THE PHARMACOKINETICS OF A THIAZOLE BENZENESULFONAMIDE $\beta_3$-ADRENERGIC RECEPTOR AGONIST AND ITS ANALOGS IN RATS, DOGS, AND MONKEYS: IMPROVING ORAL BIOAVAILABILITY


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ABSTRACT:

The pharmacokinetics and oral bioavailability of (R)-N-[4-[2-[2-hydroxy-2-(pyridin-3-yl)ethyl]amino]ethyl]phenyl]-4-[4-[4-(trifluoromethyl)phenyl]thiazol-2-yl]benzenesulfonamide (1), a 3-pyridyl thiazole benzenesulfonamide $\beta_3$-adrenergic receptor agonist, were investigated in rats, dogs, and monkeys. Systemic clearance was higher in rats (~30 ml/min/kg) than in dogs and monkeys (both ~10 ml/min/kg), and oral bioavailability was 17, 27, and 4%, respectively. Since systemic clearance was 25 to 40% of hepatic blood flow in these species, hepatic extraction was expected to be low, and it was likely that oral bioavailability was limited either by absorption or a large first-pass effect in the gut. The absorption and excretion of $^3$H-labeled 1 were investigated in rats, and only 28% of the administered radioactivity was orally absorbed. Subsequently, the hepatic extraction of 1 was evaluated in rats (30%) and monkeys (47%). The low oral bioavailability in rats could be explained completely by poor oral absorption and hepatic first-pass metabolism; in monkeys, oral absorption was either less than in rats or first-pass extraction in the gut was greater. In an attempt to increase oral exposure, the pharmacokinetics and oral bioavailability of two potential prodrugs of 1, an N-ethyl ((R)-N-[4-[2-[2-hydroxy-2-(3-pyridinyl)ethyl]amino]ethyl]phenyl]-4-[4-[4-(trifluoromethyl)phenyl]thiazol-2-yl]benzenesulfonamide (2) and a morpholine derivative ((R)-N-[4-[2-[2-(3-pyridinyl)morpholin-4-yl]ethy]phenyl]-4-[4-[4-(trifluoromethyl)phenyl]thiazol-2-yl]benzenesulfonamide; 3), were evaluated in monkeys. Conversion to 1 was low (~3%) with both derivatives, and neither entity was an effective prodrug, but the oral bioavailability of 3 (56%) compared with 1 (4%) was significantly improved. The hypothesis that the increased oral bioavailability of 3 was due to a reduction in hydroxy bonding sites in the molecule led to the design of (R)-N-[4-[2-[2-hydroxy-2-(pyridin-2-yl)ethyl]amino]ethyl]phenyl]-4-[4-(4-trifluoromethyl)phenyl]thiazol-2-yl]benzenesulfonamide (4), a 2-pyridyl $\beta_3$-adrenergic receptor agonist with improved oral bioavailability in rats and monkeys.

The prevalence of obesity is increasing throughout the world and especially in the United States (Kuczmarski et al., 1994, Wickelgren, 1998). This health risk is associated with the onset of other conditions such as noninsulin-dependent diabetes and cardiovascular disease (Pi-Sunyer, 1993). Basically, obesity is the result of an imbalance between caloric intake and energy expenditure. The challenge of reducing caloric intake in an obese population has led to a focus on pharmaceutical intervention to achieve weight loss. A number of therapeutic strategies have been explored for the pharmacological treatment of obesity (Kordik and Reitz, 1999). One potential mechanism for increasing energy expenditure is via activation of a unique $\beta_3$-adrenergic receptor subtype, the $\beta_3$-adrenergic receptor (Arch and Wilson, 1996; Dow, 1997; Weber, 1998). This receptor has been identified on the surface of rat adipocytes, and its activation results in the stimulation of lipolysis (Arch et al., 1984). In brown adipose tissue, $\beta_3$-adrenergic receptor activation leads to up-regulation of UCP1, a mitochondrial uncoupling protein, which uncouples oxidative phosphorylation from fatty acid $\beta$-oxidation, resulting in an increase in energy expenditure (Lowell and Flier, 1997). In rats, this increase is reflected in thermogenesis and weight loss due to a decrease in body lipid with no loss in muscle mass (Arch et al., 1990). Recently, it was demonstrated that chronic treatment of monkeys with selective human $\beta_3$-adrenergic receptor agonists elicits lipolysis and metabolic rate elevation (Fisher et al., 1998; Hom et al., 2001). Thus, $\beta_3$-adrenergic receptor agonists might be useful for the treatment of obesity in humans. A major barrier to the development of this class of compounds has been low oral bioavailability. Much effort has focused in the design of more orally bioavailable $\beta_3$-adrenergic receptor agonists (Naylor et al., 1998, 1999; Weber et al., 1998; Parmee et al., 1999; Shih et al., 1999; Bitu et al., 2000; Brockunier et al., 2000; Feng et al., 2000; Ok et al., 2000). In this paper, we report the pharmacokinetics of the $\beta_3$-adrenergic receptor agonist (R)-N-[4-[2-[2-hydroxy-2-(pyridin-3-yl)ethyl]amino]ethyl]phenyl]-4-[4-[4-
(trifluoromethyl)phenyl]thiazol-2-yl]benzenesulfonamide (1, Fig. 1) and its analogs (Mathvink et al., 2000b) in rats, dogs, and monkeys. We describe efforts to identify the factors that contribute to the poor oral bioavailability of 1. Based on that information, we proposed a rationale for redesign that led to (R)-N-[4-2-(2-hydroxy-2-(pyridin-2-yl)ethyl)aminoethyl]phenyl]-4-[4-(4-trifluoromethyl)phenyl]thiazol-2-yl]benzenesulfonamide (4, Figure 1), a β3-adrenergic receptor agonist with improved oral bioavailability in rats and monkeys.

