Role of P-Glycoprotein in Region-Specific Gastrointestinal Absorption of Talinolol in Rats

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Received February 26, 2010; accepted June 9, 2010

ABSTRACT:

P-Glycoprotein (PGP) is nonuniformly distributed along the gastrointestinal (GI) tract; however, the data regarding regional differences in PGP function in the intestine are controversial. The aim of this work was to investigate the role of PGP efflux in region-specific absorption of talinolol from the GI tract in rats. Plasma talinolol concentrations were measured after several modes of administration, including high (40 mg/kg) and low (4 mg/kg) dose levels, to different segments of the GI tract (stomach versus colon), and codosing with PGP inhibitors (verapamil or cyclosporine). The bioavailability (F) of talinolol after high-dose administration to the stomach was significantly greater than that achieved by the low dose (approximately 18 versus 2%). Codosing of low-dose talinolol with cyclosporine increased F by approximately 5-fold (p < 0.01). For the high dose, codosing with PGP inhibitors did not increase the extent of absorption. Talinolol demonstrated poor colonic absorption that was significantly increased by coadministration with cyclosporine (F = 0.76 versus 8.1%). Oral verapamil significantly increased systemic clearance and the steady state volume of distribution of intravenous talinolol. A semiphysiological model was developed that successfully captured the pharmacokinetic profiles of talinolol after various modes of administration. PGP-mediated efflux appears to be a major factor responsible for GI region-specific absorption of talinolol in rats, and gastroenterotenic dosage forms may provide an advantage in the delivery of talinolol and PGP substrate drugs.

Introduction

P-Glycoprotein (PGP), a plasma membrane-bound ATP-dependent efflux transporter, is a well recognized factor that can influence drug pharmacokinetics. PGP is expressed in a wide range of normal tissues and functions to minimize the exposure to potentially toxic xenobiotics. This is achieved by localization of this transporter at various body boundaries where it can reduce drug absorption and distribution and facilitate drug excretion. For example, PGP is found at the bile canalicular side of hepatocytes, at the apical surface of proximal tubules in the kidney and in the endothelial cells of the blood-brain barrier, the blood-testis barrier, and the blood-mammary tissue barrier (Lin et al., 1999; Sharom, 2007). In the intestine, the PGP efflux pump is expressed at the apical membrane of enterocytes where it acts in concert with cytochrome P450-metabolizing enzymes to limit systemic absorption of orally administered drugs (Kivistö et al., 2004).

Various segments of the gastrointestinal (GI) tract exhibit different characteristics (e.g., biochemical environment, amount of fluid, microbial flora, and expression of absorption transporters) that may result in region-specific absorption properties for certain drugs. PGP is also nonuniformly distributed along the GI tract; however, the data regarding regional differences in PGP function in the intestine are controversial. Several groups have reported that PGP expression and activity increase progressively from the proximal to distal small intestine (Yumoto et al., 1999; Tian et al., 2002; Mouly and Paine, 2003; Dahan et al., 2009). In contrast, others have found the opposite trend in PGP distribution in the small intestine (Nakayama et al., 2000; Berggren et al., 2007). Studies that include the large intestine also provide conflicting conclusions. Some studies have shown that the highest PGP levels appear in the colon (Fojo et al., 1987; Fricker et al., 1996), whereas others reported that the jejunum is the region with maximal PGP expression (Nakayama et al., 2000; Berggren et al., 2007). The differences in efflux capacity between segments along the GI tract may affect the absorption of PGP substrates. Regional differences in PGP have been primarily assessed ex vivo and do not take into account the entirety of factors influencing in vivo drug absorption. Moreover, the diversity of applied methodologies undoubtedly contributes to data inconsistency. Hence, in vivo investigation is essential to determine the role of PGP in region-specific drug absorption.

An increasing number of drugs are reported to be substrates of PGP (Raub, 2006). Ascertaining the effect of PGP on drug absorption is complex because the majority of PGP substrates are also substrates of the metabolic enzyme CYP3A4. Therefore, the systemic bioavailability of such drugs is limited by both PGP-mediated efflux in the intestine and presystemic metabolism in the intestine and liver. Talinolol, a clinically used β1 selective adrenergic antagonist, is a known PGP substrate that is not metabolized by CYP3A4. The overall metabolic clearance of talinolol is low, and 99% of the drug is

ABBREVIATIONS: PGP, P-glycoprotein; GI, gastrointestinal; AUC, area under the time-concentration curve from time 0 to infinity.
eliminated unchanged (Trausch et al., 1995). This combination of properties is rare and makes talinolol a valuable probe for studying the effects of PGP on pharmacokinetic processes. Although there was no direct evidence of involvement of active carriers in talinolol absorption at the time this study was performed; it is now recognized that talinolol is also a substrate of the organic anion-transporting polypeptide transporter (Shirasaka et al., 2009, 2010).

