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Interconversion and Tissue Distribution of Pentoxifylline and Lisofylline in Mice

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ABSTRACT The aim of this study was to assess the interconversion pharmacokinetics and tissue distribution of pentoxifylline and the active (*R*)-enantiomer of its metabolite M1, lisofylline in male CD-1 mice. Both compounds were administered intravenously at a dose of 50 mg/kg on two separate occasions. Serum and tissues were collected at different time points following drug administration. In addition, the (*S*)-enantiomer of M1 was administered to a group of mice and serum samples were obtained. Analyte concentrations were measured by chiral HPLC. All serum concentration versus time data were fitted simultaneously to a pharmacokinetic model incorporating interconversion processes of parent drug and metabolites. The estimated conversion clearance of (−)-(*R*)-M1 to pentoxifylline (CL_{21}) was six times greater than that for the reverse process (CL_{12}). The interconversion of pentoxifylline and (+)-(*S*)-M1 was faster as reflected by the values of conversion clearances CL_{13} and CL_{31} which were approximately 16 and 7 times greater in comparison with the corresponding clearances for the interconversion of pentoxifylline and (−)-(*R*)-M1. When fitting pharmacokinetic data of both parent compounds to a one-compartment model, the values of elimination clearances assessed were close to those obtained on the basis of the interconversion model. After administration of pentoxifylline, tissue-to-serum AUC ratios ranged from 0.1 for liver and lungs to 0.32 for brain tissue. Serum levels of its metabolite, (−)-(*R*)-M1 were very low, whereas its tissue levels exceeded serum concentrations. The highest value of metabolite-to-parent AUC ratio (4.98) was observed in lungs. When (−)-(*R*)-M1 was given as a parent drug, tissue-to-serum AUC ratios in liver, kidney, and lungs were very close and ranged from 0.64 to 0.72. At the same time, levels of its metabolite, pentoxifylline were relatively low both in serum and all tissues studied. In consequence, metabolite-to-parent AUC ratios did not exceed the value of 0.27. In conclusion, reversible metabolism plays a modest role in the disposition of pentoxifylline and (−)-(*R*)-M1. It seems that pentoxifylline has less favourable pharmacokinetic properties than (−)-(*R*)-M1 due to lower concentrations attained in target organs. High levels of (−)-(*R*)-M1 observed after pentoxifylline administration in certain tissues such as liver or lungs suggest that pentoxifylline may constitute an effective prodrug for (−)-(*R*)-M1 in these organs. *Chirality 18:644–651, 2006.*

KEY WORDS: enantioselective disposition; pharmacokinetic model; interconversion clearance, tissue-to-serum AUC ratio

Pentoxifylline, 1-(5-oxohexyl)-3,7-dimethylxanthine, is a hemorheologic agent widely used for the treatment of patients with intermittent claudication. In addition, it exerts immunomodulatory effects both in vitro and in vivo in animal models of sepsis.[1–3] Several clinical studies have demonstrated that pentoxifylline decreases plasma levels of tumour necrosis factor alpha (TNF-α) in septic shock[4] and reduces the mortality rate in premature infants with sepsis.[5] In mammals it is transformed into several metabolites, two of which (1-(5-hydroxyhexyl)-3,7-dimethylxanthine (M1) and 1-(3-carboxypropyl)-3,7-dimethylxanthine (M5)) have been reported to produce hemorheologic effects similar to the parent drug.[6] The most interesting metabolite M1 is a chiral compound and its *R*-enantiomer, (−)-(*R*)-M1 is known as lisofylline. Unlike M5[7], (−)-(*R*)-M1 has been shown to affect lipopolysaccharide-induced TNF-α release and in comparison to pen-

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toxifylline it is about 800 times more active as an inhibitor of phosphatidic acid formation.[8] It is a drug candidate that has been under investigation for acute respiratory distress syndrome (ADRS), acute lung injury (ALI), septic shock, and mucositis. Moreover, it may prevent neutropenic infections in cancer patients receiving high-dose chemotherapy, and it has also been shown to be effective in the prevention and treatment of Type 1 diabetes.[9] In contrast, the optical antipode of lisofylline, (+)-(*S*)-M1 is considered pharmacologically inactive.