Materials and Methods

Chemicals. 1, 2, 3, and 4 were obtained from Merck Research Laboratories. 3H-labeled 1 (226.9 mCi/mg) was synthesized by the Labeled Compound Synthesis Group, Merck Research Laboratories. The purity of the labeled and unlabeled compounds was >97%. HPLC grade solvents were from Fisher Scientific (Fair Lawn, NJ). Ammonium acetate, 85% lactic acid solution, PEG 20,000, and TFA were purchased from Aldrich Chemical Co. (Milwaukee, WI). Isotonic saline solution was from Radix Laboratories (Eau Claire, WI). Ammonium acetate, 85% lactic acid solution, PEG/h2210 (20:60:20, v/v/v). Three rats were dosed by oral gavage at 10 mg/kg (2 ml/kg; 5 mg/ml) in a vehicle consisting of 0.5% methylcellulose (Methocel:Tween 80 (90:10, v/v)). Serial blood samples (0.3 ml) were collected via the arterial cannula at 2 (i.v. only), 5, 15, and 30 min and at 1, 2, 4, 6, 8, 10, and 24 h post dose. Blood was transferred to heparinized microcentrifuge tubes; plasma was collected by centrifugation and stored at −20°C. Rats were euthanized after the last blood sample. The same oral dose formulation was used for compound 4. For i.v. dosing, 4 was administered at the same concentration as 1 but in a vehicle that consisted of ethanol/polyethylene glycol 4000/90% saline (20:60:20, v/v/v).

Hepatic extraction. Nine rats (N = 3/dose) had cannulas surgically implanted in the femoral artery, femoral vein, and portal vein. Rats were dosed with 1 by 30 min infusion via the femoral vein (N = 3) or via the portal vein (N = 3) at 0.2 mg/kg (1 ml/kg; 0.2 mg/ml) in a vehicle consisting of ethanol/polyethylene glycol 4000/90% saline (20:60:20, v/v/v). Three rats were dosed with 1 by oral gavage at 10 mg/kg (2 ml/kg; 5 mg/ml) in 0.5% Methocel containing 0.03 M HCl. Serial blood samples (0.3 ml) were collected from the portal vein and femoral artery at 10, 20, and 30 min after the onset of infusion and at 5, 15, and 30 min and at 1, 2, 4, 10, and 24 h postinfusion. Only arterial blood was sampled from the rats dosed via the femoral vein. Blood was transferred to heparinized microcentrifuge tubes; plasma was collected by centrifugation and stored at −20°C. The same oral dose formulation was used for compound 4. For the femoral and portal vein routes of administration for the infusion studies; however, 4 was administered as described above for 1, but the vehicle was fresh rat plasma.

