + 2CV^2 \cdot \text{MTT}_2 R_N}} \quad (5)
\]

where \(CV^2\) is the normalized variance, and \(R_N\) is the efficiency number that characterizes the elimination of solute by the liver.

\[
g_p = 1 + \frac{k_b h_{\text{out}} (1 + \frac{k_m}{k_u h_{\text{out}}})}{(k_{\text{out}} + k_m)^2}, \quad R_N = \frac{k_b h_{\text{out}}}{k_u + k_{\text{out}}} \quad (6)
\]

\[
G_i = \exp \left( \frac{1}{CV^2} \left[ \frac{\text{MTT}_i}{\text{CV}^2/2} \cdot \frac{1}{2 \text{MTT}_i \text{CV}^2} \right]^{1/2} \right) \quad (i = 1, 2) \quad (7)
\]

In the sensitivity analysis, the effects of altering the parameters defining hepatic disposition on \(E\) and \(\text{MTT}\) of propranolol was examined for R,S-propranolol by changing the individual model parameter values derived to define propranolol disposition in the perfused liver.

**Statistical Analysis.** All data are presented as mean ± S.D. Statistical analysis was performed using a two-way analysis of variance to assess the presence of significance between the control and treatment groups followed by Tukey’s post hoc test (including the Kramer extension) to identify the source of the significance within the group. Statistical significance was taken at the level \(P < 0.05\).

**Results**

Figure 2A shows a typical \(R(+)\)-propranolol and \([U-^{14}\text{C}]\) sucrose outflow perfusion concentration-time profile of the regression (data weighted, \(1/y_{\text{obs}}^2\)). \([U-^{14}\text{C}]\) sucrose was coadministered as an extracellular reference solute in the same bolus injection. The fit was obtained by the equations described in the section of data analysis. It is evident that the model gives a good fit for the data. The perfusate data appear to have at least three phases (rapid up phase, fast down phase, and slow down phase). The corresponding \(S(-)\)-propranolol and \([U-^{14}\text{C}]\) sucrose perfusate concentration-time data and model regressions are shown in Fig. 2B. The profiles are similar to the \(R(+)\) profile but with the perfusate outflow fractions being slightly lower.

Figure 3 shows a comparison of typical measured and predicted (fitted data) outflow perfusion concentration-time profiles before and after monensin treatment for the propranolol \(R(+)\) and \(S(-)\)-enantiomers and racemic propranolol using coadministered \([U-^{14}\text{C}]\) sucrose and \([^{3}H]\) water for estimation of extracellular and cellular volumes. It is apparent that monensin pretreatment greatly broadened the peak of the outflow profiles compared with controls. Data points measured and data regression lines predicted by the two-phase organ model appeared adequately fitted (Fig. 3, A–C). It is also shown in Fig. 3 that data points and predicted regression lines for both enantiomers and racemic propranolol increased following monensin administration.

Table 1 shows the nonparametric moments parameters for the drugs used in the study. No significant differences between control and monensin-treated groups were observed for hepatic extraction ratio and normalized variance for the propranolol optical isomers. However, there was a significant difference in mean transit time between control and treatment groups (Table 1). No significant differences for the nonparametric parameters were found to exist between the two propranolol enantiomers and racemic propranolol (Table 1).

Table 2 summarizes the kinetic parameters derived from the two-phase organ model for hepatic drug disposition of propranolol enantiomers and racemic propranolol. The vesicular ion-trapping constant \(K_v\) significantly decreased following monensin treatment for \(R(+)\)-propranolol, \(S(-)\)-propranolol, and racemic propranolol (\(K_v\) control/\(K_v\) treatment: 33-, 34-, and 35-fold, respectively). However, no changes following monensin treatment were observed for CL \(_{\text{int}}\), PS, or \(K_v\) values for all optical isomers.

