Role of P-glycoprotein in region-specific gastrointestinal absorption of talinolol in rats

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Running title:
Role of PGP in GI region-specific absorption of talinolol

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Text pages: 34
Tables: 4
Figures: 5
References: 40
Abstract: 227
Introduction: 646
Discussion: 1346
Abstract

P-glycoprotein (PGP) is non-uniformly distributed along the gastro-intestinal (GI) tract; however, the data regarding regional differences in PGP function in the intestine are controversial. The aim of this work is to investigate the role of PGP efflux in region-specific absorption of talinolol from the GI tract in rats. Plasma talinolol concentrations were measured following several modes of administration, including high (40 mg/kg) and low (4 mg/kg) dose levels, to different segments of the GI tract (stomach vs. colon), and co-dosing with PGP inhibitors (verapamil or cyclosporine A). The bioavailability (F) of talinolol following high dose administration to the stomach was significantly greater than that achieved by the low dose (about 18% vs. 2%). Co-administration of low dose talinolol with cyclosporine increased F by about 5-fold (p<0.01). For the high dose, co-dosing with PGP inhibitors did not increase the extent of absorption. Talinolol demonstrated poor colonic absorption that was significantly increased by co-administration with cyclosporine (F = 0.76% vs. 8.1%). Oral verapamil significantly increased systemic clearance and the steady state volume of distribution of intravenous talinolol. A semi-physiological model was developed that successfully captured the pharmacokinetic profiles of talinolol following various modes of administration. PGP-mediated efflux appears to be a major factor responsible for GI region-specific absorption of talinolol in rats and gastro-retentive 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 (Kivisto et al., 2004).

Various segments of the gastro-intestinal (GI) tract exhibit different characteristics (e.g., biochemical environment, amount of fluid, microbial flora, 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 increases 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 report that the jejunum is the region with maximal PGP expression (Nakayama et al., 2000; Berggren et al., 2007).
differences in efflux capacity between segments along the GI tract might impact 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 as 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 beta-1 selective adrenergic antagonist, is a known PGP substrate that is not metabolized by CYP3A4. The overall metabolic clearance of talinolol is low and 99% percent of the drug is 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 OATP transporter (Shirasaka et al., 2009; Shirasaka et al., 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 appear 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 following several modes of administration, including various dose levels to different regions of the GI tract, as well as co-administration with PGP inhibitors.
Materials and Methods

Chemicals

Talinolol was kindly provided by Degussa (Essen, Germany). Cyclosporine A 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 HPLC 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. Male Wistar rats (Harlan, Israel) weighing 280-400 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 1mL/kg solution of ketamine 20 mg/mL: xylazine 100 mg/mL (90:10 v/v) by intra-peritoneal 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, Becton-Dickinson, MD, USA) 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 administration, at different dose levels, with and without PGP inhibitors. All experimental groups, including doses and drug formulations, are presented in Table 1. Intravenous bolus (IV) 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 A, 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 A 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 LC–MS system comprised of Waters pump (600 controller), Waters autosampler (717plus Auto-sampler) and Waters Micro-mass ZQ mass spectrometer (Waters Corporation, Milford, MA, USA). Plasma samples (180 µL) were mixed with 200 µL NaOH (1 M) and 20 µL of internal standard (midazolam, 5 µg/mL). 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 XTerra C18 MS column (3.5 µm, 2.1 x 100 mm,
Waters Corporation, Milford, MA, USA) 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**

A noncompartmental data analysis was performed for each individual talinolol concentration-time profile. The maximum plasma drug concentration (C\text{max}) and time to reach C\text{max} (T\text{max}) were obtained directly from the experimental data. Terminal half-life, area under the concentration time curve from time zero to infinity (AUC, calculated by linear trapezoidal method), mean residence time (MRT), volume of distribution at steady state (V\text{ss}), and both systemic and oral clearances were calculated (Gibaldi and Perrier, 1982).