Absorption and excretion in rats. To determine route of excretion, silastic cannulas were implanted in the bile duct and the proximal duodenum 1 day before the experiment while the rats were under pentobarbital anesthesia. The tubing was externalized at the center of the back and protected using a Harvard Apparatus tethering system (Harvard Apparatus, Holliston, MA). The cannulas were reconnected to permit recirculation of bile. All rats had polyethylene...
cannulas implanted in the femoral artery to facilitate collection of blood; animals to be dosed intravenously had a microcannula cannula implanted in the femoral vein. The vascular cannulas were externalized at the same site as the biliary cannulas. The rats were placed in NaI metabolism cages (NaI Nunc International, Naperville, IL) where they were given access to a solution of 280 mM dextrose:150 mM NaCl:7 mM KCl ad libitum. The next day, the cannulas were attached to a dual channel swivel for collection of bile and infusion of 10 mM sodium taurocholate:280 mM dextrose:150 mM NaCl:7 mM KCl (2 ml/h). Rats were dosed with 1 by oral gavage at 10 mg/kg (N = 3) or by bolus injection into the venous cannula at 3 mg/kg (N = 3). Bile, urine, and feces were collected for 5 days; blood samples (0.3 ml) were collected at predetermined time points as described previously. The volume of bile was determined gravimetrically, assuming a density of 1.0 g/ml. Samples were collected by venipuncture from the femoral vein while the jugular vein into Vacutainer tubes containing heparin and stored on ice (BD Vacutainer Systems, Franklin Lakes, NJ). Collection was made predose and at 2 h postdose. Aliquots of the blood samples (150 μl) were transferred to microcentrifuge tubes containing heparin, and plasma was collected by centrifugation. Concentrations of 1 in blood and plasma were quantified using an LC/MS/MS method.

In vitro. Stock solutions of 1 were made at 1, 5, 20, 50, and 100 μg/ml in 0.02 M lactic acid. A stock solution of 1 (0.02MCl/ml) was made in the same vehicle. Aliquots of rat, dog, and monkey blood (1 ml) were preincubated at 37°C for 15 min and then spiked with 10 μl of the radiolabel solution and 10 μl of stock solution to give final concentrations that ranged from 10 to 1000 ng/ml (each containing 0.2 μCi). The pH of the resulting samples was 7.4. Samples were incubated for an additional 20 min at 37°C, and then plasma was prepared by centrifugation. Radioactivity content in 100-μl plasma aliquots was determined by direct liquid scintillation counting and compared with the recovery of radioactivity from a aliquot of plasma spiked with the same amount of radioactivity.

Analytical Assays. HPLC. HPLC system components included a Shimadzu SCL 10A system controller, two Shimadzu Scientific Instruments, Kyoto, Japan), and a PerkinElmer series 200 autoinjector (PerkinElmer Instruments, Norwalk, CT).

Radioactivity measurement. Radioactivity in plasma was determined by counting 0.1 to 0.2 ml aliquots in glass vials containing 6 ml of liquid scintillation cocktail (Insta-Gel XF; Packard BioScience, Meriden, CT). Blood and feces homogenates were transferred to combustion cups, dried and combusted on a Packard model 307 sample oxidizer. Radioactivity measurements were made in a Beckman LS5000TD liquid scintillation spectrometer (Beckman Coulter, Inc., Fullerton, CA). Quench corrections were automatic and by the H-number method.

LC/MS/MS. Plasma or blood concentrations of 1 (or 4) were quantified by using an LC/MS/MS method. A volume from 0.05 to 0.2-ml plasma or blood was spiked with 20 ng 5, diluted with 0.5 ml 0.1M sodium carbonate (pH 10), and extracted with 4 ml of ethyl acetate. The ethyl acetate extract was evaporated under nitrogen, reconstituted in a mobile phase consisting of 80% acetonitrile, 20% 10 mM NaH2OAc, 0.1% TFA, and injected onto a Spherisorb C8 HPLC column (4.6 × 50 mm; Waters Corp., Milford, MA) equilibrated with mobile phase at a flow rate of 1 ml/min. HPLC column effluent was analyzed directly on a Sciex API III mass spectrometer (PE Sciex, Ontario, Canada) using the heated nebulizer interface (positive ion detection). The parent/product ion combinations 625-487 and 592-574 were used for multiple reaction monitoring of 1 (or 4) and 5, respectively. A standard curve was generated from the mean of three replicates, which were made by spiking an equal volume of plasma from untreated animals with increasing amounts (0.1–1000 ng; 11 concentrations and a blank control) of 1 (or 4) and 20 ng of the internal standard (5). The peak area ratio of the 1 (or 4) response to that of the internal standard was plotted versus the amount of drug spiked to obtain the standard curve. The amount of drug in plasma was quantified by comparison of its peak area ratio in a given sample to the standard curve. The lower limit of quantification was typically between 0.5 and 2 ng/ml. Precision was assessed every time the assay was performed by using a minimum of three replicates at each concentration. At each standard concentration (0, 0.1, 0.2, 0.5, 1, 5, 10, 50, 100, 500, and 1000 ng), the percent relative standard deviation was typically less than 10%. Accuracy of the regression fit also was assessed by calculating the read back concentrations of the standards, and these typically were within 10% of their nominal values. Prodrugs 2 and 3 were quantified as described above with the exception that the parent/product ion combinations m/z 653-635 and 651-558, respectively, were used for multiple reaction monitoring.