Table 2 also compares the stereoselectivity of the \(R(+)\) and \(S(-)\)-enantiomer and the propranolol racemate. It shows that the \(S(-)\)-isomer has significantly higher PS, CL \(_{\text{int}}\), and

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**Fig. 1.** Schematic overview of hepatocellular drug transport and ion trapping (including the acidic compartment). D, drug; DH, protonated drug; \(f_{ub}\), fraction of drug unbound in perfusate; \(K_{uB}\), \(K_{out}\), and \(K_v\), permeation, efflux, and elimination rate constant, respectively; \(K_p\), equilibrium amount ratio characterizing the rapidly equilibrating intracellular binding sites (microsomal and nonspecific binding sites); \(K_{pB}\), and \(K_v\), rate constants for transport from cytosol into acidic vesicles (lysosomes and mitochondria) or from acidic vesicles into cytosol, respectively, determining the equilibrium amount ratio \(K = K_{pB}/K_v\), characterizing the slowly accessible pool for ion trapping; \(V_v\), extracellular volume (vascular + Disse space); \(V_c\), cellular water volume.
$K_b$ values than those of $R\text{-}(+/H11001)$-isomer but a comparable $K_v$ value to $R\text{-}(+/H11001)$-isomer. The kinetic parameters $PS$, $CL_{int}$, and $K_b$ values were found to be increased 1.5-, 1.4-, and 1.2-fold, respectively, for the $S\text{-}(+/H11002)$-propranolol compared with the $R\text{-}(+/H11001)$-enantiomer, reflecting a more pronounced disposition of the $S\text{-}(+/H11002)$-enantiomer in liver tissue. However, no significant difference was found to exist between $S\text{-}(+/H11002)$-propranolol and $R,S\text{-}propranolol$.

Table 3 shows the results of the in vitro drug binding and drug metabolism study. Monensin did not affect binding or metabolic activity in vitro for all three optical isomers. The calculated $CL_{int}$ and fraction of drug unbound values for the $R\text{-}(+/H11001)$-propranolol were statistically different ($P < 0.05$) from those of $S\text{-}(+/H11002)$-propranolol and $R,S\text{-}propranolol$ both in the control and treatment groups.

The determination of the intracellular pH showed no statistically significant differences before and after monensin treatment ($7.34 \pm 0.19$ and $7.27 \pm 0.06$, respectively).

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**Fig. 2.** Typical outflow profiles for the enantiomers of propranolol and $[\text{U-}^{14}\text{C}]$sucrose in the regressions. A, $R\text{-}(+/H11001)$-propranolol with sucrose. B, $S\text{-}(+/H11002)$-propranolol with sucrose. The solid circles represent enantiomer experiment data. The open circles represent sucrose experimental data. The lines represent the fits of the profiles.

$K_b$ values than those of $R\text{-}(+/H11001)$-isomer but a comparable $K_v$ value to $R\text{-}(+/H11001)$-isomer. The kinetic parameters $PS$, $CL_{int}$, and $K_b$ values were found to be increased 1.5-, 1.4-, and 1.2-fold, respectively, for the $S\text{-}(+/H11002)$-propranolol compared with the $R\text{-}(+/H11001)$-enantiomer, reflecting a more pronounced disposition of the $S\text{-}(+/H11002)$-enantiomer in liver tissue. However, no significant difference was found to exist between $S\text{-}(+/H11002)$-propranolol and $R,S\text{-}propranolol$.

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The determination of the intracellular pH showed no statistically significant differences before and after monensin treatment ($7.34 \pm 0.19$ and $7.27 \pm 0.06$, respectively).

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**Fig. 3.** Comparison of outflow perfusion concentration-time profiles and regression lines obtained from the two-phase physiologically based organ pharmacokinetic model for: A, racemic propranolol; B, $R\text{-}(+/H11001)$-propranolol; and C, $S\text{-}(+/H11002)$-propranolol in controls and following monensin treatment. Solid circles represent controls, and open circles represent treatment. Solid and dashed lines stand for fitted data in control and treatments, respectively.
Two-way analysis of variance showed there was no significant difference between the control and treatment groups for the three isomers for \( E \) and CV\(^2 \) values and significant differences for the MTT value between the control and treatment groups. Furthermore, Tukey’s post hoc test showed there was no significant difference between the three isomers for the MTT value both in the control and treatment groups.

Furthermore, Tukey’s post hoc test showed significant differences between \( E \) values. Two-way analysis of variance showed there was no significant difference between the control and treatment groups for the three isomers for CL\(_{\text{int}}\), PS, and \( K_v \) values and significant differences for the \( K_v \) value between the control and treatment groups. Further, Tukey post hoc test showed CL\(_{\text{int}}\), PS, and \( K_v \) values for \( R^+ \)-propranolol were significant differences to those of \( S^- \)-propranolol (\( P < 0.05 \)) and \( R,S \)-propranolol (\( P < 0.05 \)) but no difference for the \( K_v \) value for the three isomers both in the control and treatment groups; no significant difference was found to exist between \( S^- \)-propranolol and \( R,S \)-propranolol for all kinetic parameters.