A semi physiological model was developed to capture the pharmacokinetics of talinolol following different modes of administration (Figure 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\text{t}). These rate constants were estimated from re-fitting 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 following 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\text{tS} = 3.56\times10^{-2} \text{ min}^{-1}, k\text{tUSI} = 1.69\times10^{-2} \text{ min}^{-1}, and k\text{tLSI} = 0.70\times10^{-2} \text{ 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 (Figure 1). The rate constant for
elimination of the drug from the last intestinal compartment (k_{d,L1}) was estimated during the modeling process. Talinolol was assumed to be absorbed from intestinal compartments by a first-order process (k_a) into corresponding GI wall compartments (WUSI, WLSI, and WLI). Each intestinal wall compartment represents a pool of enterocytes that, following absorption of the drug, can secrete drug back into the GI lumen by a PGP-mediated efflux mechanism (V_{max} and K_m) or transfer it to the central compartment (A_C) by a first-order process (k_{wc}). The PGP-mediated efflux mechanism was assumed to have GI region-specific capacity to reflect an unequal distribution of the transporter in the GI tract (V_{maxUSI}, V_{maxLSI}, and V_{maxLI}). Administration of a PGP inhibitor (cyclosporine) was assumed to affect the affinity of talinolol to the transporter (K_{mc}).

Drug is eliminated from the central compartment by a linear elimination process (k_{el}) or transferred back to the intestinal wall compartments (k_{cw}). In addition, talinolol can be distributed to and from a peripheral compartment (A_P) by first-order distribution processes (k_{cp} and k_{pc}). 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 IV by the measured concentration (at 2 min) (Watanabe et al., 2009). The following equations were used to describe the pharmacokinetic model:

\[
\frac{dA_C}{dt} = -(k_{el} + k_{cp} - k_{cw}) \cdot A_C + k_{pc} \cdot A_P + k_{wc} \cdot (WUSI + WULI + WLI)
\] (1)

\[
\frac{dA_P}{dt} = k_{cp} \cdot A_C - k_{pc} \cdot A_P
\] (2)

\[
\frac{dS}{dt} = -k_{tS} \cdot S
\] (3)

\[
\frac{dUSI}{dt} = k_{tS} \cdot S - (k_{tUSI} + k_{aUSI}) \cdot USI + \frac{V_{maxUSI} \cdot WUSI}{K_m + WUSI}
\] (4)
\[
\frac{dL_S}{dt} = \frac{k_{tUSI} \cdot U_S - (k_{tLSI} + k_{aLSI}) \cdot L_S + \frac{V_{maxLSI} \cdot W_{LSI}}{K_m + W_{LSI}}}{m}
\]

\[
\frac{dL_I}{dt} = \frac{k_{tLSI} \cdot L_S - (k_{tLI} + k_{aLI}) \cdot L_I + \frac{V_{maxLI} \cdot W_{LI}}{K_m + W_{LI}}}{m}
\]

\[
\frac{dW_{USI}}{dt} = \frac{k_{c_w} \cdot A_c + k_{aUSI} \cdot U_S - k_{w_c} \cdot W_{USI} - \frac{V_{maxUSI} \cdot W_{USI}}{K_m + W_{USI}}}{m}
\]

\[
\frac{dW_{LSI}}{dt} = \frac{k_{c_w} \cdot A_c + k_{aLSI} \cdot L_S - k_{w_c} \cdot W_{LSI} - \frac{V_{maxLSI} \cdot W_{LSI}}{K_m + W_{LSI}}}{m}
\]

\[
\frac{dW_{LI}}{dt} = \frac{k_{c_w} \cdot A_c + k_{aLI} \cdot L_I - k_{w_c} \cdot W_{LI} - \frac{V_{maxLI} \cdot W_{LI}}{K_m + W_{LI}}}{m}
\]