Despite the substantial number of studies on pentoxifylline and (−)-(*R*)-M1 as anti-inflammatory agents, knowledge of the pharmacokinetic behaviour of these compounds, especially about their tissue distribution, is rather sparse. It is well known that circulating cytokine concentrations may not reflect their activity in organs, as they are produced locally, (e.g., by liver or alveolar macrophages).[10] Therefore, it seems to be of importance to assess pentoxifylline and (−)-(*R*)-M1 concentrations in organs in which these compounds may exert their therapeutic effects. Moreover, there is in vitro evidence that pentoxifylline and (−)-(*R*)-M1, as well as pentoxifylline and (+)-(*S*)-M1 may undergo metabolic interconversion.[11] Thus, irrespective of which is administered, all compounds could be anticipated to be present in blood. However, up to now there is a lack of in vivo studies assessing the role of reversible metabolism in their disposition.

The aim of the present study was to investigate the interconversion pharmacokinetics of pentoxifylline and (−)-(*R*)-M1 after intravenous administration to mice on two occasions. In order to fully explain this phenomenon, (+)-(*S*)-M1 was also administered in a separate experiment and its serum concentrations were determined. Moreover, the tissue distribution characteristics of pentoxifylline and (−)-(*R*)-M1 were assessed and special attention was paid to the enantioselective disposition of pentoxifylline.

MATERIALS AND METHODS

Chemicals

Pentoxifylline was purchased from Sigma-Aldrich (St. Louis, MO, USA). (−)-(*R*)-M1, (+)-(*S*)-M1, and 7-(2′-chloroethyl)-1,3-dimethylxanthine were obtained in the Department of Technology and Biotechnology of Drugs, Collegium Medicum, Jagiellonian University. Their purities were ascertained chromatographically. Temazepam was a gift from Polfa (Cracow, Poland). HPLC grade dichloromethane, hexane, and 2-propanol were from Merck (Darmstadt, Germany). All other chemicals were of analytical reagent grade and also were obtained from Merck (Darmstadt, Germany).

Animals

Male Crl:CD-1 mice aged eight to ten weeks and weighing 28 to 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 and 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 Animal Research Ethics Committee in Cracow, Poland.

Drug Distribution Studies

Pentoxifylline, (−)-(*R*)-M1, and (+)-(*S*)-M1 were dissolved in 0.9% sterile saline for injection to achieve a final concentrations of 17.5 mg/ml and used within one day of preparation. The animals were administered either pentoxifylline, (−)-(*R*)-M1 or (+)-(*S*)-M1 at a dose of 50 mg/kg each, by intravenous injection into a tail vein. At 5, 15, 30, 45, and 60 min after dosing 4 or 5 mice per time point were exsanguinated while under light ketamine/xylazine anesthesia. In the case of pentoxifylline and (−)-(*R*)-M1 liver, brain, kidney, and lungs were harvested. Serum and other samples were stored at −80°C until assayed.

Analytical Method

The concentrations of pentoxifylline, (−)-(*R*)-M1, and (+)-(*S*)-M1 in serum and tissues were measured by a chiral HPLC method. Briefly, the tissues were homogenized in 0.1 M PBS (1:4 w/v). In order to obtain wide linearity, to 0.2 ml of serum or 1 ml of tissue homogenate two internal standards with different concentrations were added: temazepam (20 μl of 1 μg/ml in methanol) and 7-(2′-chloroethyl)-1,3-dimethylxanthine (20 μl of 5 μg/ml in methanol) for low (<1 μg/ml or μg/g) and high (>1 μg/ml or μg/g) analyte concentrations, respectively. The samples were acidified with 40 μl 1 M HCL and extracted with 5 ml of dichloromethane. After centrifugation (1000 *g,* 15 min), the organic layer was transferred to a new tube, then evaporated to dryness at 37°C under a gentle stream of nitrogen. The residue was dissolved in 100 μl of mobile phase, and 50 μl of this solution were injected into the HPLC system.

The HPLC system (Thermo Separation Products, San Jose, CA, USA) consisted of a P100 isocratic pump, a Rheodyne 7125 injector (Rheodyne, Cotati, CA, USA) with a 50-μl sample loop, a UV100 variable-wavelength UV/VIS detector operating at 275 nm, and a SP4400 (ChromJet) integrator. All analyses were performed at ambient temperature on a 250 × 4.6 mm Chiralpak AD column (Daicel Corp., Japan) with 10 μm particles, protected with a 20 × 4.6 mm LC-Si guard-column (Supelco Inc., Bellefonte, PA, USA). The mobile phase consisting of 100 μl of diethylamine in hexane/2-propanol (78:22 v/v) was pumped at a flow rate of 1 ml/min. Under these conditions, the approximate retention times were: 7-(2′-chloroethyl)-1,3-dimethylxanthine: 11.70 min; temazepam: 17.60 min; pentoxifylline: 22.30 min; (−)-(*R*)-M1: 25.30 min; and (+)-(*S*)-M1: 28.72 min. No interferences were observed at the retention times of interest.