The aim of this work was to evaluate the role of PGP in region-specific absorption of talinolol from the GI tract of rats. Rats are an established preclinical model for evaluation of drug absorption, and the GI tracts of rats and humans seem to be similar in their absorption properties for many drugs (Fagerholm et al., 1996; Chiou and Barve, 1998; Zhao et al., 2003; Cao et al., 2006). Our experimental strategy involved assessment of the pharmacokinetics of talinolol after several modes of administration, including various dose levels to different regions of the GI tract, as well as coadministration with PGP inhibitors.

Materials and Methods

Chemicals. Talinolol was kindly provided by Degussa (Essen, Germany). Cyclosporine was purchased from HELM AG (Hamburg, Germany). Midazolam and verapamil hydrochloride were purchased from Sigma-Aldrich (Rehovot, Israel). All other chemicals were of analytical reagent grade, and solvents were high-performance liquid chromatography-grade.

Animals. All surgical and experimental procedures were reviewed and approved by the Animal Experimentation Ethics Committee of The Hebrew University Hadassah Medical School (Jerusalem, Israel). Male Wistar rats (Harlan, Jerusalem, Israel) weighing 280 to 400 g were kept under a 12-h light/dark cycle with free access to water and food (standard rat chow diet, pellets 19520; Koffolk, Tel Aviv, Israel).

Anesthesia was initiated with a 1 ml/kg solution of 20 mg/ml ketamine-100 mg/ml xylazine (90:10, v/v) by intraperitoneal injection and maintained by pure ketamine as needed. In all rats, the right jugular vein was cannulated with polyethylene tubing (PE-50, Intramedic polyethylene tubing; BD Biosciences, Sparks, MD) to allow for blood sampling. An additional PE-50 cannula was inserted into the cecum of animals that received drugs directly to the large intestine. The cannula was fixed to the abdominal muscles using silk 4-0 ligatures and was exteriorized at the back of the neck. After surgery, animals were transferred to metabolic cages and allowed to recover overnight (12–16 h); during this period, animals were fasted and water was available ad libitum.

Experimental Procedure. Talinolol was administered to rats by several modes of delivery, at different dose levels, and with and without PGP inhibitors. All experimental groups, including doses and drug formulations, are presented in Table 1. Intravenous bolus doses were delivered through the jugular vein cannula followed by 0.2 ml of heparinized saline (50 IU/ml) to ensure delivery of the entire dose. Oral doses were delivered by a gavage needle. Delivery to the cecum was conducted through the surgically implanted cannula. In all experimental groups that received verapamil or cyclosporine, the PGP inhibitor was administered as a bolus (oral or intracecal) immediately before talinolol administration. Verapamil hydrochloride was administered as a 10 mg/ml solution in water. Cyclosporine was administered as a 150 mg/ml solution in 53% ethanol, 42% water, and 5% propylene glycol.

Systemic blood samples were collected into heparin-containing test tubes at predetermined time intervals. Plasma was separated by centrifugation for 8 min at 1000 g and stored at −20°C until analysis.

Analytical Procedure. Talinolol plasma concentrations were analyzed using an liquid chromatography-mass spectrometry system composed a Waters pump (600 controller), a Waters autosampler (717plus Autosampler), and a Waters Micromass ZQ mass spectrometer (Waters, Milford, MA). Plasma samples (180 μl) were mixed with 200 μl of NaOH (1 M) and 20 μl of internal standard (5 μg/ml midazolam). Both materials were extracted with 4 ml of ethyl acetate and evaporated to dryness using a vacuum evaporator. The residue was reconstituted with 70 μl of 20% acetonitrile and 80% water. The volume of injection was 20 μl. The mobile phase consisted of acetonitrile-water (20:80) containing 0.1% (v/v) formic acid, and the flow was set to 0.25 ml/min. Separation was achieved with an X Terra MS C18 column (3.5 μm, 2.1 × 100 mm; Waters) that was kept at 35°C. Retention times for talinolol and midazolam were 5.5 and 7 min. The detection masses (m/z) were 364.3 and 326.9, and the limit of quantification for talinolol was 0.1 ng/ml.