In vitro microsomal protein (MP) binding and metabolism of the enantiomers of propranolol and racemic propranolol (mean ± S.D., \( n = 6 \))

Two-way analysis of variance showed there was no significant difference between the control and treatment groups for the three isomers for \( f_{\text{u,MP}} \) and \( K_v \) values.

A sensitivity analysis on the effects of changing the individual model parameter values for \( R,S \)-propranolol suggests that hepatic extraction is significantly affected by metabolism (\( K_{\text{g,}} \), \( P < 0.05 \)) and permeability (\( P < 0.05 \)), and blood flow (\( P < 0.05 \)) but not intracellular binding (\( K_v \)) and ion trapping (\( K_{\text{v,i}} \)) (Table 5). In contrast, the mean transit time is significantly affected by \( K_{\text{g,}} \) (\( P < 0.01 \)) and \( K_{\text{v,i}} \) (\( P < 0.05 \); Table 5). Both the simulated control and monensin-treated groups had similar predicted hepatic extraction and mean transit time values as those obtained from the nonparametric moment analysis (Table 5).

An analysis of the outflow perfusion-concentration-time profiles in the control and the monensin-treated groups (Fig. 3C) yielded a significant difference in \( K_v \) (Table 2), and such a difference also leads to model-predicted differences in mean transit time that are consistent with moment estimates (Table 5).

**Discussion**

In this study, a physiologically based two-phase organ pharmacokinetic model was used to account for vascular dispersion, hepatic permeability, ion trapping by subcellular acidic organelles, intracellular binding, and intrinsic metabolic clearance of propranolol in the perfused rat liver (Roberts et al., 1988). Resolution of the relative concentration of each transport process by the model followed the conduct of impulse-response profiles of propranolol in control and monensin-treated livers, the propranolol being administered at a sufficiently low dose to avoid a nonlinearity in plasma protein binding (Ludden, 1991), liver binding (Anderson et al., 1978; Miyachi et al., 1993), or in hepatic metabolism, recognizing that a range of Michaelis constants need to be incorporated under saturable conditions (Ishida et al., 1992).

The model gave a good fit of the data with and without monensin treatment (Fig. 3) and yielded predicted values of hepatic extraction and mean transit time, consistent with model-independent moment estimates (Table 5). The individual propranolol enantiomer perfusate concentration-time profile is similar in shape to that observed for the racemate that we have reported earlier (Siebert et al., 2004). It is evident that monensin increases the peak propranolol outflow concentration and abolishes the initial subsequent rapid decline in outflow concentrations after dosing (Fig. 3). As is evident from the binding and transport kinetic data derived
is the pH of lysosomes (Daniel, 2003), and pHv distribution ratio (v/i) for propranolol can be estimated from the individual organelle volume fraction and concentration ratio above using the equation:

\[
K_v = \frac{f_{\text{org}} \times v + f_{\text{cyt}} \times i}{v + i}
\]

Comparison of predicted and observed model-derived \( K_v \) (equilibrium amount ratio characterizing the vesicular ion-trapping sites) for propranolol optical isomers

<table>
<thead>
<tr>
<th>Drug</th>
<th>pk_a</th>
<th>Lysosomes/Intracellular Concentration Ratio (v/i)</th>
<th>Mitochondria/Intracellular Concentration Ratio (m/i)</th>
<th>Predicted ( K_v )</th>
<th>Observed Model-Derived ( K_v )</th>
</tr>
</thead>
<tbody>
<tr>
<td>( R^+ )-Propranolol</td>
<td>9.45</td>
<td>736</td>
<td>3.96</td>
<td>8.16</td>
<td>8.20 ± 0.64</td>
</tr>
<tr>
<td>( S^- )-Propranolol</td>
<td>9.45</td>
<td>736</td>
<td>3.96</td>
<td>8.16</td>
<td>8.54 ± 1.06</td>
</tr>
<tr>
<td>( R,S )-Propranolol</td>
<td>9.45</td>
<td>736</td>
<td>3.96</td>
<td>8.16</td>
<td>8.79 ± 1.79</td>
</tr>
</tbody>
</table>