Pharmacokinetic Calculations. Pharmacokinetic parameters after i.v. and p.o. dosing were analyzed by noncompartmental methods (Gibaldi and Perrier, 1982). Plasma AUC<sub>inf</sub> values (x being the time of the last plasma concentration measured) were estimated by using the log-linear trapezoidal method. The terminal phase was determined by visual inspection of the log-transformed concentration-time data. The terminal phase rate constant (k<sub>10</sub>) was obtained from linear regression analysis of the log-transformed terminal phase concentration-time data. AUC<sub>inf</sub> was estimated by dividing the last plasma concentration value measured by the terminal phase rate constant; this residual area was added to AUC<sub>0-<inf>∞</inf></sub> to obtain AUC<sub>0-<inf>∞</inf></sub>. In all cases, extrapolated areas (AUC<sub>0-<inf>∞</inf></sub>) accounted for less than 30% and typically less than 10% of the total area under the plasma concentration-time curves (AUC<sub>0-<inf>∞</inf></sub>). Plasma clearance (CL<sub>p</sub>), terminal phase half-life (t<sub>1/2</sub>), volume of distribution at steady state...
(Vdᵦ), oral bioavailability (F), and hepatic extraction (Eₒ) were estimated based on eqs. 1 through 6 shown below:

\[ C_l_p = \frac{Dose}{AUC_{iv,n}} \]  
(1)

\[ t_{1/2} = ln2/C_l_p \]  
(2)

\[ Vdᵦ = (\text{Dose} \cdot AUMC_{iv,n})/AUC_{iv,n} \]  
(3)

\[ F = AUC_{iv,d}/AUC_{iv,n} \]  
(4)

\[ f_a/f_s = AUC_p/AUC_{iv,n} \]  
(5)

\[ Eₒ = 1 - f_a \]  
(6)

\( Cl_b \) blood (systemic) clearance; p.v., portal vein; NC, not calculated.

* Values represent mean ± S.D.

# Results

The pharmacokinetics and oral bioavailability of 1 (Fig. 1) were investigated in Sprague-Dawley rats, beagle dogs, and rhesus monkeys dosed orally at 10 mg/kg and intravenously at doses that ranged from 0.2 to 3 mg/kg. Pharmacokinetic data are summarized in Table 1. Following i.v. dosing, the total plasma clearance (\( Cl_p \)) was higher in rats (62 ml/min/kg) than in dogs (20 ml/min/kg) and monkeys (19 ml/min/kg). In rats and monkeys, the increase in plasma AUC value was proportional to the increase in dose over the range from 0.2(0.3) to 3 mg/kg. A single i.v. dose was evaluated in dogs. The volume of distribution at steady state (\( Vdᵦ \)) was generally large and ranged from 23 l/kg in rats and 12 l/kg in dogs to 6 l/kg in monkeys. The high plasma clearance values obtained with 1 prompted examination of the blood-to-plasma partition ratio.

The blood-to-plasma concentration ratios of \(^3\)H-labeled 1, determined in vitro in rat, dog, and monkey blood, were approximately 1.6 to 2 over a drug concentration range of 10 to 1000 ng/ml. This estimate was supported by a time course study of the distribution of 1 in blood collected from i.v.-dosed rats over a 24-h period, in which the blood-to-plasma concentration ratio was ~2 over a range from 13 to 5000 ng/ml.

After oral dosing, 1 was absorbed with a \( T_{max} \) that ranged from 2 to 3 h in rats, dogs, and monkeys. Dose-normalized plasma AUC values for the parent drug were higher in dogs (11 \( \mu \)g \cdot min / kg / mg / ml) than in rats (3 \( \mu \)g \cdot min / kg / mg / ml) or monkeys (2 \( \mu \)g \cdot min / kg / mg / ml). The oral bioavailability in rats and dogs was 17% and 27%, respectively, and was lower in monkeys (4%).