For IV administration, \(A_c(0)\) was set equal to dose and initial conditions for Equations 2-9 were set to zero. For oral administration, \(S(0)\) was set equal to the oral dose level and initial conditions for Equations 1, 2, and 4-9 were set to zero. For cecal infusion, initial conditions for Equations 1-9 were set to zero, and a zero-order input rate constant was added to Equation 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 ANOVA was applied to assess differences between more than two groups followed by Tukey Multiple Comparisons Test where appropriate. Comparisons between two groups were conducted using the two tailed t-test. A p-value of less than 0.05 was considered statistically significant. Data are presented as mean±SEM, unless stated otherwise.
Model fitting and parameter estimation were performed using nonlinear regression analysis with the ADAPT 5 computer program (Biomedical Simulations Resource, USC, Los Angeles, CA) and the maximum likelihood method. The variance model was defined as:

$$VAR_i = (\sigma_1 + \sigma_2 \cdot Y(\theta, t_i))^2$$  \hspace{1cm} (10)

where $VAR_i$ is the variance of the $i$th data point, $\sigma_1$ and $\sigma_2$ are the variance model parameters, and $Y(\theta, t_i)$ 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 (IV) alone or in combination with oral verapamil or oral cyclosporine. Concentration-time profiles are presented in Figure 2 and the corresponding pharmacokinetic parameters obtained by noncompartmental analysis are presented in Table 2. AUC values were utilized to calculate bioavailability following various modes of enteral talinolol administration. The value of systemic clearance obtained for IV talinolol administered without PGP inhibitors is in agreement with previously reported values (Tronde et al., 2003). Both systemic clearance and $V_{ss}$ of talinolol following co-administration with oral verapamil were significantly higher than the corresponding values after IV 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 following co-administration with PGP inhibitors was based on the AUC data of the corresponding IV group (either IV talinolol with oral verapamil or IV talinolol with oral cyclosporine). Two dose levels of 40 and 4 mg/kg administered by different modes of enteral administration were evaluated. In general, talinolol pharmacokinetics following 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 following enteral administration at a high dose (40 mg/kg) are shown in Figures 3 and 4. Two absorption peaks occurring at 1 and 4 h were evident in most individual pharmacokinetic profiles following oral bolus administration, which is also reflected in the mean data (Figure 3). No double peaks were found after cecal infusion administration.
Talinolol demonstrated a significant GI region-specific absorption: oral bolus administration produced much higher plasma concentrations (Figure 3) in comparison to intracecal administration of the same dose (Figure 4). The corresponding bioavailability values were 18 and 0.78% (Table 3). Mean plasma concentration-time profile of talinolol following a low oral dose (4 mg/kg) is shown in Figure 5. The double peak phenomenon was less evident following the low dose oral administration. A decrease in plasma concentration was more than dose proportional in comparison to the high dose, and the bioavailability was approximately eight times lower than that obtained following 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 cecum was not performed as the plasma concentrations were predicted to be below the assay quantification 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 A. Plasma concentration-time profiles of high dose talinolol administered orally with or without PGP inhibitors are shown in Figure 3. Administration with cyclosporine results in a pharmacokinetic profile that is very similar to the profile obtained following administration of talinolol alone. Administration with verapamil resulted in lower plasma concentrations in comparison to talinolol alone; however, these differences were not statistically significant due to high inter-animal variability. These lower concentrations do not necessarily indicate a decrease in bioavailability. In fact, when the AUC data from corresponding IV groups were used, the bioavailability values following all three modes of administration were similar (Table 3).

Plasma concentration-time profiles of low dose talinolol administered orally with or without PGP inhibitors are shown in Figure 5. Cyclosporine at a dose of 100 mg/kg was a significantly more potent enhancer of bioavailability for the low-dose talinolol than verapamil.
5 mg/kg. Administration with cyclosporine increased the extent of absorption about 5-fold (p<0.01) in comparison to 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 about a 5-fold higher bioavailability in comparison to cecum infusion without cyclosporine (Figure 4 and Table 3). Comparison of terminal half-lives (calculated by a noncompartmental approach) from all experimental groups (Tables 2 and 3) performed by ANOVA 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 following various modes of administration. The data sets for co-administration of talinolol and verapamil were excluded from the modeling process, as no reliable mechanistic explanation of verapamil effects on talinolol pharmacokinetics could be proposed. The model fits are shown in Figures 2-5 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_{cw} = k_{wc}). In addition, the same absorption rate constant was assumed for all intestinal segments (k_{aUSI} = k_{aLSI} = k_{aLI}), 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 change in talinolol affinity to the transporter (K_m). Two separate values were estimated for oral and intracecal cyclosporine administration (K_{mC1} and K_{mC2}), 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 data sets. Additional modifications to the model (including biliary excretion, transporter mediated uptake from GI lumen, and GI region-specific absorption processes) did not improve model performance. On the other hand, a simpler model, where GI transit compartments were directly connected to the central distribution compartment and PGP mediated efflux (back into intestine) was driven by a systemic talinolol concentrations, was unable to satisfactorily describe the PK 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. Due to 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 as 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, as well as drug excretion from the systemic circulation into the gut lumen.

