JPP Journal of Pharmacy and Pharmacology

JPP 2007, 59: 495–501

Received August 10, 2006
Accepted December 4, 2006
DOI 10.1211/jpp.59.4.0003
ISSN 0022-3573

Pharmacokinetic modelling of pentoxifylline and lisofylline after oral and intravenous administration in mice

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Abstract

The aim of this study was to develop pharmacokinetic models for pentoxifylline (PTX) and the *R*(–)-enantiomer of the PTX metabolite 1, lisofylline (LSF), in order to identify some factors influencing the absorption of these compounds from the intestines and to clarify mechanisms involved in their non-linear pharmacokinetics. Serum samples were collected after oral and intravenous administration of PTX and LSF to male CD-1 mice at two different doses. In addition, both compounds under investigation were coadministered with a modulator of drug transporters, verapamil, and an inhibitor of cytochrome P450 (CYP) 3A4, ketoconazole. Pharmacokinetic analysis revealed that a one-compartment model with Michaelis–Menten type absorption and elimination best described the pharmacokinetics of PTX, whereas the LSF concentration–time data were adequately fitted to a two-compartment model with a first-order absorption and Michaelis–Menten type elimination process. Both coadministered compounds significantly decreased the area under the concentration–time curve from 0 to 60 min calculated for PTX and increased the value of this parameter for LSF. The results of this study indirectly suggest that saturation of drug transport across intestinal cells and elimination from the central compartment may be responsible for the non-linear pharmacokinetics of PTX, whereas in the case of LSF, the dose dependency in the pharmacokinetics is solely related to the elimination from the central compartment. It seems that the observed changes in PTX and LSF concentrations after coadministration with verapamil and ketoconazole may be clinically significant, especially after chronic treatment, however further studies are necessary to assess the importance of these interactions in humans.

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Funding: This work was supported by the Polish Ministry of Education and Science (grant no. 2 P05F 032 30) and Collegium Medicum, Jagiellonian University (grant no. CR-92/2003).

Introduction

Before the drug reaches systemic circulation it must cross the intestinal mucosa. Multiple pathways are involved in the membrane transport process, including passive diffusion and carrier-mediated mechanisms (Doherty & Charman 2002). In addition, intestinal transporters such as organic anion transporting polypeptides (OATPs in humans; Oatps in rodents) or oligopeptide transporter 1 facilitate drug absorption, while P-glycoprotein or multidrug resistance-associated protein 2 promote efflux from intestinal tissue into the lumen (reviewed by Pang 2003). Thus, low oral bioavailability of many compounds may be related not only to the activity of intestinal and hepatic CYP enzymes, especially those of the CYP3A family, which are responsible for the oxidative metabolism of many clinically used drugs, but also to the intestinal efflux transporters, among which P-glycoprotein has been probably the most extensively studied. Not surprisingly, saturation of the intestinal metabolic enzymes or drug transporters responsible for either absorptive or secretory processes often leads to dose-dependent pharmacokinetics of drugs that are administered orally. Moreover, the absorption of substrates of CYP3A and/or membrane transporters from the gastrointestinal tract may change considerably in the presence of modulators of these proteins (Zhang & Benet 2001; Lin 2003).

Pentoxifylline (PTX) is a hemorheologic agent commonly used in patients with chronic peripheral arterial disease. Due to its anti-inflammatory effects it has also been under investigation for the treatment of sepsis and septic shock (Zeni et al 1996; Lauterbach et al

1999). The *R*(–)-enantiomer of the PTX active metabolite 1 (M1), known as lisofylline (LSF), is a lysophosphatidic acid acyltransferase inhibitor identified as a drug candidate for the prevention of treatment-related toxicity in cancer patients (Margolin et al 1997) and in bone marrow transplant recipients (List et al 2000). Further clinical development of LSF for the treatment of acute lung injury and acute respiratory distress syndrome (Levetown 2002), and the prevention of autoimmune disorders, including Type 1 diabetes and β cell protection in islet transplantation, has recently been proposed (Yang et al 2005). It has been shown that in-vitro metabolism of PTX to LSF is reversible (Lillibridge et al 1996), however serum concentrations of both compounds as metabolites observed in-vivo in mice following intravenous administration of PTX or LSF are rather low (Wyska et al 2006).