Pharmacokinetic Model. Noncompartmental data analysis was performed for each individual talinolol concentration-time profile. The maximum plasma drug concentration (C_max) and time to reach C_max (T_max) were obtained directly from the experimental data. Terminal half-life, area under the concentration-time curve from 0 to infinity (AUC) were calculated by the linear trapezoidal method, mean residence time, volume of distribution at steady state (V ss), and both systemic and oral clearances were calculated (Gibaldi and Perrier, 1982).

A semiphysiological model was developed to capture the pharmacokinetics of talinolol after different modes of administration (Fig. 1). The model incorporates four GI lumen transit compartments that describe the physiological movement of the drug inside the GI tract: stomach (S), upper small intestine (USI), lower small intestine (LSI), and large intestine (LI). The GI lumen compartments are connected by first-order transit rate constants (k). These rate constants were estimated from refitting a previously described GI transit model (Kagan and Hoffman, 2008a) to recovery data of an unabsorbable marker (phenol red) from different regions of the GI tract after oral administration to rats (Sawamoto et al., 1997). The previous model was modified such that no time delay was estimated for material transfer from the small to the large intestine. A reasonably good description of experimental transit data was obtained (data not shown), and the estimated rate constants were fixed for subsequent modeling (k = 3.56 × 10⁻² min⁻¹, kUSI = 1.69 × 10⁻² min⁻¹, and kLI = 0.70 × 10⁻² min⁻¹). These rate constants are similar to the previously estimated values and permitted the exclusion of the empirical time-lag parameter from the current model (Fig. 1). The rate constant for elimination of the drug from the last intestinal compartment (k LI) was estimated during the modeling process. Talinolol was assumed to be absorbed

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### Table 1

<table>
<thead>
<tr>
<th>No.</th>
<th>Animals</th>
<th>Dose mg/kg</th>
<th>Administration Mode</th>
<th>Formulation</th>
<th>P-Glycoprotein Inhibitor</th>
</tr>
</thead>
<tbody>
<tr>
<td>6</td>
<td>9</td>
<td>4 p.o.</td>
<td>B</td>
<td>C</td>
<td>Verapamil 5 p.o.</td>
</tr>
<tr>
<td>6</td>
<td>4 p.o.</td>
<td>A</td>
<td>B</td>
<td>C</td>
<td>Cyclosporine 100 p.o.</td>
</tr>
<tr>
<td>4</td>
<td>4 p.o.</td>
<td>B</td>
<td>C</td>
<td>C</td>
<td>Verapamil 5 p.o.</td>
</tr>
<tr>
<td>6</td>
<td>40 p.o.</td>
<td>B</td>
<td>C</td>
<td>C</td>
<td>Cyclosporine 100 p.o.</td>
</tr>
<tr>
<td>5</td>
<td>40 p.o.</td>
<td>B</td>
<td>C</td>
<td>C</td>
<td>Verapamil 5 p.o.</td>
</tr>
<tr>
<td>6</td>
<td>40 p.o.</td>
<td>B</td>
<td>C</td>
<td>C</td>
<td>Cyclosporine 100 p.o.</td>
</tr>
<tr>
<td>6</td>
<td>4 p.o.</td>
<td>C</td>
<td>C</td>
<td>C</td>
<td>Verapamil 5 p.o.</td>
</tr>
<tr>
<td>5</td>
<td>4 p.o.</td>
<td>C</td>
<td>C</td>
<td>C</td>
<td>Cyclosporine 100 p.o.</td>
</tr>
</tbody>
</table>

*Formulations of talinol: A, 2 mg/ml solution in 40% sodium phosphate buffer 0.2 M, pH 7; 40% propylene glycol and 20% ethanol; B, 8 mg/ml solution in 25% polyethylene glycol 400, 15% ethanol, and 60% water; and C, 1 mg/ml solution in 25% polyethylene glycol 400, 15% ethanol, and 60% water.*
from intestinal compartments by a first-order process \( (k_d) \) into corresponding GI wall compartments (WUSI, WLSI, and WLI). Each intestinal wall compartment represents a pool of enterocytes that, after absorption of the drug, can secrete drug back into the GI lumen by a PGP-mediated efflux mechanism (\( V_{\text{maxWUSI}}, V_{\text{maxWLSI}}, \) and \( V_{\text{maxWLI}} \)). Administration of a PGP inhibitor (cyclosporine) was assumed to affect the affinity of talinolol to the transporter (\( K_{\text{mCP}} \)).