The pharmacokinetics of talinolol following 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 about 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 the high dose talinolol had no apparent effect on the extent of systemic absorption as the efflux mechanism was apparently saturated by talinolol and functioned at its maximum capacity. On the other hand, following administration with low-dose talinolol, cyclosporine was able to reduce the efflux and increase bioavailability. Verapamil demonstrated a trend to 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%) as compared to that found following oral administration (17.8%). The extent of absorption in humans was markedly decreased when talinolol was perfused in the distal small intestine in comparison to a more proximal location (Gramatte et al., 1996). Similarly, the bioavailability of talinolol from rectal capsules is reduced by 80% as compared to an orally administered immediate release formulation (Weitschies et al., 2005). Low absorption from the large intestinal regions might be due to a poor absorptive permeability or more efficient excretion of the drug back to the GI lumen. For example, atenolol (a relatively hydrophilic drug that is not metabolized nor a PGP substrate) has been shown to have a much lower bioavailability when administered into the cecum as compared to oral administration (4.6% vs. 19.5%) (Kagan and Hoffman, 2008a). Atenolol is mainly absorbed by paracellular passive diffusion, and this absorption route is limited in the large intestine (Rojanasakul et al., 1992; Mummaneni and Dressman, 1994; Rouge et al., 1996). In this study, administration with cyclosporine increased the bioavailability of talinolol following intracecal administration by 10-fold. Thus, a negligible absorption of talinolol from the distal intestine might be primarily attributed to a very efficient efflux by the PGP pump. The experimental data was further supported by modeling results suggesting that the PGP-mediated efflux capacity of the distal intestine is higher than in proximal regions.

Intestinal PGP function appears to be higher in rats as compared to humans. The oral bioavailability of talinolol in humans is about 55% at a usual therapeutic dose level of 100 mg (Trausch et al., 1995), and is reported to increase with increasing dose levels (Tubic et al., 2006a). The dose range tested in this study was considerably greater, but even with the highest
dose level, the bioavailability was less than 20%. The issue of species differences in PGP function should be investigated in further studies.

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 as compared to 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 following co-administration 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 following co-administration 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 following 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 a 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). Recently, talinolol was shown to be a substrate of OATP absorption transporters (Shirasaka et al., 2009; Shirasaka et al., 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 more frequent administration 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 about 2-fold higher than from the controlled released tablet (Tubic et al., 2006b). Even a relatively small delay in release (achieved by enteric coating) was shown to reduce absorption by about 50% in comparison to 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 the proximal part of the GI tract, as provided by gastro-retentive 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 many rats following oral administration, and many profiles demonstrated double peaks. Direct intestinal secretion is a major component of talinolol clearance (Gramatte et al., 1996; Wetterich et al., 1996). Hence, this continuous absorption phenomena (in the presence of negligible colonic bioavailability) can probably be explained by an “entero-enteral 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 following IV administration in some rats. Similar type of behavior can be seen for talinolol administered IV with either verapamil or cyclosporine (Figure 2, time points 120 and 150 min).

In conclusion, PGP-mediated efflux was demonstrated to be a major factor responsible for GI 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 gastro-retentive dosage forms) or co-administration 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 calculating bioavailability values. A semi-physiological model was developed that successfully captured the observed pharmacokinetics of talinolol, as well as system-based phenomena, and might be further utilized for investigating the absorption of other PGP substrate drugs.
Acknowledgments

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References


Legends for figures

Figure 1. Pharmacokinetic model for GI absorption and disposition of talinolol in rats.