Following oral administration, the bioavailability of PTX and LSF is low in both humans (Beermann et al 1985; Smith et al 1986; Bursten et al 1998) and animals (Raju et al 1993; Marsella et al 2000; De Boever et al 2005). In addition, both PTX and M1 exhibit non-linear pharmacokinetics in humans (Smith et al 1986). However, to date, no attempts have been made to explain the reasons for these phenomena.

The aim of the present study was to develop pharmacokinetic models for PTX and LSF to identify some factors influencing the absorption of these compounds from the intestines and to clarify mechanisms involved in their non-linearity. In order to achieve this goal, two different doses of both compounds were given orally and intravenously to mice. In addition, PTX and LSF were coadministered with a potent CYP3A inhibitor, ketoconazole, and a known inhibitor of drug transport, verapamil.

Materials and Methods

Chemicals

PTX, verapamil hydrochloride and ketoconazole were purchased from Sigma-Aldrich (St Louis, MO, USA). LSF was obtained from the Department of Technology and Biotechnology of Drugs, Collegium Medicum, Jagiellonian University, Poland. All other chemicals were of high-performance liquid chromatography or analytical reagent grade and were purchased from Merck (Darmstadt, Germany).

Animals

Male Crl:CD-1 mice, 8–10 weeks old, 28–33 g, bred in-house from progenitors obtained from Charles River Laboratories (Sulzfed, Germany) were used in this study. Animals were housed under controlled environmental conditions with a 12-h dark/light cycle. They were fasted overnight prior to drug administration but had free access to water. All animal procedures were approved by the Animal Research Ethics Committee in Kraków, Poland.

Drug administration

All compounds under investigation were directly dissolved in 0.9% sterile saline and used within 1 day of preparation. The only exception was ketoconazole, which was first suspended in ethanol. The animals were administered PTX or LSF (50 or 100 mg kg^{-1}) by oral gavage (22 G; FST, Heidelberg, Germany). Verapamil and ketoconazole were also given orally at 25 mg kg^{-1}, 15 min and 60 min, respectively, before the 50 mg kg^{-1} oral dose of PTX or LSF. The dose and time of dosing of both coadministered compounds were chosen on the basis of pilot study results. Control animals received an equivalent volume of 0.9% saline for the pretreatment. Before the dose (time 0) and at 5, 10, 15, 30, 45 and 60 min after dosing, three or four mice per time point were exsanguinated while under light ketamine/xylazine anaesthesia. Additionally, at 5 and 30 min, brain, liver, kidney and lungs were harvested. Both compounds studied were also given intravenously at a dose of 50 and 150 mg kg^{-1} and blood samples were collected. Serum and other samples were stored at −80°C until assayed. The concentrations of PTX and LSF in serum and tissues were measured by a chiral high-performance liquid chromatography method described previously (Wyska et al 2006).

Pharmacokinetic analysis

One- and two-compartment pharmacokinetic models with Michaelis–Menten type saturable absorption and/or elimination from the absorption and/or central compartment were tested. PTX or LSF serum concentrations for both oral and intravenous routes of administration and all dose levels were simultaneously fitted to obtain a single set of parameters. Bioavailability was modelled as a parameter (F) using the final models. The maximum concentration (C_{max}) and the time to reach peak concentration (t_{max}) were obtained directly from the concentration–time data. The terminal elimination rate constant (λ_z) was assessed by linear regression. Terminal half-life ($t½$) was calculated as $\ln 2/\lambda_z$. The area under the concentration–time curve from 0 to 60 min ($AUC_{0–60}$) was calculated by the linear trapezoidal rule. Non-compartmental and model-dependent analyses were performed using WinNonlin version 3.3 (Pharsight Corp., Mountain View, CA, USA). The final pharmacokinetic models for PTX and LSF were selected on the basis of visual inspection of the fitting, examination of residuals, parameter precision, Akaike Information Criteria, Schwarz Criteria and analysis of the correlation matrix.

Statistical analysis

Statistical analysis was performed using a one-way analysis of variance with the Tukey post-hoc comparison or Student's *t*-test where appropriate (Statistica version 7.0; StatSoft, Inc., Tulsa, OK, USA). Comparison of the AUC between the treatment groups was performed using a *Z*-test (Bailer 1988). A value of $P < 0.05$ was considered statistically significant.