The method was validated for murine serum, liver, brain, kidney, and lungs. The calibration curves were linear in the tested pentoxifylline, (−)-(*R*)-M1, and (+)-(*S*)-M1 concentration ranges, that is, from 0.05 to 1 μg/ml (low concentrations) and from 1 to 60 μg/ml (high concentrations) for serum and from 0.075 to 1 μg/g (low concentrations) and from 1 to 40 μg/g (high concentrations) for tissues. The assay was reproducible with low intra- and interday

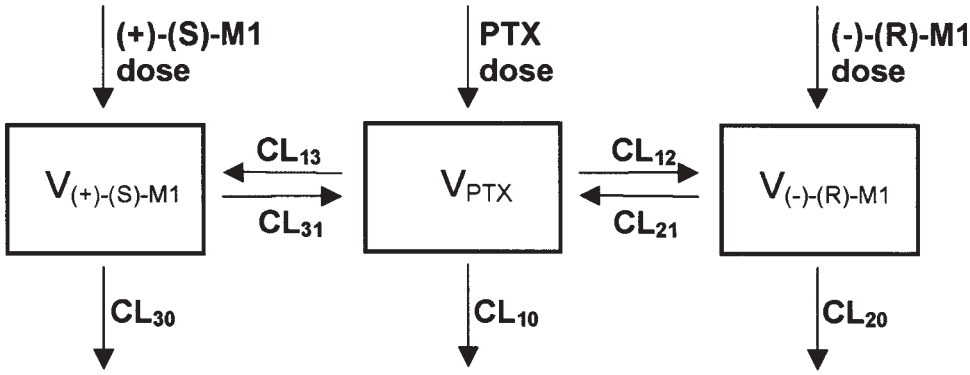

Fig. 1. Pharmacokinetic model for interconversion of pentoxifylline, (−)-(*R*)-M1, and (+)-(*S*)-M1 (abbreviations defined in the text).

variations (CV less than 10%) and the recovery ranged from 68–98%, depending on the type of tissue.

Pharmacokinetic Analysis

The initial serum concentration (C_0) was estimated by back extrapolation to $t = 0$ of the logarithmic plots. The maximum concentration (C_{max}) and the time to reach peak concentration (t_{max}) were obtained directly from the concentration versus time data. The terminal elimination rate constant (λ_z) was assessed by linear regression. Terminal half-life ($t_{0.5}$) was calculated as $\ln 2/\lambda_z$. Areas under the concentration-time curve (AUC) were calculated by the linear trapezoidal rule from the time of dosing to infinity. The extrapolated terminal area was calculated as C_n/λ_z, where C_n is the last data point.

Serum concentration versus time profiles of pentoxifylline, (−)-(*R*)-M1, (+)-(*S*)-M1, and their metabolites were simultaneously fitted to the interconversion model (Fig. 1) using $1/(Y_{predicted})^2$ as the weighting scheme in WinNonlin v. 3.3 (Pharsight Corp., Mountain View, CA). Linear disposition of all compounds under investigation was assumed based on the results of preliminary data analyses where alternative models with Michaelis–Menten type saturable conversion/elimination processes were tested. Thus, pharmacokinetic parameters were obtained by solving the following differential equations:

$$V_{PTX}\frac{dC^{\text{PTX}}_{\text{PTX}}}{dt} = -(\text{CL}_{10}+\text{CL}_{12}+\text{CL}_{13})C^{\text{PTX}}_{\text{PTX}} + \text{CL}_{21}C^{\text{PTX}}_{(R)\text{-M1}} + \text{CL}_{31}C^{\text{PTX}}_{(S)\text{-M1}}, \quad (1)$$

$$V_{(R)\text{-M1}}\frac{dC^{\text{PTX}}_{(R)\text{-M1}}}{dt} = -(\text{CL}_{20}+\text{CL}_{21})C^{\text{PTX}}_{(R)\text{-M1}} + \text{CL}_{12}C^{\text{PTX}}_{\text{PTX}}, \quad (2)$$

$$V_{(S)\text{-M1}}\frac{dC^{\text{PTX}}_{(S)\text{-M1}}}{dt} = -(\text{CL}_{30}+\text{CL}_{31})C^{\text{PTX}}_{(S)\text{-M1}} + \text{CL}_{13}C^{\text{PTX}}_{\text{PTX}}, \quad (3)$$