The absorption and excretion of 1 were investigated in rats. \(^3\)H-labeled 1 was given orally and intravenously to bile duct-cannulated rats, and the excretion of radioactivity in bile, urine, and feces was monitored. Data are presented in Table 2. Five days after i.v. dosing, 63±13% of the administered dose was recovered in bile, 11±2% in urine, and 9±3% in feces. After oral dosing, 22±9% of the dose was recovered in bile, 6±3% in urine, and 75±20% in feces. Most of the radioactivity was excreted within 24 h post i.v. and p.o. dosing. Pharmacokinetic data from both studies are shown in Table 1.

Pharmacokinetic studies also were carried out in portal and femoral vein-cannulated rats and rhesus monkeys to evaluate hepatic extraction in these species. Animals were dosed via infusion into the portal or femoral vein, and systemic circulation was sampled at specified intervals postdose. Data are presented in Table 1. Hepatic extraction was calculated by comparing plasma AUC values obtained in rats and monkeys after i.v. (20.0 and 44.3 \( \mu \)g \cdot min / kg / mg / ml) and portal vein (14.0 and 23.7 \( \mu \)g \cdot min / kg / mg / ml) infusions. Hepatic extraction of 1 was 30 and 47%, respectively.

To determine whether \( N \)-alkyl derivatives of 1 could serve as prodrugs and improve oral absorption, particularly in monkeys, the pharmacokinetics of the \( N \)-ethyl (2) and morpholine (3) derivatives of
TABLE 2

Disposition of $^3$H-labeled 1 in bile duct-cannulated rats

<table>
<thead>
<tr>
<th>Values represent mean ± S.D.</th>
<th>i.v. Dose</th>
<th>Oral Dose</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(3 mg/kg)*</td>
<td>(10 mg/kg)</td>
</tr>
<tr>
<td>% of dose recovered</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bile 0–120 h:</td>
<td>63 ± 13</td>
<td>22 ± 9</td>
</tr>
<tr>
<td>Urine 0–120 h:</td>
<td>11 ± 2</td>
<td>6 ± 3</td>
</tr>
<tr>
<td>Feces 0–120 h:</td>
<td>9 ± 3</td>
<td>75 ± 20</td>
</tr>
<tr>
<td>Total: 0–120 h:</td>
<td>83 ± 8</td>
<td>103 ± 26</td>
</tr>
</tbody>
</table>

*N = 3 rats/dose

1 were examined in two monkeys dosed intravenously at 3 mg/kg. Chemical structures and pharmacokinetic parameters are shown in Fig. 1 and Table 3, respectively. Conversion of these potential prodrugs to 1 was evaluated by comparing the dose-normalized AUC values of 1 obtained after dosing 1 (16 μg · min · kg/mg · ml), 2 (0.4 μg · min · kg/mg · ml), or 3 (0.3 μg · min · kg/mg · ml) i.v. at 3 mg/kg. Conversion to 1 was low (<3%) with both derivatives. The $C_{max}$, $V_{dss}$, and $t_{1/2}$ values were 124 ml/min/kg, 22.3 l/kg, and 3.3 h for 2 and 64 ml/min/kg, 3.5 l/kg, and 2.4 h for 3. After oral administration of 2 and 3 at 10 mg/kg, dose-normalized plasma AUC values of 1 (1.1 and 1.8 μg · min · kg/mg · ml, respectively) were not higher than observed with 1 (2.7 μg · min · kg/mg · ml) administered orally at the same dose; therefore, neither entity was an effective prodrug. Dose-normalized plasma AUC values for intact 2 (0.5 μg · min · kg/mg · ml) and 3 (8.9 μg · min · kg/mg · ml) however, differed substantially, and oral bioavailabilities were estimated at 6% and 56%, respectively.

The pharmacokinetics of 1 were evaluated in rats, and data are shown in Table 4. The $C_{max}$, $V_{dss}$, and $t_{1/2}$ values were 20.1 ml/min/kg, 5.8 l/kg, and 5.7 h for 4 compared with 62 ml/min/kg, 23 l/kg, and 8 h for 1. After oral dosing at 10 mg/kg, $C_{max}$ (395 ng/ml) was 3-fold higher than 1 (126 ng/ml), and the oral bioavailability (30%) was approximately twice that of 1 (17%). The hepatic extraction of 4 also was evaluated in rats by comparing systemic AUC values following femoral and portal vein infusions. These data also are presented in Table 4. The hepatic extraction of 4 was 33%, and the plasma clearance of 4 was not dependent on the vehicle in which the drug was administered (ethanol/PEG 400/saline versus plasma).