Results

Following intravenous administration of PTX and LSF at two different doses, a more than proportional increase in $AUC_{0–60}$

was observed. The values of this parameter following the doses of 50 and 150 mg kg^{-1} were 975.01 and 4405.20 mg min L^{-1} for PTX, and 1017.57 and 5064.02 mg min L^{-1} for LSF. When the oral dose of LSF was doubled, the calculated AUC_{0-60} increased by almost three times, that is from 103.09 to 300.62 mg min L^{-1} for the 50 and 100 mg kg^{-1} doses, respectively. In the case of PTX, a two-times higher oral dose caused only a slight alteration in the value of this parameter: it increased from 273.97 to 309.24 mg min L^{-1}. These findings clearly indicate that both compounds exhibit a dose-dependent pharmacokinetic behaviour over the dose range tested.

Pharmacokinetic analysis revealed that a one-compartment model with Michaelis–Menten type absorption and elimination best described the pharmacokinetics of PTX, whereas LSF concentration–time data were adequately fitted to a two-compartment model with a first-order absorption and Michaelis–Menten type elimination process. Both models consider loss of drug due to presystemic metabolism represented by a first-order rate constant. A schematic representation of the proposed models is given in Figure 1. Figure 2 shows the observed and model-predicted concentrations of two different oral and intravenous doses of both compounds when fitted simultaneously to the appropriate models. Pharmacokinetic parameters and their respective coefficients of variation (CV) are listed in Tables 1 and 2. As shown in these tables, the PTX volume of distribution and first-order rate constant representing presystemic elimination (k) are greater than those of LSF by 45% and 37%, respectively. In contrast to PTX, the absorption of LSF was linear, with an absorption half life of 28.88 min. The relatively low value of K_m (15.08 mg L^{-1}) estimated for LSF indicates that saturation of its elimination from the central compartment may occur even at the lowest dose administered, whereas in the case of PTX, an intravenous dose of greater than 50 mg kg^{-1} is necessary to reveal non-linearity in the pharmacokinetics of this drug. The bioavailability parameter F for both compounds was assessed by fitting oral and intravenous data simultaneously to the proposed models after presystemic metabolism had been excluded. In the case of PTX, the value of F was allowed to vary with the dose to account for non-linearity in the absorption process. The estimated value of F for LSF was 16% (16.13 CV%), whereas for

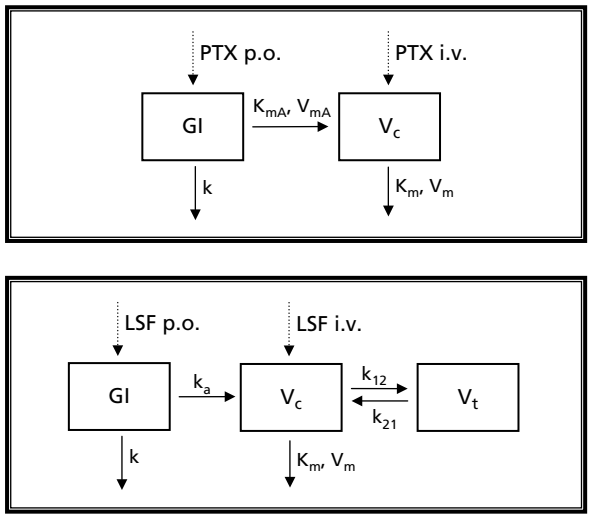

Figure 1 Proposed pharmacokinetic models for oral and intravenous pentoxifylline (PTX) and lisofylline (LSF) selected on the basis of goodness-of-fit criteria. GI, gastrointestinal tract; V_c and V_t, volume of the central and tissue compartments, respectively; k, first-order presystemic elimination rate constant; k_a, first-order absorption rate constant; V_{mA}, maximal absorption rate; K_{mA}, amount of drug at which the absorption rate is half-maximal; V_m, maximal elimination rate; K_m, drug concentration at which the elimination rate is half-maximal; k_{12} and k_{21}, distribution rate constants.