$$V_{(R)\text{-M1}}\frac{dC^{(R)\text{-M1}}_{(R)\text{-M1}}}{dt} = -(\text{CL}_{20}+\text{CL}_{21})C^{(R)\text{-M1}}_{(R)\text{-M1}} + \text{CL}_{12}C^{(R)\text{-M1}}_{\text{PTX}}, \quad (4)$$

$$V_{PTX}\frac{dC^{(R)\text{-M1}}_{\text{PTX}}}{dt} = -(\text{CL}_{10}+\text{CL}_{12}+\text{CL}_{13})C^{(R)\text{-M1}}_{\text{PTX}} + \text{CL}_{21}C^{(R)\text{-M1}}_{(R)\text{-M1}} + \text{CL}_{31}C^{(R)\text{-M1}}_{(S)\text{-M1}}, \quad (5)$$

$$V_{(S)\text{-M1}}\frac{dC^{(R)\text{-M1}}_{(S)\text{-M1}}}{dt} = -(\text{CL}_{30}+\text{CL}_{31})C^{(R)\text{-M1}}_{(S)\text{-M1}} + \text{CL}_{13}C^{(R)\text{-M1}}_{\text{PTX}}, \quad (6)$$

$$V_{(S)\text{-M1}}\frac{dC^{(S)\text{-M1}}_{(S)\text{-M1}}}{dt} = -(\text{CL}_{30}+\text{CL}_{31})C^{(S)\text{-M1}}_{(S)\text{-M1}} + \text{CL}_{13}C^{(S)\text{-M1}}_{\text{PTX}}, \quad (7)$$

$$V_{PTX}\frac{dC^{(S)\text{-M1}}_{\text{PTX}}}{dt} = -(\text{CL}_{10}+\text{CL}_{12}+\text{CL}_{13})C^{(S)\text{-M1}}_{\text{PTX}} + \text{CL}_{21}C^{(S)\text{-M1}}_{(R)\text{-M1}} + \text{CL}_{31}C^{(S)\text{-M1}}_{(S)\text{-M1}}, \quad (8)$$

$$V_{(R)\text{-M1}}\frac{dC^{(S)\text{-M1}}_{(R)\text{-M1}}}{dt} = -(\text{CL}_{20}+\text{CL}_{21})C^{(S)\text{-M1}}_{(R)\text{-M1}} + \text{CL}_{12}C^{(S)\text{-M1}}_{\text{PTX}}, \quad (9)$$

where $C^{\text{PTX}}_{\text{PTX}}$, $C^{\text{PTX}}_{(R)\text{-M1}}$, and $C^{\text{PTX}}_{(S)\text{-M1}}$ are serum concentrations of pentoxifylline and its metabolites, (−)-(*R*)-M1 and (+)-(*S*)-M1 after administration of pentoxifylline, $C^{(R)\text{-M1}}_{(R)\text{-M1}}$, $C^{(R)\text{-M1}}_{\text{PTX}}$, and $C^{(R)\text{-M1}}_{(S)\text{-M1}}$ are serum concentrations of (−)-(*R*)-M1 and its metabolites, pentoxifylline and (+)-(*S*)-M1 after administration of (−)-(*R*)-M1, $C^{(S)\text{-M1}}_{(S)\text{-M1}}$, $C^{(S)\text{-M1}}_{\text{PTX}}$, and $C^{(S)\text{-M1}}_{(R)\text{-M1}}$ are serum concentrations of (+)-(*S*)-M1 and its metabolites, pentoxifyl-