The pharmacokinetics of 4 were evaluated subsequently in rhesus monkeys. Data are presented in Table 4. The $C_{max}$, $V_{dss}$, and $t_{1/2}$ values were 8 ml/min/kg, 3 l/kg, and 7 h for 4 compared with 19 ml/min/kg, 6 l/kg, and 9 h for 1. After oral dosing at 10 mg/kg, $C_{max}$ (374 ng/ml) was approximately 12-fold higher than 1 (32 ng/ml), and the oral bioavailability (23%) was 4-fold higher than that of 1 (~5%).

Discussion

The pharmacokinetics and oral bioavailability of 1 were investigated in rats, dogs, and monkeys. Moderate to high plasma clearance values ($C_{lp}$) in these species were attributed in part to partitioning into red blood cells since blood-to-plasma ratios were estimated to be ~2. Therefore, blood clearance was 2-fold lower than plasma clearance and approximately 25 to 40% of hepatic blood flow in each species. On this basis, if 1 were well absorbed and cleared mainly in the liver, the oral bioavailability would be expected to exceed 60%. After oral dosing, however, 1 was absorbed relatively slowly with $T_{max}$, ranging from 2 h in rats to 3 h in dogs and monkeys. Plasma AUC values for the parent drug were higher in dogs (10.8 μg · min · kg/mg · ml) than rats (2.7 μg · min · kg/mg · ml) or monkeys (2.0 μg · min · kg/mg · ml), and, consequently, oral bioavailabilities were 27, 17, and 4%, respectively. This suggested that 1 was either not well absorbed or underwent extraction in the gut. Low oral bioavailability was a major issue in rats and monkeys but not in dogs; therefore, studies focused on identifying the factors that contributed to low oral bioavailability in these two species.

To verify that the generally low oral bioavailability was due to poor oral absorption, the absorption and excretion of 1 were investigated in rats. $^3$H-labeled 1 was administered orally and intravenously to bile duct-cannulated rats, and the excretion of radioactivity in bile, urine, and feces was monitored. Five days after i.v. dosing, 63 ± 13% of the administered radioactivity was recovered in bile and 11 ± 2% in urine, indicating that biliary excretion of compound-related material was a primary route of elimination for this compound. The excretion of 9 ± 3% of the i.v. dose into the feces of bile duct-cannulated rats suggested that 1 underwent efflux from the gastrointestinal tract. Since 1 has been identified as a substrate for P-glycoprotein (Tang et al., 2002), it is likely that efflux into the gastrointestinal tract was mediated by this transporter.

After oral dosing, 22 ± 9% of the radioactivity was recovered in bile and 6 ± 3% in urine, indicating that only 28% of the administered radioactivity was absorbed. The remainder of the oral dose (75 ± 20%) was recovered in feces. These results supported the conclusion that 1 was not well absorbed and suggested that P-glycoprotein-mediated efflux might contribute, at least in part, to the low oral bioavailability. The metabolite profile was determined in bile collected from rats dosed orally with 1. The metabolites that were identified were primarily hepatic oxidation products and included the pyrindine N-oxide derivative, a primary amine resulting from N-dealkylation and loss of the pyridinyl-2-hydroxyethyl group; a carboxyblyc acid derived from N-dealkylation and loss of pyridinyl-2-hydroxyethylyamine; and the corresponding taurine and isothiocyanic acid conjugates (Tang et al., 2002).