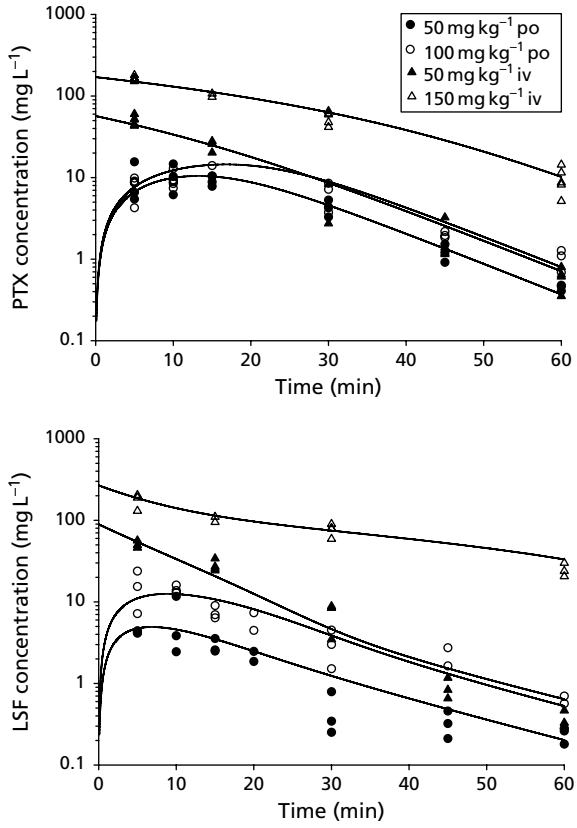

Figure 2 Observed (symbols) and pharmacokinetic model-predicted (lines) serum concentration–time profiles of pentoxifylline (PTX) and lisofylline (LSF) after oral and intravenous administration of two different doses of both compounds to mice.

Table 1 Estimated model parameters for pentoxifylline in mice

Parameter	Final estimate	CV (%)
V_c (L kg^{-1})	0.88	8.78
k (min^{-1})	0.08	10.91
V_{mA} (mg min^{-1} kg^{-1})	2.29	26.61
K_{mA} (mg kg^{-1})	25.60	68.82
V_m (mg min^{-1} kg^{-1})	5.36	10.73
K_m (mg L^{-1})	70.17	18.04

V_c, volume of the central compartment; k, first-order presystemic elimination rate constant; V_{mA}, maximal absorption rate; K_{mA}, amount of drug at which the absorption rate is half-maximal; V_m, maximal elimination rate; K_m, drug concentration at which the elimination rate is half maximal.

Table 2 Estimated model parameters for lisofylline in mice.

Parameter	Final estimate	CV (%)
V_c (L kg^{-1})	0.480	34.30
k (min^{-1})	0.051	20.20
k_a (min^{-1})	0.024	15.68
k_{12} (min^{-1})	0.081	84.99
k_{21} (min^{-1})	0.087	37.41
V_m (mg min^{-1} kg^{-1})	2.33	17.80
K_m (mg L^{-1})	15.08	42.47

V_c, volume of the central compartment; k, first-order presystemic elimination rate constant; k_a, first-order absorption rate constant; k_{12} and k_{21}, distribution rate constants; V_m, maximal elimination rate; K_m, drug concentration at which the elimination rate is half-maximal.

PTX it decreased with the dose administered and, for doses of 50 and 100 mg kg^{-1}, the values of this parameter were 37% (9.48 CV%) and 18% (8.83 CV%), respectively.

Based on the concentrations of LSF as a metabolite determined after PTX administration (data not shown), metabolite-to-parent ratios in serum did not differ significantly with increasing doses of the parent drug. For example, at 30 min they were 0.037 ± 0.002 and 0.033 ± 0.006 for oral doses of PTX, and 0.042 ± 0.007 and 0.044 ± 0.008 for intravenous doses of PTX. In the case of PTX as a metabolite, the corresponding values were similar for oral LSF (0.39 ± 0.044 vs 0.43 ± 0.036) but differed after bolus doses of this compound and were 0.44 ± 0.08 and 0.19 ± 0.03 ($P < 0.05$) for doses of 50 and 150 mg kg^{-1}, respectively. To determine the mechanisms of the non-linearity revealed by the pharmacokinetic modelling, both compounds under investigation were coadministered with a modulator of drug transporters, verapamil, and a strong inhibitor of CYP3A4, ketoconazole. Figure 3 shows the concentration–time profiles of PTX and LSF administered orally at a dose of 50 mg kg^{-1} alone and in the presence of verapamil or ketoconazole. The values of the pharmacokinetic parameters calculated by non-compartmental methods are summarized in Table 3. It is apparent that both coadministered compounds significantly decreased the $AUC_{0–60}$ calculated for PTX, and exerted an opposite effect (although not significant in verapamil-pretreated mice) on the value of this parameter for LSF. The influence of these drugs on PTX and LSF C_{max} was similar, however the differences did not reach statistical significance. As presented in Table 3, verapamil did not influence the t_{max} of any compound under investigation, whereas ketoconazole shortened the t_{max} of PTX from 10 to 5 min. The t½ was reduced by up to half for LSF and prolonged approximately four and three times for PTX in the presence of verapamil and ketoconazole, respectively, when compared with control animals. Thus, it seems that, irrespective of which compound was coadministered, the absorption of PTX from the gastrointestinal tract was slow (flip-flop pharmacokinetics), as indicated by the comparably long PTX t½ values in both pretreatment groups.