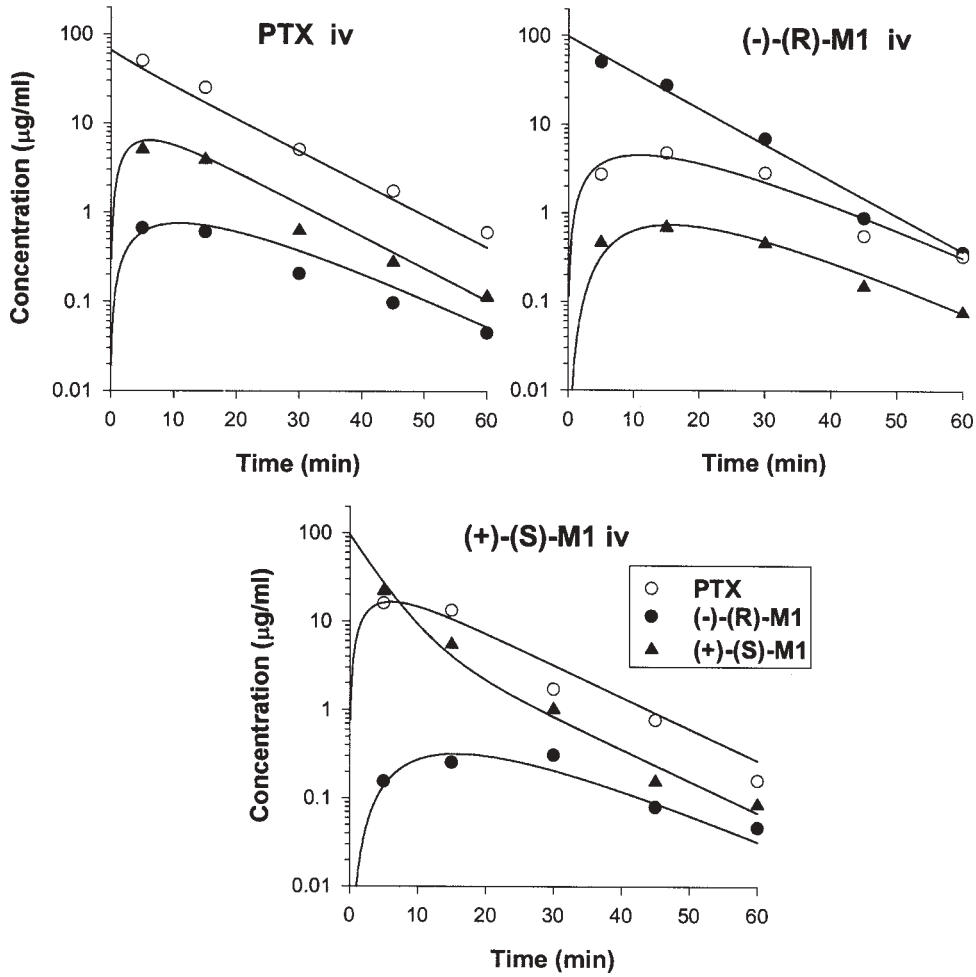

Fig. 2. Observed (symbols) and model predicted (solid lines) serum concentrations of pentoxifylline, (−)-(*R*)-M1, and (+)-(*S*)-M1 as parent drugs or metabolites after intravenous administration of 50 mg/kg of each compound to mice. Each symbol represents the mean for four mice, except at 5 min ($n = 5$).

line and (−)-(*R*)-M1 after administration of (+)-(*S*)-M1, CL_{12} is the conversion clearance of pentoxifylline to (−)-(*R*)-M1, CL_{21} is the conversion clearance of (−)-(*R*)-M1 to pentoxifylline, CL_{13} is the conversion clearance of pentoxifylline to (+)-(*S*)-M1, CL_{31} is the conversion clearance of (+)-(*S*)-M1 to pentoxifylline, CL_{10}, CL_{20}, and CL_{30} are sums of all clearance processes for pentoxifylline, (−)-(*R*)-M1, and (+)-(*S*)-M1, respectively, excluding interconversion. Initial conditions, when $t = 0$, $C^{\text{PTX}}_{\text{PTX}}(0) = \frac{D_{\text{PTX}}}{V_{\text{PTX}}}, C^{\text{PTX}}_{(R)\text{-M1}}(0) = 0, C^{\text{PTX}}_{(S)\text{-M1}}(0) = 0, C^{(R)\text{-M1}}_{(R)\text{-M1}}(0) = \frac{D_{(R)\text{-M1}}}{V_{(R)\text{-M1}}}, C^{(R)\text{-M1}}_{\text{PTX}}(0) = 0, C^{(R)\text{-M1}}_{(S)\text{-M1}}(0) = 0, C^{(S)\text{-M1}}_{(S)\text{-M1}}(0) = \frac{D_{(S)\text{-M1}}}{V_{(S)\text{-M1}}}, C^{(S)\text{-M1}}_{\text{PTX}}(0) = 0$, and $C^{(S)\text{-M1}}_{(R)\text{-M1}}(0) = 0$. D_{PTX}, $D_{(R)\text{-M1}}$, and $D_{(S)\text{-M1}}$ are doses of pentoxifylline, (−)-(*R*)-M1, and (+)-(*S*)-M1 administered, and V_{PTX}, $V_{(R)\text{-M1}}$, and $V_{(S)\text{-M1}}$ are volumes of distribution for pentoxifylline, (−)-(*R*)-M1, and (+)-(*S*)-M1, respectively. In order to assess the role of metabolic interconversion in the disposition of the compounds under investigation, concentration versus time profiles of pentoxifylline and (−)-(*R*)-M1 as parent drugs were also fitted to a traditional one-compartment pharmacokinetic model. Goodness of fit was evaluated by the standard procedures.[12]