Drug is eliminated from the central compartment by a linear elimination process \( (k_e) \) or transferred back to the intestinal wall compartments \( (k_{\text{dUSI}}, k_{\text{dLSI}}, \) and \( k_{\text{dLI}} \)). To reduce the number of estimated parameters, the volume of the central compartment \( (V_c) \) was fixed to 726 ml/kg, calculated by dividing the dose administered intravenously by the measured concentration (at 2 min) (Watanabe et al., 2009). Equations 1 to 9 were used to describe the pharmacokinetic model:

\[
d\frac{dA_c}{dt} = -(k_d + k_p - k_w) \cdot A_c + k_p \cdot A_p + k_w \cdot (WUSI + WULI + WLI)
\]

(1)

\[
d\frac{dA_p}{dt} = k_g \cdot A_c - k_p \cdot A_p
\]

(2)

\[
d\frac{dS}{dt} = -k_a \cdot S
\]

(3)

\[
d\frac{dUSI}{dt} = k_a \cdot S - (k_{dUSI} + k_{USI}) \cdot USI + \frac{V_{\text{maxUSI}} \cdot WUSI}{K_m + WUSI}
\]

(4)

\[
d\frac{dLSI}{dt} = k_{USI} \cdot USI - (k_{dLSI} + k_{LSI}) \cdot LSI + \frac{V_{\text{maxLSI}} \cdot WLSI}{K_m + WLSI}
\]

(5)

\[
d\frac{dLI}{dt} = k_{LSI} \cdot LSI - (k_{dLI} + k_{LI}) \cdot LI + \frac{V_{\text{maxLI}} \cdot WLI}{K_m + WLI}
\]

(6)

\[
d\frac{dWUSI}{dt} = k_w \cdot A_c + k_{dUSI} \cdot USI - k_w \cdot WUSI - \frac{V_{\text{maxUSI}} \cdot WUSI}{K_m + WUSI}
\]

(7)

\[
d\frac{dWLSI}{dt} = k_w \cdot A_c + k_{dLSI} \cdot LSI - k_w \cdot WLSI - \frac{V_{\text{maxLSI}} \cdot WLSI}{K_m + WLSI}
\]

(8)

\[
d\frac{dWLI}{dt} = k_w \cdot A_c + k_{dLI} \cdot LI - k_w \cdot WLI - \frac{V_{\text{maxLI}} \cdot WLI}{K_m + WLI}
\]

(9)

For intravenous administration, \( A_c(0) \) was set equal to dose and initial conditions for eqs. 2 to 9 were set to zero. For oral administration, \( S(0) \) was set equal to the oral dose level, and initial conditions for eqs. 1, 2, and 4 to 9 were set to zero. For cecal infusion, initial conditions for eqs. 1 to 9 were set to zero, and a zero-order input rate constant was added to eq. 6. Modeling was performed by simultaneous fitting of mean concentration-time profiles for talinolol administered alone or in combination with cyclosporine.

**Data Analysis.** The noncompartmental analysis was performed using WinNonlin (version 5.2; Pharsight, Mountain View, CA). An analysis of variance was applied to assess differences between more than two groups followed by a Tukey multiple comparisons test where appropriate. Comparisons between two groups were conducted using the two-tailed \( t \)-test, and \( p < 0.05 \) was considered statistically significant. Data are presented as means ± S.E.M., unless stated otherwise.

Model fitting and parameter estimation were performed using nonlinear regression analysis with the ADAPT 5 computer program (Biomedical Simulations Resource, University of Southern California, Los Angeles, CA) and the maximum likelihood method. The variance model was defined in eq. 10 as

\[
\text{VAR}_{i} = (\sigma_{1} + \sigma_{2} \cdot Y(\theta, t))^{2}
\]

(10)

where \( \text{VAR}_{i} \) is the variance of the \( i \)-th data point, \( \sigma_{1} \) and \( \sigma_{2} \) are the variance model parameters, and \( Y(\theta, t) \) is the \( i \)-th predicted value from the pharmacokinetic model. The goodness of fit was assessed by system convergence, Akaike information criterion, estimator criterion value for the maximum likelihood method, correlation coefficients, examination of residuals, and visual inspection.

**Results.**

Talinolol was administered to rats intravenously alone or in combination with oral verapamil or oral cyclosporine. Concentration-time profiles are presented in Fig. 2, and the corresponding pharmacokinetic parameters obtained by noncompartmental analysis are presented in Table 2. AUC values were used to calculate bioavailability after various modes of enteral talinolol administration. The value of systemic clearance obtained for talinolol administered intravenously without PGP inhibitors is in agreement with previously reported values (Tronde et al., 2003). Both systemic clearance and \( V_{\text{USI}} \) of talinolol after coadministration with oral verapamil were significantly higher than the corresponding values after intravenous dosing without verapamil (Table 2).