Pretreatment with verapamil or ketoconazole did not affect the levels of PTX and LSF attained in brain, liver, kidney and lungs in comparison with mice pretreated with saline when measured at 5 and 30 min after oral administration. However, statistical analysis revealed significantly higher PTX tissue-to-serum concentration ratios in liver and kidneys at 30 min after drug dosing in mice pretreated with verapamil in comparison with both control and ketoconazole groups (Figure 4).

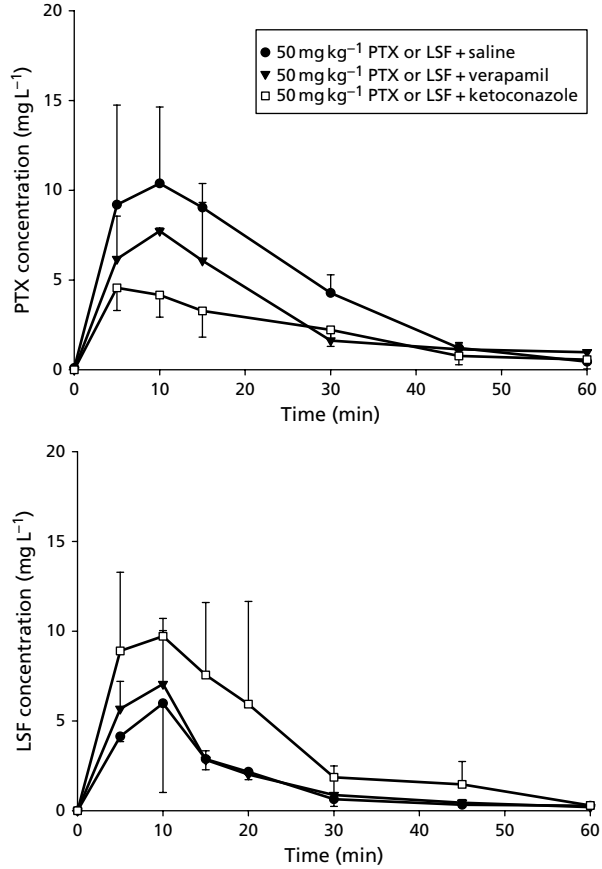

Figure 3 Pentoxifylline (PTX) and lisofylline (LSF) serum concentration–time profiles after oral administration (50 mg kg^{-1}) alone or concomitantly with verapamil or ketoconazole both at the oral doses of 25 mg kg^{-1}. Values are mean ± s.d., n = 3–4.

Discussion

In most previous pharmacokinetic studies of PTX in human subjects and animals, a single dose of the drug was administered and concentration–time data were described by traditional one- or two-compartment models or, even more frequently, non-compartmental analysis was utilized (Beermann et al 1985; Miller et al 1998; De Boever et al 2005). In one study, PTX in solution was given orally to humans over a wide range of doses, however no attempts were made to explain the reasons for the observed dose-dependent increase in C_{max} and AUC of the parent drug and M1 (Smith et al 1986). As far as LSF is concerned, there are only very limited data regarding the pharmacokinetic behaviour of this compound in mice and humans (Rice et al 1994; Bursten et al 1998), and, for the

Table 3 Pharmacokinetic parameters of pentoxifylline and lisofylline given orally at a dose of 50 mg kg^{-1} to mice pretreated with saline, verapamil or ketoconazole, calculated by non-compartmental analysis