\( V_{\text{ves}}/V_{\text{cyt}} \) concentration ratio = \( (1 + 10^{\text{pH}_{\text{cyt}} - 7.27})/(1 + 10^{\text{pH}}_{\text{ves}} - 7.27}) \) (Goldstein et al., 1974), where \( \text{pH}_{\text{cyt}} \geq 7.27 \) is the cytosolic pH (Le Couteur et al., 1993), \( \text{pH}_{\text{ves}} \geq 4.4 \) is the pH of lysosomes (Daniel, 2003), and \( \text{pH}_{\text{ves}} \approx 6.67 \) is the mitochondria pH in the fasted state (Soholl et al., 1980).

Given that the fraction of lysosomes \( (f_{\text{org}}) \) and mitochondria \( (f_{\text{cyt}}) \) to cytosol is 1 and 20% (Rhoades and Pflanzer, 1996), the overall unbound drug vesicles/intracellular distribution ratio \( (v/i) \) propranolol can be estimated from the individual organelle volume fraction and concentration ratio above using the equation: \( K_v = f_{\text{org}} \times v + f_{\text{cyt}} \times i \).

Data fitting results using the physiologically based two-phase organ pharmacokinetic model.

in Tables 2 and 3, monensin’s effect can be attributed almost exclusively to its reducing the distribution of propranolol into acidic cell organelles.

A further clarification on the disposition of propranolol isomers in the liver is possible from analysis of outflow profiles and the amount of propranolol isomer remaining in the liver over time. Under hypoxic conditions, the metabolism of propranolol is considerably compromised (Elliott et al., 1993) so that it becomes possible to sacrifice liver perfusions at various times and measure propranolol isomer concentrations showed that the S-isomer has higher tissue levels than the R-isomer, consistent with the high binding to liver proteins as suggested by the in vitro studies (Table 3) and previously reported data (Anderson et al., 1978). An analysis of propranolol tissue levels over time revealed that the logarithm of the propranolol tissue concentrations decline in a linear manner over time further, confirming that the propranolol concentrations used in this work were below those causing saturation.

Stereo-selectivity in the disposition of propranolol enantiomers in the perfused liver is evident for PS, Cl_{in}, and K_{b} but not K_{v}. Ion trapping accounts for 47.4% of the hepatic sequestration for both \( R^+ \)- and \( S^- \)-enantiomers. Propranolol has been shown to be stereoselective in both its response and in its metabolism, the \( S^- \)-enantiomer being about 100 times more effective as a beta blocker than the \( R^+ \)-enantiomer (Barrett and Cullum, 1968; Marier et al., 1998). Enantiomers usually vary in their biological and pharmacological effects, and \( \beta \)-blockers (such as atenolol or propranolol) with a single chiral center vary in their stereoselectivity to bind to the \( \beta_1 \) or \( \beta_2 \) adrenergic receptors. Generally the cardiac activity is attributable to the \( S^- \)-enantiomer, which has a much higher binding affinity than its \( R^+ \)-counterpart (Barrett and Cullum, 1968; Pearson et al., 1989; Stoschitzky et al., 1993; Marier et al., 1998). It has also been suggested that the pharmacokinetics of the enantiomers in vivo in rabbits are comparable at lower doses but stereoselective at higher doses, due to hepatic saturation. Drug distribution and propranolol enantiomer plasma binding is not stereoselective or dose-dependent (Marier et al., 1998).

The modeling of perfusate impulse-response data in our study has shown that there is a preferential uptake of the \( S^- \)-propranolol into rat heart tissue when compared with \( R^+ \)-propranolol (Kawashima et al., 1976).

The sensitivity of effects of changes in model parameters describing the disposition of propranolol in rat liver controls on hepatic extraction ratio (E) and mean transit time (MTT) for \( R,S \)-propranolol (mean ± S.D., n = 6)

Model-independent moment-derived estimates from Table 1 are shown in italics for comparison.