Bioavailability, typically calculated by comparing dose-normalized AUCs between various modes of administration, is based on the assumption that the clearance of the drug is the same for all groups (Gibaldi and Perrier, 1982). Given that verapamil significantly increases talinolol clearance, the calculation of oral talinolol bioavailability after coadministration with PGP inhibitors was based on the AUC data of the corresponding intravenous group (either intravenous
talinolol with oral verapamil or intravenous talinolol with oral cyclo-
sporine). Two dose levels of 40 and 4 mg/kg administered by different
modes of enteral administration were evaluated. In general, talinolol
pharmacokinetics after enteral administration was characterized by
high variability between animals. In addition, several concentration-
time profiles demonstrated two distinct concentration peaks, which is
a known phenomenon for talinolol pharmacokinetics (Weitschies et
al., 2005; Tubic et al., 2006b). Mean plasma concentration-time
profiles of talinolol after enteral administration at a high dose (40
mg/kg) are shown in Figs. 3 and 4. Two absorption peaks occurring at
1 and 4 h were evident in most individual pharmacokinetic profiles
after oral bolus administration, which is also reflected in the mean
data (Fig. 3). No double peaks were found after cecal infusion ad-
ministration. Talinolol demonstrated a significant GI region-specific
absorption: oral bolus administration produced much higher plasma
concentrations (Fig. 3) in comparison with intracecal administration
of the same dose (Fig. 4). The corresponding bioavailability values
were 18 and 0.76% (Table 3). The mean plasma concentration-time
profile of talinolol after a low oral dose (4 mg/kg) is shown in Fig. 5.
The double peak phenomenon was less evident after the low-dose oral
administration. A decrease in plasma concentration was more than
dose proportional in comparison with the high dose, and the bioavail-
ability was approximately eight times lower than that obtained after
administration of the high dose. This nonlinearity in absorption might
be attributed to the function of PGP. The administration of a low dose
of talinolol directly into the cecum was not performed because the plasma concentrations were predicted to be below the assay quanti-
fication limits.

To further investigate the effect of PGP on region-specific absorption, talinolol was administered orally in combination with two commonly used PGP inhibitors, verapamil and cyclosporine. Plasma concentration-time profiles of high-dose talinolol administered orally with or without PGP inhibitors are shown in Fig. 3. Administration with cyclosporine results in a pharmacokinetic profile that is very similar to the profile obtained after administration of talinolol alone. Administration with verapamil resulted in lower plasma concentrations in comparison with talinolol alone; however, these differences were not statistically significant because of high interanimal variability. These lower concentrations do not necessarily indicate a decrease in bioavailability. In fact, when the AUC data from corresponding intravenous groups were used, the bioavailability values after all three modes of administration were similar (Table 3).

### Table 2

| Data are presented as mean (S.E.M.). |
|-----------------|-----------------|-----------------|
| **Control**     | **Verapamil (p.o.)** | **Cyclosporine (p.o.)** |
| **4 mg/kg**     | **2 mg/kg**     | **2 mg/kg**     |
| \( t_{1/2} \) (min) | 64.3 (8.0) | 74.7 (7.5) | 71.4 (17.4) |
| AUC (\( \mu g \cdot min/ml \)) | 75.5 (9.1) | 19.7 (3.7) | 33.9 (7.6) |
| Cl (\( ml/min \cdot kg \)) | 58.6 (9.7) | 119.3 (19.6)* | 69.3 (16.7) |
| \( V_{ss} \) (ml/kg) | 1689 (441) | 4668 (995)* | 2311 (920) |
| MRT (min) | 27.7 (4.8) | 39.0 (5.8) | 29.5 (6.6) |
| Cl, systemic clearance; MRT, mean residence time. |
| * Statistically significant difference from control group (\( p < 0.05 \)). |
Pharmacokinetic parameters of talinolol after various modes of enteral administration with and without PGP inhibitors obtained by noncompartmental analysis