Parameter	Pentoxifylline			Lisofylline		
	Saline	Verapamil	Ketoconazole	Saline	Verapamil	Ketoconazole
t_{max} (min)	10	10	5	10	10	10
C_{max} (mg L^{-1})	10.38 (4.26)	9.17 (2.53)	4.57 (1.27)	5.98 (4.96)	7.06 (2.97)	9.72 (0.99)
t½ (min)	10.12	40.46	32.76	24.20	19.54	11.18
AUC_{0-60} (mg min L^{-1})	273.97 (23.47)	186.24* (22.10)	138.28* (14.00)	103.09 (16.39)	111.92 (10.49)	224.99* (35.85)

C_{max} and AUC_{0-60} values are reported as mean (s.d.). *$P < 0.05$, significantly different compared with mice pretreated with saline (*Z*-test).

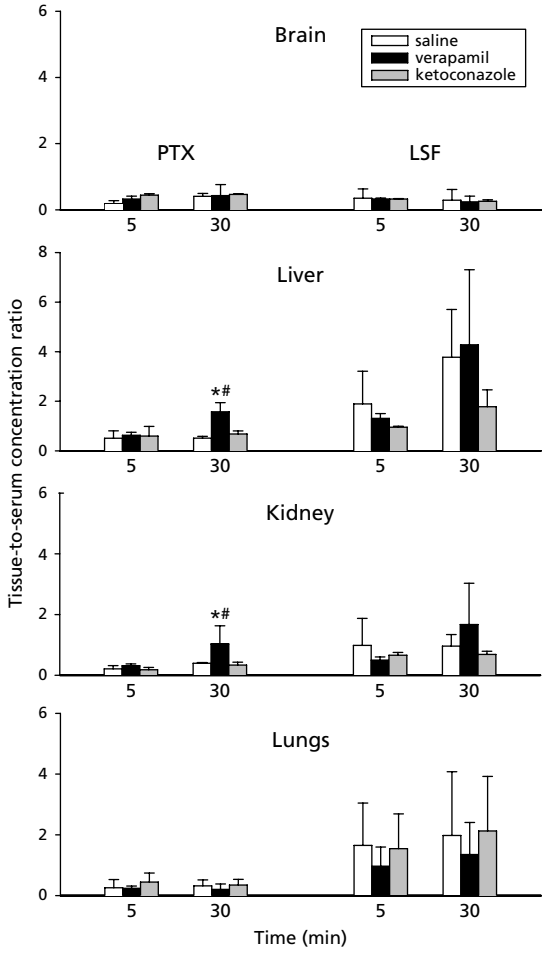

Figure 4 Tissue-to-serum concentration ratios observed at two time points after oral administration of pentoxifylline (PTX) or lisofylline (LSF) to mice at a dose of 50 mg kg^{-1} each in the absence and presence of verapamil and ketoconazole. Values are mean ± s.d., n = 3–4. *$P < 0.05$, significantly different compared with saline; #$P < 0.05$, significantly different compared with ketoconazole.

latter, linear pharmacokinetics of LSF was reported (Bursten et al 1998). Non-compartmental analysis performed in the present study revealed a disproportionate increase in AUC of both compounds with increasing oral and intravenous doses. We believe our study is the first to demonstrate the dose-dependent pharmacokinetics of LSF in mice.

It is well known that PTX and LSF undergo metabolic interconversion (Lillibridge et al 1996). The developed models did not encompass this phenomenon for simplicity reasons and due to the fact that our previous studies in which PTX, LSF and its optical antipode (S)-M1 were given to mice intravenously on three separate occasions indicated that interconversion plays a modest role in the pharmacokinetics of both compounds (Wyska et al 2006).

The proposed pharmacokinetic models for PTX and LSF explained, at least in part, the reasons for the non-linearity in the pharmacokinetics of both compounds. Interestingly, despite structural similarities, both compounds when given in increasing oral and intravenous doses revealed different pharmacokinetic behaviour. The occurrence of dose-dependent PTX absorption from the gastrointestinal tract may suggest the saturation of unspecified transport mechanisms. The hypothesis that saturable transport may be involved in PTX absorption may be further confirmed by the fact that oral administration of a two-fold higher dose of this drug did not influence maximal serum PTX concentrations and only slightly increased the AUC. In turn, Michaelis–Menten type elimination from the central compartment for both drugs under investigation may indicate saturation of both transport and metabolism. In the case of PTX, the possibility of the former was further confirmed by a significantly higher tissue-to-serum concentration ratio at 30 min after drug dosing in eliminating organs such as the liver and kidneys of mice pretreated with verapamil.