<table>
<thead>
<tr>
<th>Effects of Change in Model Parameter</th>
<th>E</th>
<th>MTT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Observed control (with acidic vesicles) data</td>
<td>0.96 ± 0.03</td>
<td>119 ± 20.3</td>
</tr>
<tr>
<td>Simulated control data</td>
<td>0.92 ± 0.03</td>
<td>124 ± 20.8</td>
</tr>
<tr>
<td>Observed treated (no acidic vesicles) data</td>
<td>0.93 ± 0.06</td>
<td>79.5 ± 15.3</td>
</tr>
<tr>
<td>Simulated( ^a ) treated data ( K_v = 0.25 )</td>
<td>0.92 ± 0.03</td>
<td>73.8 ± 23.5( ^d )</td>
</tr>
<tr>
<td>Simulated( ^a ) with ( K_v ) reduced by 50%</td>
<td>0.92 ± 0.03</td>
<td>80.9 ± 17.6( ^a )</td>
</tr>
<tr>
<td>Simulated( ^a ) with ( k_{b} ) reduced by 50%</td>
<td>0.81 ± 0.06( ^b )</td>
<td>115 ± 20.1</td>
</tr>
<tr>
<td>Simulated( ^a ) with ( Q_b ) reduced by 50%</td>
<td>0.78 ± 0.06( ^b )</td>
<td>96.8 ± 24.3</td>
</tr>
<tr>
<td>Simulated( ^a ) with PS( ^a ) reduced by 50%</td>
<td>0.74 ± 0.08( ^b )</td>
<td>91.7 ± 27.7</td>
</tr>
</tbody>
</table>

\( ^a \) Data obtained from nonparametric moments analysis (Table 1).

\( ^b \) Simulation using eq. 4 or 5.

\( ^c \) \( K_v \), ion-trapping parameter.

\( ^d \) Tukey’s post hoc differences between observed data and various simulations \( (P < 0.01) \).

\( ^e \) \( K_{b} \), intracellular binding.

\( ^f \) Tukey’s post hoc differences between observed data and various simulations \( (P < 0.05) \).

\( ^g \) \( k_{b} \), metabolism.

\( ^h \) Q, blood flow.

\( ^i \) PS, permeability-surface area product.
the higher binding affinity of the S(-)-enantiomer for liver tissue and the rapidly equilibrating binding sites. A similar finding was evident on analysis of an in vitro hepatic microsomal protein binding of propranolol study (Table 3). The fraction unbound of S(-)-propranolol was found to be lower than that of R(+) -propranolol (Table 3).

Table 2 shows that the derived intrinsic elimination clearance of the S(-)-enantiomer is almost 1.4-fold that of the R(+) -isomer. This could be explained by the fact that one of the binding sites in question is a microsomal protein, and this binding is a prerequisite for elimination (i.e., higher microsomal binding facilitates faster elimination by microsomal metabolism). The in vitro metabolism data also showed that the in vitro CLint and unbound drug fraction values for the R(+) -propranolol were significantly different (smaller CLint and larger unbound drug fraction) compared with those of S(-)-propranolol (Table 3). Thus, one source of propranolol stereoselectivity is indeed attributable to the microsomal binding differences between these two propranolol enantiomers.

Given that only 70% microsomal protein homogenized from liver tissue is metabolically active and the average production from microsomal protein of 1-g liver tissue amounts to about 50 mg (Roberts and Rowland, 1986), the calculated CLint values obtained from the in vitro microsomal protein metabolism study (Table 3) can be converted to total liver CLint values for R(+) -propranolol, S(-)-propranolol, and R,S-propranolol (7.85 ± 0.71, 10.0 ± 1.42, and 9.25 ± 1.45 ml/min/g liver, respectively). These in vitro results are comparable with the derived CLint values in an in situ isolated perfused liver study - R(+) -propranolol, 8.54 ± 1.79; S(-)-propranolol, 12.1 ± 1.56; and R,S-propranolol, 11.8 ± 2.44 ml/min/g liver; Table 2]. The use of our earlier model, which does not explicitly recognize the ion trapping of drugs by acidic organelles (Weiss et al., 2000), with the propranolol optical isomers data resulted in a fit with similar model selection criteria (compared with the present model), but with significantly larger CLint values [R(+) -propranolol, 34.3 ± 2.17; S(-)-propranolol, 43.7 ± 5.01; and R,S-propranolol, 42.2 ± 4.87 ml/min/g liver; P < 0.001; Fig. 4] relative to those predicted from in vitro microsomal data. Therefore, it is apparent that the contribution of ion trapping in subcellular compartments to intracellular drug distribution must be taken into account to obtain a CLint value that is consistent with in vitro metabolic values.