RESULTS

Mean serum concentration versus time profiles of pentoxifylline, (−)-(*R*)-M1, (+)-(*S*)-M1 and their metabolites after intravenous administration of each compound on three separate occasions to mice are presented in Figure 2. At the dose level used, monoexponential disposition of pentoxifylline and (−)-(*R*)-M1 occurred, whereas (+)-(*S*)-M1 levels declined in a biphasic manner. Solid lines in Figure 2 representing the predicted concentrations from the interconversion model show that the proposed model adequately described the experimental data. The relatively low values of CV (coefficient of variation) are also indicative of good model fitting (Table 1). Analysis of pharmacokinetic parameters listed in Table 1 revealed that the conversion clearance of (−)-(*R*)-M1 to pentoxifylline (CL_{21}) was six times greater than that for the reverse process (CL_{12}). The values of this parameter were 9.12

TABLE 1. Pharmacokinetic parameters of pentoxifylline, (–)-(*R*)-M1, and (+)-(*S*)-M1 after intravenous administration of 50 mg/kg of each compound to mice in independent experiments (CV: coefficient of variation)

Parameter	Value	CV [%]
V_{PTX} [ml/kg]	757.03	19.08
$V_{(R)\text{-M1}}$ [ml/kg]	511.18	24.93
$V_{(S)\text{-M1}}$ [ml/kg]	519.01	50.52
CL_{10} [ml/min/kg]	53.09	21.10
CL_{20} [ml/min/kg]	38.61	20.91
CL_{30} [ml/min/kg]	74.69	40.74
CL_{12} [ml/min/kg]	1.50	23.96
CL_{21} [ml/min/kg]	9.12	23.79
CL_{13} [ml/min/kg]	24.99	37.21
CL_{31} [ml/min/kg]	62.18	37.30

and 1.50 ml/min/kg, respectively. The interconversion of pentoxifylline and (+)-(*S*)-M1 was faster as reflected by the values of the conversion clearances CL_{13} and CL_{31} describing this phenomenon which were approximately 16 and 7 times greater in comparison with the corresponding clearances estimated for the interconversion of pentoxifylline and (–)-(*R*)-M1. Central volumes of distribution were quite similar for (–)-(*R*)-M1 and (+)-(*S*)-M1 and equalled 511.18 and 519.01 ml/kg, respectively, whereas pentoxifylline volume of distribution was larger in comparison to those assessed for both enantiomers studied, namely 757.03 ml/kg.

Pharmacokinetic parameters such as volume of central compartment (V_c, ml/kg) and clearance (CL, ml/min/kg) of pentoxifylline and (–)-(*R*)-M1, obtained when their concentration versus time data were fitted separately to a one-compartment model were: 654.88 and 53.83, and 526.63 and 49.26 for pentoxifylline and (–)-(*R*)-M1, respectively. Thus, according to this model the values of elimination half-lives ($t_{0.5}$) for pentoxifylline and (–)-(*R*)-M1 were 8.45 and 7.37 min. When comparing the values of pharmacokinetic parameters of pentoxifylline estimated using both the interconversion and one-compartment models it seems that the application of traditional approaches leads to underestimation of both clearance and, to a lesser extent, volume of distribution (Table 1). The "true" value of pentoxifylline clearance calculated as the sum of both irreversible and reversible processes was 78.68 ml/min/kg. Interestingly, in the case of (–)-(*R*)-M1 both parameters do not seem to be influenced by the occurrence of metabolic interconversion.

Pentoxifylline and (–)-(*R*)-M1 concentrations measured after pentoxifylline administration and concentrations of both compounds measured when (–)-(*R*)-M1 was given as a parent drug could be detected in most tissues tested for up to 60 min following intravenous dosing. The results of noncompartmental analysis are listed in Tables 2 and 3. As shown in Table 2, when pentoxifylline was administered as a parent drug, maximum tissue concentrations (C_{max}) of this drug occurred at the first sampling time, that is, 5 min, with the highest values of this parameter observed in brain and kidney (21.81 ± 4.51 and 18.53 ± 6.68 μg/g, respectively). Serum levels of the metabolite, (–)-(*R*)-M1 was lower in comparison with those observed in the investigated tissues. Only brain (–)-(*R*)-M1 concentrations did not reach values determined in serum at all sampling times and at 60 min they dropped below the limit of quantification. Similarly to the parent drug, (–)-(*R*)-M1 peak concentrations occurred at the first sampling time. Interestingly, after administration of pentoxifylline, the highest concentrations of (–)-(*R*)-M1 were observed in lungs, where they averaged 30.01 ± 7.70 μg/g at 5 min after dosing.

