Disposition Kinetics of Propranolol Isomers in the Perfused Rat Liver

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Received April 20, 2004; accepted June 3, 2004

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 (Keiding and Steineas, 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 stereochernistry 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-yl]-2-furyl]-2-furyl]-9-hydroxy-\( \beta \)-methoxy-

ABBREVIATIONS: [U-\( ^{14} \)C]DMO, dimethylxazolidine-2,4-dione; RBC, red blood cell; HPLC, high-performance liquid chromatography; \( f_{ub} \), unbound fraction; \( \text{Cl}_{\text{int}} \), intrinsic elimination clearance; \( K_{\text{eq}} \), equilibrium amount ratio characterizing the vesicular ion-trapping sites (ion-trapping parameter); \( K_{\text{up}} \), equilibrium amount ratio characterizing the intracellular binding sites; \( V_{\text{in}} \), extracellular reference space; \( \text{PS} \), permeability-surface area product; \( V_{\text{c}} \), cellular water volume; \( \text{pH}_{\text{in}} \), intracellular \( \text{pH} \).
a,2,8-tetramethyl-1,6-dioxaspiro[4,5]decane-7-butryc sodium salt), S(−)-propranolol (1-(1-methyl ethyl) amino)-3-(1-naphtale
nyloxy)-2-propanol), R(+) -propranolol, and RS-propranolol al
were obtained from Sigma-Aldrich (St. Louis, MO). Dimethylo
xazoline-2,4-dione (U-14C,DMO), [U-14C]sucrose, and [3H]water
were purchased from Amersham Biosciences UK, Ltd. (Little Chalfont, Buckinghamshire, UK).

In Situ Perfusion of the Isolated Rat Liver. Perfusion of the isolated rat liver used in this study was performed as described elsewhere (Cheung et al., 1996). Briefly, male Wistar rats weighing 200 to 250 g were anesthetized using an intraperitoneal injection of xylazine/ketamine (10/80 mg/kg). The laparatomized rats were hepar
inized with 200 units of heparin injected into the inferior vena
cava. The bile duct and the portal vein were cannulated (PE-10; Clay Adams, Parsippany, NJ) and using an intravenous 16-gauge cathe
ter, respectively. The liver was then perfused with 4-morpholinepro
panesulfonic acid (MOPS) buffer containing 2% bovine serum alu
min and 15% washed canine red blood cells (RBCs), adjusted to pH 7.40, and oxygenated via a silastic tubing lung, ventilated with an
atmosphere of 100% pure oxygen. A peristaltic pump was used as a noncirculating perfusion system. The animals were sacrificed by
intracardiac perfusion with 0.9% NaCl solution. The isolated rat liver used in this study was performed as described
by Cheung et al. (1996). The injection was timed to coincide with the start of a computer
ment of liver viability was by macroscopic appearance, measurement of bile flow, oxygen consumption, and portal resistance pressure
(Cheung et al., 1996).

Perfusions were adjusted to a flow rate of 15 ml/min and given a 10-min period to stabilize before the injection of the first bolus.
Aliquots (50 µl) of perfusion medium containing a particular pro
pranolol optical isomer [0.06–0.11 µmol] as determined by high
performance liquid chromatography (HPLC) assay, [U-14C]sucrose (1.5 × 10^6 dpm) or [U-14C]DMO (1.5 × 10^6 dpm), and [3H]water (3 × 10^6 dpm) were injected into the liver with outlet samples collected via a fraction collector over 4 min (1 s × 20, 4 s × 5, 10 s × 5, 30 s × 5). The injection was timed to coincide with the start of a computer
controlled fraction collector (samples were collected over 4 min: 20 × 1 s, 5 × 4 s, 5 × 10 s, 4 × 30 s). Up to six bolus injections in
randomized order were administered per liver. The total perfusion
time was less than 2 h. Rats in the treatment group received a
10-min perfusion of 0.5 mM (final concentration) monensin/methanol
in buffer/RBC, and controls were perfused for 10 min with buffer/
RBC.

The collected samples were centrifuged, and 75-µl aliquots of the supernatant containing [3H]water, [U-14C]sucrose, or [3H]DMO
were taken for scintillation counting (MINAXI beta TRI-CARB 4000
series liquid scintillation counter; PerkinElmer Life and Analytical Sciences, Boston, MA). The remaining was vortexed and treated with
trichloroacetic acid/mobile phase (1:2) for HPLC analysis to deter
mination of the efflux rate constant. The unbound fraction of 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 (Millipore YM-30, 30,000 molecular weight cut-off; Millipore Corporation) and then centrifuged at 3000g for 10 min. The ultra
filtrate (in triplicate) was assayed by HPLC. The unbound fraction
(fun) was determined as the ratio of the free concentration to total
concentration of solute.