Figure 2. Plasma concentration-time profiles of talinolol following IV administration to rats with or without PGP inhibitors. Symbols represent mean experimental data and lines are model fitted profiles: (◊ and solid line) talinolol IV 4 mg/kg, (×) talinolol IV 2 mg/kg and oral verapamil 5 mg/kg, (◻ and dashed line) talinolol IV 2 mg/kg and oral cyclosporin A 100 mg/kg. Error bars represent SEM (n=4-6).

Figure 3. Plasma concentration-time profiles of high-dose talinolol (40 mg/kg) following oral administration to rats with or without PGP inhibitors. Symbols represent mean experimental data and lines are model fitted profiles: (○ and solid line) talinolol alone, (×) talinolol in combination with oral verapamil 5 mg/kg, (● and dashed line) talinolol in combination with oral cyclosporine A 100 mg/kg. Error bars represent SEM (n=5-6).

Figure 4. Plasma concentration-time profiles of high-dose talinolol (40 mg/kg) following intracecal infusion over 4 h to rats with or without PGP inhibitor: (□ and solid line) talinolol alone, (■ and dashed line) talinolol in combination with cecal bolus of cyclosporine A 100 mg/kg. Data are presented as mean ±SEM (n=5).

Figure 5. Plasma concentration-time profiles of low-dose talinolol (4 mg/kg) following oral administration to rats with or without PGP inhibitors: (○ and solid line) talinolol alone, (×) talinolol in combination with oral verapamil 5 mg/kg, (● and dashed line) talinolol in combination with oral cyclosporine A 100 mg/kg. Data are presented as mean ±SEM (n=5-9).
Table 1. Experimental groups for evaluating talinolol pharmacokinetics in rats.

<table>
<thead>
<tr>
<th>Number of animals</th>
<th>Dose (mg/kg)</th>
<th>Administration mode</th>
<th>Formulation</th>
<th>P-glycoprotein inhibitor</th>
<th>Dose (mg/kg)</th>
<th>Administration mode</th>
</tr>
</thead>
<tbody>
<tr>
<td>6</td>
<td>4</td>
<td>IV</td>
<td>A</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>6</td>
<td>2</td>
<td>IV</td>
<td>A</td>
<td>Verapamil</td>
<td>5</td>
<td>PO</td>
</tr>
<tr>
<td>4</td>
<td>2</td>
<td>IV</td>
<td>A</td>
<td>Cyclosporine A</td>
<td>100</td>
<td>PO</td>
</tr>
<tr>
<td>6</td>
<td>40</td>
<td>PO</td>
<td>B</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>5</td>
<td>40</td>
<td>PO</td>
<td>B</td>
<td>Verapamil</td>
<td>5</td>
<td>PO</td>
</tr>
<tr>
<td>6</td>
<td>40</td>
<td>PO</td>
<td>B</td>
<td>Cyclosporine A</td>
<td>100</td>
<td>PO</td>
</tr>
<tr>
<td>5</td>
<td>40</td>
<td>Cecum infusion over 4h</td>
<td>B</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>5</td>
<td>40</td>
<td>Cecum infusion over 4h</td>
<td>B</td>
<td>Cyclosporine A</td>
<td>100</td>
<td>Cecum bolus</td>
</tr>
<tr>
<td>6</td>
<td>4</td>
<td>PO</td>
<td>C</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>5</td>
<td>4</td>
<td>PO</td>
<td>C</td>
<td>Verapamil</td>
<td>5</td>
<td>PO</td>
</tr>
<tr>
<td>9</td>
<td>4</td>
<td>PO</td>
<td>C</td>
<td>Cyclosporine A</td>
<td>100</td>
<td>PO</td>
</tr>
</tbody>
</table>

Formulations of talinolol:

A - 2 mg/mL solution in 40% sodium phosphate buffer 0.2 M pH7, 40% propylene glycol, 20% ethanol;

B - 8 mg/mL solution in 25% polyethylene glycol 400, 15% ethanol, 60% water;

C - 1mg/mL solution in 25% polyethylene glycol 400, 15% ethanol, 60% water.
Table 2. Pharmacokinetic parameters of talinolol following IV administration with and without PGP inhibitors (verapamil and cyclosporine A administered orally) obtained by noncompartmental analysis.