In contrast to pentoxifylline, when (–)-(*R*)-M1 was given as a parent compound, its tissue levels were close to those in serum with the exception of brain where C_{max} was only 17.87 ± 2.76 μg/g (Table 3). The concentrations of pentoxifylline as (–)-(*R*)-M1 metabolite in serum and the tissues under investigation were relatively low and followed exactly the same pattern as in the case when pentoxifylline was administered as a parent drug. Thus, the highest values of pentoxifylline C_{max} were observed in serum, brain, and kidney. Following (–)-(*R*)-M1 administration, C_{max} of (–)-(*R*)-M1 occurred very quickly, that is, within 5 min in all tissues studied, while the maximum concentrations of its metabolite, pentoxifylline in serum, liver, and brain reached the highest values 15 min after dosing (Table 3). It is worth noting that after pentoxifylline administration, kidney and brain concentrations declined approximately in parallel with serum concentrations of the parent drug, whereas in lungs and liver pentoxifylline levels were maintained longer than in

TABLE 2. Model independent pharmacokinetic parameters of pentoxifylline and its metabolite (–)-(*R*)-M1 in serum and tissues after intravenous administration of 50 mg/kg pentoxifylline

	Pentoxifylline (parent)			(–)-(*R*)-M1 (metabolite)		
Tissue	t_{max} [min]	C_{max} (±SD) [μg/g]	$t_{0.5}$ [min]	t_{max} [min]	C_{max} (±SD) [μg/g]	$t_{0.5}$ [min]
Serum	NA	71.17[a]	8.44	5.0	0.66 ± 0.15[b]	12.22
Liver	5.0	6.39 ± 3.29	14.36	5.0	6.19 ± 0.66	13.86
Brain	5.0	21.81 ± 4.51	10.16	5.0	0.49 ± 0.09	7.45
Kidney	5.0	18.53 ± 6.68	11.33	5.0	5.34 ± 1.96	11.00
Lungs	5.0	7.19 ± 6.29	16.62	5.0	30.01 ± 7.70	11.65

NA: not applicable.
[a]C_0(μg/ml): the initial concentration.
[b]In μg/ml.

TABLE 3. Model independent pharmacokinetic parameters of (–)-(*R*)-M1 and its metabolite pentoxifylline in serum and tissues after intravenous administration of 50 mg/kg (–)-(*R*)-M1

	(–)-(*R*)-M1 (parent)			Pentoxifylline (metabolite)		
Tissue	t_{max} [min]	C_{max} (±SD) [μg/g]	$t_{0.5}$ [min]	t_{max} [min]	C_{max} (±SD) [μg/g]	$t_{0.5}$ [min]
Serum	NA	68.61[a]	7.26	15.0	4.75 ± 0.48[b]	9.62
Liver	5.0	38.89 ± 9.30	9.44	15.0	1.89 ± 0.51	11.98
Brain	5.0	17.87 ± 2.76	8.79	15.0	2.44 ± 0.32	10.73
Kidney	5.0	41.67 ± 9.76	11.66	5.0	3.42 ± 0.18	18.56
Lungs	5.0	42.16 ± 4.31	9.75	5.0	1.00 ± 0.24	8.46

NA: not applicable.
[a]C_0(μg/ml): the initial concentration.
[b]In μg/ml.

serum (terminal half-lives, $t_{0.5}$ were 16.62 and 14.36 min versus 8.44 min, respectively). After (–)-(*R*)-M1 administration, its serum levels declined more rapidly than those in all tissues studied, however, no retention of the compound in any specific tissue was observed (Tables 2 and 3).