The impact of monensin on hepatic drug metabolism was assessed by
incubation of 0.06 to 0.11 µmol of each propranolol isomer with microsomal protein (0.35 mg/ml) at 37°C. Samples were then col
lected at 0.5, 10, and 20 min. The concentration in supernatant after
centrifugation determined by HPLC and the logarithm of the con
centration remaining in solution plotted against time to obtain a
slope and an extrapolated initial concentration (at time 0). Linearity of
the relationship and an extrapolated initial concentration being
much less than the reported Michaelis-Menten constant for propra
nolol (Ishida et al., 1992) was used to confirm linear kinetics. The
intrinsic elimination clearance (CLin) was estimated as the product of
the slope and the dose divided by the extrapolated initial concen
tration.

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 inter-sinusoidal mixing also called vascular dispersion (Roberts et al., 1988), transfer
across a permeability barrier, and the intracellular distruction 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 (Kv, char
acterizing the vesicular ion-trapping sites) and intracellular binding
(Kw, characterizing the intracellular binding sites) for model catic
n 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 propanolol have been described in detail previously (Siebert et al., 2004).

f, = \frac{a + bK_m}{a + bK_m + K_U(a + bK_m + k_f + K_m) + (b/K_m)K_Uk_f + K_mK_U} \tag{1}

where the permeation rate constant, k_f = f_p/SV/V_b, is the permea
tion clearance per extracellular volume (V_b), PS is the permeability
surface area product, and K_out is the efflux rate constant. The equi
librium amount ratio K_v = K_v/K_m characterizes the slowly accessible
pool for ion trapping, K_v is defined as a rapidly equilibrating intra
cellular binding sites (microsomal and nonspecific binding), and K_m
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_v
= CLin/V_C is the intrinsic elimination clearance normalized per
cellular volume V_C (Hung et al., 2001). Data were fitted and calcu
lated using Scientist (MicroMath Inc., Salt Lake City, UT).

Intracellular pH (pHi) was calculated from the concentration out
an adaptation of the method of Le Couteur et al. (1993). Briefly, the relationship

pH = \log p \left[ 10^{10^{10}} - 10^{10^{10}} \right] \tag{2}

where p is the distribution ratio of DMO described as

\frac{MTT_{DMO} - MTT_{DMO \_sucrose}}{MTT_{water} - MTT_{DMO \_sucrose}} \tag{3}
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-14C]sucrose perfusate concentration time data and model regressions are shown in Fig. 2B. The profiles are similar to the $R(\cdot)$ 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 $R(\cdot)$- and $S(-)$-enantiomers and racemic propranolol using coadministered [U-14C]sucrose and [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 $K_s$ significantly decreased following monensin treatment for $R(\cdot)$-propranolol, $S(-)$-propranolol, and racemic propranolol ($K_{\text{cv}}$ control/$K_{\text{cv}}$ treatment: 33-, 34-, and 35-fold, respectively). However, no changes following monensin treatment were observed for CL$\text{int}$, PS, or $K_b$ values for all optical isomers.

Table 2 also compares the stereoselectivity of the $R(\cdot)$ and $S(-)$-enantiomer and the propranolol racemate. It shows that the $S(-)$-isomer has significantly higher PS, CL$\text{int}$, and
Kb values than those of R-(H11001)-isomer but a comparable Ky value to R-(H11001)-isomer. The kinetic parameters PS, CLint, and Kb values were found to be increased 1.5-, 1.4-, and 1.2-fold, respectively, for the S-(H11002)-propranolol compared with the R-(H11001)-enantiomer, reflecting a more pronounced disposition of the S-(H11002)-enantiomer in liver tissue. However, no significant difference was found to exist between S-(H11002)-propranolol and R,S-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 CLint and fraction of drug unbound values for the R-(H11001)-propranolol were statistically different (P < 0.05) from those of S-(H11002)-propranolol and R,S-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 ± 0.19 and 7.27 ± 0.06, respectively).

Fig. 2. Typical outflow profiles for the enantiomers of propranolol and [U-14C]sucrose in the regressions. A, R(+) propranolol with sucrose. B, S(−)-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.

Kb values than those of R(+) -isomer but a comparable Ky value to R(+) -isomer. The kinetic parameters PS, CLint, and Kb values were found to be increased 1.5-, 1.4-, and 1.2-fold, respectively, for the S(−)-propranolol compared with the R(+) -enantiomer, reflecting a more pronounced disposition of the S(−)-enantiomer in liver tissue. However, no significant difference was found to exist between S(−)-propranolol and R,S-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 CLint and fraction of drug unbound values for the R(+) -propranolol were statistically different (P < 0.05) from those of S(−)-propranolol and R,S-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 ± 0.19 and 7.27 ± 0.06, respectively).