In addition, the vesicular ion-trapping constant Kv significantly decreased following monensin treatment for R(+) -propranolol, S(-)-propranolol, and racemic propranolol (Kv control/Kv treatment: 33-, 34-, and 35-fold, respectively). Kv is defined by the relative rates of permeation into and out of the acidic lysosomal and mitochondria organelles. Ion trapping greatly reduces the permeation out of the organelles leading to an apparent large distribution space and a large Kv. Monensin abolishes the ion trapping leading to small Kv values. Thus, the Kv value becomes a sensitive ion-trapping marker. Table 4 shows that the observed Kv values are very similar to theoretical values based on the likely ion trapping of the enantiomers and racemate by acidic organelles. No differences in Kv values (i.e., extent of ion trapping) was found between R(+) - and S(-)-propranolol, consistent with both enantiomers having the same pKav value.

The relative contribution of ion-trapping, microsomal and nonspecific binding, and distribution of unbound drug to overall sequestration of propranolol in the liver can be estimated from a formula described in detail previously (Siebert et al., 2004). Ion trapping, microsomal binding, and unbound drug distribution account, respectively, for 47.4, 47.1, and 5.5% of the sequestration of propranolol in the liver. Thus, ion trapping equals intracellular binding as a key determinant of propranolol hepatic sequestration.

Sensitivity analyses (Table 5) suggest that propranolol extraction is mainly defined by metabolism, permeability, and blood flow, each contributing to a similar extent. In contrast, its mean transit time is mainly defined by ion trapping, intracellular binding, PS, and blood flow (Table 5). No significant differences between control and monensin-treated groups were observed for hepatic extraction ratio for the propranolol optical isomers (Table 1). This finding is consis-

![Fig. 4. Comparison of intrinsic elimination clearance (CLint) values obtained from the in vitro microsomal protein metabolism study, derived from the modified two-phase physiologically based organ pharmacokinetic model (with an additional ion-trapping parameter, Kv) and derived from the earlier two-phase organ model (without Kv) for the enantiomers of propranolol and racemic propranolol fitting in an in situ isolated perfused liver study.](image-url)
tent with hepatic drug extraction being related to lipophilicity (Hung et al., 2001).

A major limitation in the present analysis is the need to restrict our modeling to sufficiently low concentrations of propranolol to avoid saturation of metabolism or binding processes. This restriction was imposed by the inability of the physiologically based pharmacokinetic model used in this work to be applied to nonlinear data. Clinically, propranolol is normally given in doses, which saturate both metabolism (von Bahr et al., 1982) and protein binding (Ludden, 1991). In addition, the binding of propranolol in the liver is also saturable (Anderson et al., 1978; Miyauuchi et al., 1993). Modeling of the nonlinearity of the hepatic elimination of propranolol is further complicated by the multiple metabolic pathways for propranolol and that some of the cytochrome P450 isozymes have a low affinity for propranolol, whereas others have a high affinity (Ishida et al., 1992). Work is now in progress to examine whether the parameters generated in this study can be used with nonlinear propranolol data following the modeling of saturable metabolism and binding effects.

In conclusion, our study has shown that, firstly, ion trapping contributes significantly to the hepatic disposition of propranolol. This contribution is most clearly demonstrated by the approximately 34-fold decrease of $K_r$ for $(R^+)$- and $S^-$-enantiomers and racemic propranolol following monensin treatment. Thus, $K_v$ is a highly sensitive indicator of ion-trapping. Secondly, the physiologically more active $S^-$-isomer showed higher hepatic $CL_{int}$, $PS$, and $K_v$ values than those of the $(R^+)$-isomer. Thirdly, there is no difference in ion-trapping between $(R^+)$- and $S^-$-propranolol, consistent with these two enantiomers having the same p$K_a$ value. Finally, monensin treatment did not affect $CL_{int}$, $PS$, $K_v$, or drug fraction unbound values in in situ liver perfusions or in in vitro microsomal metabolism studies for all optical isomers, implying that abolishing ion trapping does not affect the hepatic metabolism, permeability, and microsomal protein binding of propranolol.

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References