<table>
<thead>
<tr>
<th></th>
<th>Oral Bolus</th>
<th>Oral Bolus, Verapamil (p.o.)</th>
<th>Oral Bolus, Cyclosporine (p.o.)</th>
<th>Cecal Infusion Over 4 h, Cyclosporine (Cecal Bolus)</th>
<th>Oral Bolus</th>
<th>Oral Bolus, Verapamil (p.o.)</th>
<th>Oral Bolus, Cyclosporine (p.o.)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>40 mg/kg</td>
<td>40 mg/kg</td>
<td>40 mg/kg</td>
<td>40 mg/kg</td>
<td>4 mg/kg</td>
<td>4 mg/kg</td>
<td>4 mg/kg</td>
</tr>
<tr>
<td>( t_{1/2} ) (min)</td>
<td>89.1 (7.6)</td>
<td>63.5 (10.9)</td>
<td>92.6 (16.9)</td>
<td>86.3 (24.4)</td>
<td>90.1 (9.9)</td>
<td>63.8 (8.5)</td>
<td>64.3 (11.1)</td>
</tr>
<tr>
<td>AUC (( \mu \text{g} \cdot \text{min/ml} ))</td>
<td>134.5 (27.9)</td>
<td>71.2 (4.2)</td>
<td>119.9 (28.5)</td>
<td>5.7 (1.3)</td>
<td>54.7 (11.8)</td>
<td>1.4 (0.2)</td>
<td>19.0 (0.2)</td>
</tr>
<tr>
<td>( T_{\text{max}} ) (min)</td>
<td>160 (30)</td>
<td>192 (35)</td>
<td>230 (29)</td>
<td>240 (33)</td>
<td>252 (22)</td>
<td>170 (10)</td>
<td>252 (35)</td>
</tr>
<tr>
<td>( C_{\text{max}} ) (ng/ml)</td>
<td>745 (181)</td>
<td>372 (48)</td>
<td>600 (165)</td>
<td>23.6 (5.0)</td>
<td>208 (40)</td>
<td>10.6 (2.1)</td>
<td>118 (0.8)</td>
</tr>
<tr>
<td>( C_{\text{max}} ) (ml/min/kg)</td>
<td>371 (77)</td>
<td>569 (32)</td>
<td>546 (214)</td>
<td>9062 (2419)</td>
<td>944 (255)</td>
<td>3052 (364)</td>
<td>2167 (213)</td>
</tr>
<tr>
<td>MRT (min)</td>
<td>195 (13)</td>
<td>202 (24)</td>
<td>236 (27)</td>
<td>276 (30)</td>
<td>305 (20)</td>
<td>180 (8.2)</td>
<td>230 (28)</td>
</tr>
<tr>
<td>( F ) (%)</td>
<td>17.8 (3.7)</td>
<td>18.0 (1.1)</td>
<td>17.7 (4.2)</td>
<td>0.76 (0.17)</td>
<td>8.1 (1.7)</td>
<td>1.9 (0.3)</td>
<td>4.8 (0.5)</td>
</tr>
</tbody>
</table>

Plasma concentration-time profiles of low-dose talinolol administered orally with or without PGP inhibitors are shown in Fig. 5. Cyclosporine at a dose of 100 mg/kg was a significantly more potent enhancer of bioavailability for low-dose talinolol than verapamil at 5 mg/kg. Administration with cyclosporine increased the extent of absorption approximately 5-fold (\( p < 0.01 \)) in comparison with talinolol alone. Administration with verapamil doubled the bioavailability of talinolol; however, this value did not reach statistical significance.

Administration of high-dose talinolol into the cecum with cyclosporine resulted in a much higher plasma concentration (\( p < 0.05 \), except at the time point 120 min) and approximately a 10-fold higher bioavailability in comparison with cecum infusion without cyclosporine (Fig. 4; Table 3). Comparison of terminal half-lives (calculated by a noncompartmental approach) from all experimental groups (Tables 2 and 3) performed by analysis of variance did not show any significant difference among the groups.

The proposed pharmacokinetic model provided a good description of the observed phenomena in talinolol concentration-time profiles after various modes of administration. The datasets for coadministration of talinolol and verapamil were excluded from the modeling process because no reliable mechanistic explanation of verapamil effects on talinolol pharmacokinetics could be proposed. The model fits are shown in Figs. 2 to 5, and model parameters are presented in Table 4. In the final model, several assumptions were made to improve the precision in parameter estimation. The rate constants describing drug transfer between the central and intestinal wall compartments were set to be equal (\( k_{\text{in}} = k_{\text{out}} \)). In addition, the same absorption rate constant was assumed for all intestinal segments (\( k_{\text{ali}} = k_{\text{ali,SI}} = k_{\text{ali,L}} \)), which is a reasonable assumption for compounds with intermediate lipophilicity (Weitschies et al., 2005). The time course of cyclosporine concentrations in the GI wall is unknown; therefore, the time course of inhibition of PGP function could not be evaluated. An average inhibitory effect was modeled as a change in talinolol affinity to the transporter (\( K_{\text{al}} \)). Two separate values were estimated for oral and intracecal cyclosporine administration (\( K_{\text{alC1}} \) and \( K_{\text{alC2}} \)); and final estimates suggest a 2- and 10-fold decrease in affinity. Modeling also suggests that PGP efflux capacities are similar in the upper and lower small intestine and are approximately 2-fold higher in the large intestine. Given the variability in the experimental data, the proposed model reasonably captured all datasets. Additional modifications to the model (including biliary excretion, transporter-mediated uptake from the GI lumen, and GI region-specific absorption processes) did not improve model performance. On the other hand, a simpler model, in which GI transit compartments were directly connected to the central distribution compartment and PGP-mediated efflux (back into intestine) was driven by systemic talinolol concentrations, was unable to satisfactorily describe the pharmacokinetic profiles (data not shown).