<table>
<thead>
<tr>
<th>Talinolol dose, mg/kg</th>
<th>4</th>
<th>2</th>
<th>2</th>
</tr>
</thead>
<tbody>
<tr>
<td>PGP inhibitor</td>
<td>- (control)</td>
<td>Verapamil (PO)</td>
<td>Cyclosporine A (PO)</td>
</tr>
<tr>
<td>T(_{1/2}), min</td>
<td>64.3 (8.0)</td>
<td>74.7 (7.5)</td>
<td>71.4 (17.4)</td>
</tr>
<tr>
<td>AUC, µg·min/mL</td>
<td>75.5 (9.1)</td>
<td>19.7 (3.7)</td>
<td>33.9 (7.6)</td>
</tr>
<tr>
<td>Cl, ml/min·kg</td>
<td>58.6 (9.7)</td>
<td>119.3 (19.6)*</td>
<td>69.3 (16.7)</td>
</tr>
<tr>
<td>Vss, mL/kg</td>
<td>1689 (441)</td>
<td>4668 (995)*</td>
<td>2311 (920)</td>
</tr>
<tr>
<td>MRT, min</td>
<td>27.7 (4.8)</td>
<td>39.0 (5.8)</td>
<td>29.5 (6.6)</td>
</tr>
</tbody>
</table>

* Statistically significant difference from control group (p<0.05). Data are presented as mean (SEM). T\(_{1/2}\) – terminal half-life, AUC – area under concentration-time curve, Cl – systemic clearance, Vss – steady state volume of distribution, MRT – mean residence time.
Table 3. Pharmacokinetic parameters of talinolol following various modes of enteral administration with and without PGP inhibitors obtained by noncompartmental analysis.

<table>
<thead>
<tr>
<th>Mode of talinolol administration</th>
<th>Oral bolus</th>
<th>Oral bolus</th>
<th>Oral bolus</th>
<th>Cecal infusion over 4h</th>
<th>Cecal infusion over 4h</th>
<th>Oral bolus</th>
<th>Oral bolus</th>
<th>Oral bolus</th>
</tr>
</thead>
<tbody>
<tr>
<td>Talinolol dose</td>
<td>40</td>
<td>40</td>
<td>40</td>
<td>40</td>
<td>40</td>
<td>4</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>PGP inhibitor</td>
<td>-</td>
<td>Verapamil (PO)</td>
<td>Cyclosporine A (PO)</td>
<td>-</td>
<td>Cyclosporine A (cecal bolus)</td>
<td>-</td>
<td>Verapamil (PO)</td>
<td>Cyclosporine A (PO)</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>
<td>125.7 (24.2)</td>
</tr>
<tr>
<td>AUC, (\mu 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>1.9 (0.2)</td>
<td>7.2 (1.5)</td>
</tr>
<tr>
<td>(T_{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>
<td>340 (67)</td>
</tr>
<tr>
<td>(C_{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>11.8 (0.8)</td>
<td>29.8 (7.2)</td>
</tr>
<tr>
<td>Cl(_{PO}), 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>
<td>850 (205)</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>
<td>365 (66)</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>
<td>10.6 (2.3)</td>
</tr>
</tbody>
</table>

Data are presented as mean (SEM). T_{1/2} – terminal half-life, AUC – area under concentration-time curve, Cl_{PO} – oral clearance, MRT – mean residence time, F – bioavailability.
Table 4. Final pharmacokinetic model estimated parameters for talinolol in rats