To evaluate whether gut extraction contributed to the poor absorption of 1, it was necessary to determine hepatic extraction. Since $F = f_{ jours} · f_{ abs}$ and $F$ and $f_{ abs}$ have been determined experimentally, at least in the rat, determination of $f_{ jours}$ would allow us to assess $f_{ abs}$ indirectly ($f_{ abs} = F/(f_{ jours} · f_{ abs}))$. The hepatic first-pass effect was evaluated in rats and monkeys following portal or systemic infusion of drug. The increase in plasma
AUC values was proportional to the systemic dose over the concentration range from 0.2(0.3) to 3 mg/kg in both rats and monkeys. Hepatic extraction (\(E_h\)) was calculated by comparison of the plasma AUC values obtained by each route of administration. On this basis, the hepatic extraction of 1 in the rat and monkey was 30 and 47%, respectively. Results from the study in rats suggested that hepatic clearance was the primary contributor to the first-pass effect because \(f_{gh} = (F(f_{gh} \cdot f_{abs}) = 87\%)\) was high, and consequently, extraction by the gut was low. Therefore, the primary factor that limits the oral bioavailability of 1 in the rat is poor oral absorption. By using the same reasoning, the product \(f_{gh} \cdot f_{abs}\) could be estimated in the monkey, since both the oral bioavailability (4%) and hepatic extraction (47%) have been determined experimentally; thus, in the monkey, \(f_{gh} \cdot f_{abs} = F/f_h = 8\%\), which is considerably lower than the 24% \(f_{gh} \cdot f_{abs}\) estimated in the rat. Two factors can explain this result; either 1) oral absorption in monkeys is less than in rats or 2) the contribution from intestinal first-pass metabolism is greater in monkeys. Studies were not performed to substantiate either hypothesis.

Attempts were made to improve the oral bioavailability of 1 by synthesizing potential prodrugs. To determine whether N-alkylation of 1 was a viable prodrug strategy, the pharmacokinetics of the N-ethyl (2) and morpholine (3) derivatives of 1 were examined in i.v.-dosed monkeys. An estimate of the extent of conversion of the prodrugs to 1 was made by comparing plasma AUC values of 1 after i.v. administration of either 1, 2, or 3. Conversion to 1 was low (<3%) with both derivatives. After oral administration of 2 and 3, the plasma AUC value of 1 (1.1 and 1.8 \(\mu g \cdot min^{-1} \cdot kg^{-1} \cdot ml\), respectively) was not higher than that observed when 1 (2.7 \(\mu g \cdot min^{-1} \cdot kg^{-1} \cdot ml\)) was given at the same dose; therefore, neither derivative was an effective prodrug.

The oral bioavailability of intact 3, however, was high (56%). The increased oral bioavailability of 3 in comparison to 1 was assumed to be due to improved absorption, a result that demonstrated the potential for improving the oral bioavailability of this class of compounds.

Consideration of the prodrug pharmacokinetic data led to the hypothesis that increased absorption might be achieved by decreasing the number of hydrogen bonding sites within the ethanolamine functional group. One strategy was to change the linkage to the pyridine moiety from the 3- to the 2-position so that the pyridyl-nitrogen atom was positioned to hydrogen bond with the ethanolamine hydroxyl group as shown in Fig. 2. This analog (4) was synthesized, and its pharmacokinetics were evaluated in rats and monkeys. Plasma clearance values were lower, and oral bioavailability was higher in both species. To determine whether the increased oral bioavailability of 4 was due to improved absorption or decreased clearance, hepatic extraction of 4 was evaluated in rats. The hepatic extraction of 4 (33%) was similar to that of 1 (30%). Having obtained values for \(F\) (30%) and \(f_{gh}\) (1 - \(E_h\) = 67%) in rats, \(f_{gh} \cdot f_{abs}\) could be estimated as described previously. This estimate \((F(f_{gh} \cdot f_{abs} = 45\%)\) suggested that, at least in rats, the absorption of 4 was approximately 2-fold greater than that of 1 (24\%).

In conclusion, the oral bioavailability of 1, a \(\beta_1\)-adrenergic receptor agonist, is limited by poor oral absorption in rats and monkeys. The oral bioavailability of this compound was improved by making a slight modification to its structure, resulting in the 2-pyridyl analog 4, that was designed to increase intramolecular hydrogen bonding interactions and thereby minimize intermolecular interactions that may limit the oral absorption of this compound class.\(^2\)

### References


\(^2\) Subsequently, the disposition of 1 and 4 were evaluated in brain and plasma after i.v. dosing in CF1 mdr1a \(+/-\) and \(-/-\) mice (data not shown). Plasma concentrations of both drugs were similar in each strain of mice, but the concentrations in brain were 5- to 7-fold higher in the \(-/-\) strain. This data suggested that both 1 and 4 were P-glycoprotein substrates and that the increased oral absorption of 4 was not due to a difference in P-glycoprotein substrate selectivity but rather to an improvement in the intrinsic membrane permeability of this analogue.