Discussion

In this study, the role of PGP in GI region-specific drug absorption was investigated using talinolol as a model molecule. Intestinal PGP and CYP3A enzymes work together to minimize absorption of xenobiotics. Most PGP substrates can also undergo first-pass metabolism, which might confound the interpretation of in vivo experiments. Because of its negligible metabolic clearance, talinolol allows for selective assessment of PGP function without interference of metabolic degradation. The mode of drug administration can affect the pharmacokinetics of PGP substrates, and our model also provides insights for optimizing the delivery of such drugs. The in vivo approach is advantageous because it provides a realistic evaluation of a variety of factors that might affect pharmacokinetics, such as drug delivery site, transit kinetics of the drug along the intestine, and drug excretion from the systemic circulation into the gut lumen.

The pharmacokinetics of talinolol after oral administration is nonlinear, and the extent of absorption is highly dependent on the dose level. The bioavailability of the high oral dose of talinolol was approximately 8-fold higher than the bioavailability of the low dose. This significant difference might be attributed to saturation of PGP-mediated intestinal efflux, which allowed a greater amount of the drug to reach the systemic circulation after high-dose administration. On the other hand, after low-dose administration, the efflux mechanism...
was able to function more efficiently. This hypothesis was further substantiated by experiments with PGP inhibitors. The addition of either verapamil or cyclosporine to high-dose talinolol had no apparent effect on the extent of systemic absorption because the efflux mechanism was apparently saturated by talinolol and functioned at its maximum capacity. On the other hand, after administration with low-dose talinolol, cyclosporine was able to reduce the efflux and increase bioavailability. Verapamil demonstrated a trend for a similar effect, which was not statistically significant. These results are in line with a competitive inhibition mechanism as reported for both cyclosporine and verapamil (Maki et al., 2003).

Talinolol exhibits region-specific absorption properties with almost negligible absorption from the large intestine (less than 1%) compared with that found after oral administration (17.8%). The extent of absorption in humans was markedly decreased when talinolol was administered by 10-fold. Thus, negligible absorption from the large intestine (less than 1%) compared with that found after oral administration (17.8%).

Many drugs require administration more frequently than once daily to be effective and therefore could benefit from administration in a controlled-release drug delivery system. However, a common property of conventional controlled-release technologies is that a large portion of the drug load is released in the colon where the dosage form stays for a relatively long period of time. Here, the colonic delivery of talinolol led to a very low absorption. This finding agrees with the observations of Schwarz et al. (1998). A decrease in plasma concentrations is usually interpreted as decreased bioavailability. It was proposed that the verapamil inhibitory effect on certain absorptive carrier systems probably prevails over its effect on PGP (Schwarz et al., 2001). Talinolol was shown to have a much lower bioavailability when administered after coadministration of a high dose with verapamil was observed in this study, whereas the opposite trend was found for low-dose talinolol. A decrease in plasma talinolol concentrations after coadministration with verapamil has been shown in mice (Schwarz et al., 2001) and in humans (Schwarz et al., 1999). In rats, low-dose (4 mg/kg) verapamil increased plasma concentrations of talinolol after oral administration (20 mg/kg), whereas a higher verapamil dose (20 mg/kg) led to a decrease in plasma talinolol concentrations (Spahn-Langguth et al., 1998). A decrease in plasma concentrations is usually interpreted as decreased bioavailability. It was proposed that the verapamil inhibitory effect on certain absorptive carrier systems probably prevails over its effect on PGP (Schwarz et al., 2001). Talinolol was recently shown to be a substrate of organic anion-transporting polypeptide absorption transporters (Shirasaka et al., 2009, 2010). On the other hand, our data show that verapamil might influence both the volume of distribution and clearance, which might result in a decrease in plasma concentrations without affecting the extent of absorption.