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(+) -propranolol; and C, S(−)-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.
TABLE 1
Nonparametric moments for the enantiomers of propranolol and racem 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 $K$ 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 isomers for the MTT value both in the control and treatment groups.

<table>
<thead>
<tr>
<th>Drug</th>
<th>Hepatic Extraction Ratio ($K$)</th>
<th>Mean Transit Time (MTT)</th>
<th>Normalized Variance (CV$^2$)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control$^a$</td>
<td>Treatment$^a$</td>
<td>Control</td>
</tr>
<tr>
<td>$R^+($)-Propranolol</td>
<td>0.96 ± 0.05</td>
<td>0.93 ± 0.07</td>
<td>109 ± 24.3</td>
</tr>
<tr>
<td>$S^-(−)$-Propranolol</td>
<td>0.97 ± 0.02</td>
<td>0.90 ± 0.09</td>
<td>121 ± 18.5</td>
</tr>
<tr>
<td>$R,S^-$-Propranolol</td>
<td>0.96 ± 0.03</td>
<td>0.93 ± 0.06</td>
<td>119 ± 20.3</td>
</tr>
</tbody>
</table>

$^a$ Before monensin treatment.
$^b$ After monensin treatment.

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 (Luddon, 1991), liver binding (Anderson et al., 1978; Miyauchi 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.
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 output 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 in the liver at those times. The overall output profiles obtained in our laboratory using red blood cell free perfusate yielded similar-shaped profiles as found for control livers but with a much slower terminal phase. Therefore, hypoxia did not appear to affect either the peak output concentration or subsequent rapid decline and contrasts with the data obtained in Tables 2 and 3, monensin’s effect can be attributed almost exclusively to its reducing the distribution of propranolol into acidic cell organelles.

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 β-blockers (such as atenolol or propranolol) with a single chiral center vary in their stereoselectivity to bind to the β_{1} or β_{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 is comparable at lower doses but stereoselective at higher doses, due to hepatic saturation of S(-)-propranolol clearance and that propranolol enantiomer plasma binding is not stereoselective or dose-dependent (Marier et al., 1998). In the dog, a larger distribution volume of S(-)-propranolol has been suggested (Bai et al., 1983), and it has been shown that there is a larger uptake of S(-)-propranolol into rat heart tissue when compared with R(+-)-propranolol (Kawashima et al., 1976).

The modeling of perfuse impulse-response data in our study has shown that there is a preferential uptake of the S(-)-propranolol enantiomer by the intracellular binding sites relative to R(+-)-propranolol (Table 2). The kinetic parameters PS and K_{v} were found to be increased about 1.4-fold for the S(-) compared with the R(+-)-enantiomer, reflecting

### Table 4

<table>
<thead>
<tr>
<th>Drug</th>
<th>pK_{a}</th>
<th>Lysosomes/Intracellular Concentration Ratio (l/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>

a Vesicle/cytosol concentration ratio = (1 + 10^{pK_{a}-pH})/(1 + 10^{10-pK_{a}-pH}) (Goldstein et al., 1974), where pH \approx 7.27 is the cytosolic pH (Le Couteur et al., 1993), pH_{l} \approx 4.4 is the pH of lysosomes (Daniel, 2003), and pH_{v} \approx 6.67 is the mitochondria pH in the fasted state (Scholl et al., 1980).

b Given that the fraction of lysosomes (f_{lys}) and mitochondria (f_{mito}) to cytosol is 1 and 20% (Rhoades and Pflanzer, 1996), the overall bound drug vesicles/intracellular distribution ratio (v/i) for propranolol can be estimated from the individual organ volume fraction and concentration ratio above using the equation: K_{v} = f_{lys} \times l/i + f_{mito} \times m/i.

c Data fitting results using the physiologically based two-phase organ pharmacokinetic model.
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 hepatic 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 tissue CLint values for R(+) -propranolol, S(-)-propranolol, and R,S-propranolol (7.85 ± 0.71, 10.0 ± 1.42, and 9.25 ± 1.46 ml/min/g liver, respectively). These in vitro results are comparable with the derived CLint values in an 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 pKa 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)
tient 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; Miyauchi 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 Kᵣ for R⁺- and S(-)-enantiomers and racemic propranolol following monensin treatment. Thus, Kᵣ is a highly sensitive indicator of ion-trapping. Secondly, the physiologically more active S(-)-isomer showed higher hepatic CLᵣ, PS, and Kᵣ 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 pKᵣ value. Finally, monensin treatment did not affect CLᵣ, PS, Kᵣ or drug fraction unbound values in situ liver perfusates or 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.

Acknowledgments

We acknowledge the support of the National Health and Medical Research Council of Australia and the Queensland and New South Wales Lions Kidney and Medical Research Foundation. We are also grateful to Paul Mills and Melanie Thompson for assistance in generating the data for propranolol disposition in hypoxic livers.

References