The significant changes in the pharmacokinetics of both PTX and LSF when coadministered with verapamil or ketoconazole may suggest the involvement of both CYP and/or transmembrane transporters in their absorption, distribution and elimination. It has been shown in in-vitro studies that CYP3A4 is involved in the formation of lisofylline 4,5-diol (M3) from LSF. In addition, this compound is transformed to PTX by CYP1A2 and to a minor extent by CYP2E1 (Lee & Slattery 1997), and to M7 also by CYP1A2 (Peterson et al 2004). In turn, most of the PTX reduction to M1 takes place in erythrocytes by enzymes of the carbonyl reductase type (Nicklasson et al 2002). Other important PTX metabolic pathways are oxidation to carboxymetabolite M5 by carboxylases (Hinze 1972) and oxidation to metabolite M6 via CYP1A2 (Peterson et al 2004). It has been shown that concomitant administration of PTX and the known inhibitor of CYP1A2, ciprofloxacin, to mice resulted in considerably increased PTX and M1 levels in comparison with mice receiving PTX alone (Peterson et al 2004). Similar interaction was observed in rats

after coadministration of PTX and cimetidine (Luke et al 1986).

Both verapamil and ketoconazole are inhibitors of both CYP and drug transporters such as P-glycoprotein (Achira et al 1999) and OATP (Cvetkovic et al 1999). Ketoconazole is a weak inhibitor of CYP1A2 (von Moltke et al 1996) and a potent inhibitor of CYP3A4. It has been shown that the IC50 values for the inhibition of CYP3A4 activity in human intestinal and liver microsomes were 0.02 and 20 μM for ketoconazole and verapamil, respectively. In turn, verapamil is a stronger inhibitor of P-glycoprotein than ketoconazole with a 10-times lower IC50 value (Achira et al 1999). The ability of both drugs to inhibit OATP-mediated transport is similar (Cvetkovic et al 1999).

There is no information in the literature concerning the involvement of drug transporters in the pharmacokinetics of PTX and LSF. The decreased concentrations of PTX following oral administration in animals pretreated with both coadministered compounds observed in the present study provide indirect evidence that unidentified uptake transporters may facilitate PTX absorption, whereas inhibition of CYP isozymes (probably CYP3A4 and/or CYP1A2) in the liver and intestines by ketoconazole and, to a lesser extent, by verapamil solely contributed to higher concentrations of LSF after oral administration in comparison with saline-treated animals. The latter observation may be further confirmed by the decreased metabolite-to-parent ratio after intravenous administration of LSF at the highest dose of 150 mg kg^{-1}, and by the fact that no statistically significant differences were observed in tissue-to-serum concentration ratios for this compound in mice pretreated with verapamil (Figure 4).

An unexpected finding of our study was that coadministration of ketoconazole and, to a lesser extent, verapamil led to a reduction in PTX serum concentrations after oral administration. Such an interaction has not been reported previously. It has been shown, however, that ketoconazole has no significant effect on the pharmacokinetics of other xanthine derivatives such as theophylline (Heusner et al 1987) and caffeine (Wahlländer & Paumgartner 1989), drugs metabolized primarily by CYP1A2. Only in one study were decreased theophylline concentrations in asthmatic patients receiving ketoconazole observed (Murphy et al 1987). It is quite possible that the mechanism of this interaction between theophylline and ketoconazole in humans is similar to that observed in the present study for PTX and ketoconazole or verapamil in mice. It seems that the observed reduction in PTX concentrations after coadministration with both drugs may be clinically significant, especially after chronic treatment.

Conclusions

The results of the present study indirectly suggest that saturation of drug transport across intestinal cells and elimination from the central compartment may be responsible for the nonlinear pharmacokinetics of PTX after oral administration to mice, whereas in the case of LSF the dose dependency in the pharmacokinetics is solely related to the elimination from the central compartment. These observations were further confirmed by the fact that pretreatment with known inhibitors of membrane transporters and CYP led to a decrease in PTX and an increase in LSF absorption after oral administration, as well as to an elevated tissue-to-serum PTX concentration ratio in eliminating organs, that is the liver and kidneys of verapamil-pretreated mice. Further studies are necessary to assess the existence and clinical significance of these interactions in humans.

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