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Description</th>
<th>Units</th>
<th>Estimate</th>
<th>%CV</th>
</tr>
</thead>
<tbody>
<tr>
<td>$k_{el}$</td>
<td>First-order elimination rate constant from central compartment</td>
<td>min$^{-1}$</td>
<td>9.70×10$^{-2}$</td>
<td>12</td>
</tr>
<tr>
<td>$k_{pc}$</td>
<td>First-order distribution rate constant to central compartment</td>
<td>min$^{-1}$</td>
<td>2.24×10$^{-2}$</td>
<td>40</td>
</tr>
<tr>
<td>$k_{cp}$</td>
<td>First-order distribution rate constant from central compartment</td>
<td>min$^{-1}$</td>
<td>4.46×10$^{-3}$</td>
<td>18</td>
</tr>
<tr>
<td>$k_{aUSI} = k_{aLSI} = k_{aLI}$</td>
<td>First-order absorption rate constant</td>
<td>min$^{-1}$</td>
<td>1.22×10$^{-2}$</td>
<td>107</td>
</tr>
<tr>
<td>$k_{cw} = k_{wc}$</td>
<td>First-order transfer rate constant between central and GI wall compartments</td>
<td>min$^{-1}$</td>
<td>9.92×10$^{-3}$</td>
<td>68</td>
</tr>
<tr>
<td>$k_{S}$</td>
<td>First-order transit rate constant</td>
<td>min$^{-1}$</td>
<td>3.56×10$^{-2}$</td>
<td>a</td>
</tr>
<tr>
<td>$k_{USI}$</td>
<td>First-order transit rate constant</td>
<td>min$^{-1}$</td>
<td>1.69×10$^{-2}$</td>
<td>a</td>
</tr>
<tr>
<td>$k_{LSI}$</td>
<td>First-order transit rate constant</td>
<td>min$^{-1}$</td>
<td>0.70×10$^{-2}$</td>
<td>a</td>
</tr>
<tr>
<td>$k_{LI}$</td>
<td>First-order elimination rate constant from last intestinal segment</td>
<td>min$^{-1}$</td>
<td>1.14×10$^{-2}$</td>
<td>16</td>
</tr>
<tr>
<td>$V_c$</td>
<td>Volume of the central compartment</td>
<td>L/kg</td>
<td>7.53×10$^{-2}$</td>
<td>a</td>
</tr>
<tr>
<td>$K_m$</td>
<td>Affinity of talinolol to PGP in the absence of cyclosporine</td>
<td>ng</td>
<td>2.46×10$^{5}$</td>
<td>57</td>
</tr>
<tr>
<td>$K_{mC1}$</td>
<td>Affinity of talinolol to PGP following oral administration of cyclosporine</td>
<td>ng</td>
<td>5.21×10$^{3}$</td>
<td>61</td>
</tr>
<tr>
<td><strong>$K_{mC2}$</strong></td>
<td>Affinity of talinolol to PGP following cecal administration of cyclosporine</td>
<td>ng</td>
<td>$2.51 \times 10^6$</td>
<td>69</td>
</tr>
<tr>
<td>--------------</td>
<td>--------------------------------------------------------------------------------</td>
<td>------</td>
<td>-------------------</td>
<td>--------</td>
</tr>
<tr>
<td><strong>$V_{maxUSI}$</strong></td>
<td>Maximal capacity of PGP-mediated efflux in the upper small intestine</td>
<td>ng/min</td>
<td>$1.98 \times 10^5$</td>
<td>121</td>
</tr>
<tr>
<td><strong>$V_{maxLSI}$</strong></td>
<td>Maximal capacity of PGP-mediated efflux in the lower small intestine</td>
<td>ng/min</td>
<td>$2.07 \times 10^5$</td>
<td>101</td>
</tr>
<tr>
<td><strong>$V_{maxLI}$</strong></td>
<td>Maximal capacity of PGP-mediated efflux in the large intestine</td>
<td>ng/min</td>
<td>$4.07 \times 10^5$</td>
<td>107</td>
</tr>
</tbody>
</table>

%CV reflects precision of the estimated parameters and not inter-animal variability

- fixed value
Figure 4

Talinolol plasma concentration (ng/mL) vs. Time (min).

- Solid line with squares: Drug A
- Dashed line with squares: Drug B
- Solid line with circles: Drug C

Error bars indicate variability in concentration.