The mechanism by which verapamil affects talinolol pharmacokinetics is not completely understood. In Caco-2 cell systems, talinolol demonstrated a significantly greater permeability in basolateral-to-apical experiments compared with the apical-to-basolateral direction, and this difference was minimized by the addition of verapamil (Wetterich et al., 1996; Tronde et al., 2003; Augustijns and Mols, 2004). A trend toward a decrease in plasma concentrations of talinolol after coadministration of a high dose with verapamil was observed in this study, whereas the opposite trend was found for low-dose talinolol. A decrease in plasma talinolol concentrations after coadministration with verapamil has been shown in mice (Schwarz et al., 2001) and in humans (Schwarz et al., 1999). In rats, low-dose (4 mg/kg) verapamil increased plasma concentrations of talinolol after oral administration (20 mg/kg), whereas a higher verapamil dose (20 mg/kg) led to a decrease in plasma talinolol concentrations (Spahn-Langguth et al., 1998). A decrease in plasma concentrations is usually interpreted as decreased bioavailability. It was proposed that the verapamil inhibitory effect on certain absorptive carrier systems probably prevails over its effect on PGP (Schwarz et al., 2001). Talinolol was recently shown to be a substrate of organic anion-transporting polypeptide absorption transporters (Shirasaka et al., 2009, 2010). On the other hand, our data show that verapamil might influence both the volume of distribution and clearance, which might result in a decrease in plasma concentrations without affecting the extent of absorption.

Many drugs require administration more frequently than once daily to be effective and therefore could benefit from administration in a controlled-release drug delivery system. However, a common property of conventional controlled-release technologies is that a large portion of the drug load is released in the colon where the dosage form stays for a relatively long period of time. Here, the colonic delivery of talinolol led to a very low absorption. This finding agrees with previous reports that bioavailability of talinolol from immediate-release tablets is approximately 2-fold higher than that from the controlled-release tablets (Tubic et al., 2006b). Even a relatively small delay in release (achieved by enteric coating) was shown to reduce absorption by approximately 50% in comparison with that of an immediate-release formulation (Weitschies et al., 2005). Thus, conventional controlled-release technologies that carry a significant part of the drug to distal regions of the GI tract are not suitable for the delivery of PGP substrates. On the other hand, continuous delivery to
of the proximal part of the GI tract, as provided by gastroretentive dosage forms (Hoffman et al., 2004; Streubel et al., 2006; Kagan and Hoffman, 2008b), might be beneficial for these drugs. An alternative approach would be administration of PGP substrate drugs with compounds that have PGP inhibitory properties; however, this might increase the potential for adverse effects.

Prolonged talinolol absorption was evident for rats after oral administration, and many profiles demonstrated double peaks. Direct intestinal secretion is a major component of talinolol clearance (Graßmann et al., 1996); hence, this continuous absorption phenomenon (in the presence of negligible colonic bioavailability) can probably be explained by an “enteroenteral cycling,” i.e., continuous excretion of talinolol into the lumen from the systemic circulation with subsequent reabsorption. This cycle can also explain a type of plateau in concentrations that was evident after intravenous administration in some rats. A similar type of behavior can be seen for talinolol administered intravenously with either verapamil or cyclosporine (Fig. 2, time points 120 and 150 min).

In conclusion, PGP-mediated efflux was demonstrated to be a major factor responsible for region-specific absorption of talinolol in rats. Two alternative approaches can be proposed to enhance systemic bioavailability of PGP substrate drugs: either region-specific drug delivery to the upper GI tract (that can be accomplished by gastroretentive dosage forms) or coadministration with PGP inhibitors that can significantly improve absorption, especially from the distal intestine. A drug-drug interaction with verapamil can affect the clearance of talinolol and this should be taken into account when one is calculating bioavailability values. A semiphysiological model was developed that successfully captured the observed pharmacokinetics of talinolol, as well as system-based phenomena, and might be further used for investigating the absorption of other PGP substrate drugs.

Acknowledgments. We thank Dr. Werner Weitschies from the University of Greifswald (Greifswald, Germany) for help in obtaining talinolol. We also thank Yael Ratz from the Hebrew University of Jerusalem for technical assistance and Dr. John M. Harrold from the University at Buffalo, SUNY for help in developing the mathematical model. Professor Amnon Hoffman is affiliated with the David R. Bloom Center for Pharmacy at the Hebrew University of Jerusalem.

References