Figure 3 illustrates tissue-to-serum AUC ratios of pentoxifylline and (–)-(*R*)-M1 after their intravenous administration. In the case of pentoxifylline these ratios were very low and ranged from 0.1 for liver and lungs to 0.32 for brain tissue. In turn, following (–)-(*R*)-M1 dosing they were relatively high for liver, kidney, and lungs (0.64–0.72), whereas for brain this ratio was two-fold lower in comparison to other tissues studied. Following administration of pentoxifylline as the parent drug, (–)-(*R*)-M1/pentoxifylline AUC ratio was low and similar for serum and brain (0.018 and 0.027, respectively) and much higher for other analyzed tissues. In the case of lungs it reached especially high value of 4.98 (Fig. 4). At the same time, (+)-(*S*)-M1/pentoxifylline AUC ratio reached extremely high values in liver (5.66), moderately high in kidneys and lungs (1.70 and 1.38), whereas in serum this ratio did not exceed 0.1 (Fig. 4, inset). In contrast, when (–)-(*R*)-M1 was given as the parent compound, pentoxifylline/(–)-(*R*)-M1 AUC ratio did not differ considerably between serum and other tissues under investigation.

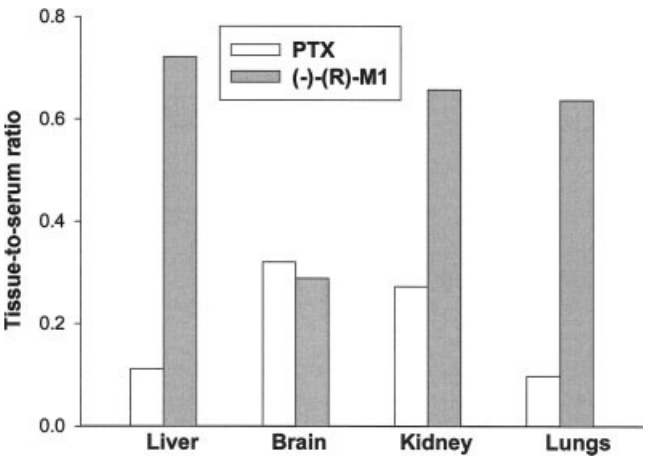

Fig. 3. The ratios of pentoxifylline and (–)-(*R*)-M1 AUCs calculated in tissues and serum (tissue-to-serum ratios) in mice receiving intravenous dose of these compounds in separate experiments.

DISCUSSION

The results of the present study indicated that pentoxifylline, (–)-(*R*)-M1, and (+)-(*S*)-M1 are very rapidly eliminated from murine serum. The observed monoexponential decay of pentoxifylline concentrations over time is in agreement with the results of other studies in mice[13] and rats.[14] In humans, a two-compartment pharmacokinetic model may be used to describe pharmacokinetics of pentoxifylline[15,16] and (–)-(*R*)-M1.[17] Interestingly, after administration of (+)-(*S*)-M1 as a parent compound a biexponential serum concentration versus time profile was observed. The slower rate of distribution equilibrium between the tissues and blood after administration of (+)-(*S*)-M1 in comparison to pentoxifylline and (–)-(*R*)-M1 indicates that, besides passive diffusion, enantioselective transport mechanisms may be involved in the disposition of both enantiomers studied. However, no significant improvement in fit was obtained by incorporating the tissue compartment for (+)-(*S*)-M1 in the model presented in Figure 1. The inclusion of this compartment led to an increase in the Akaike Information Criterion from 77.66 to 103.01 and in the Schwartz Criterion from 95.27 to

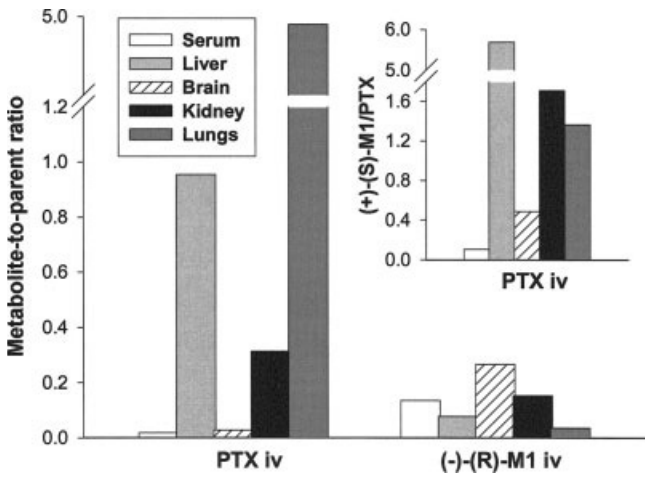

Fig. 4. The ratios of metabolite to parent AUCs in serum and all investigated tissues following intravenous administration of 50 mg/kg pentoxifylline or 50 mg/kg (–)-(*R*)-M1 to mice. The inset shows the ratio of (+)-(*S*)-M1 to pentoxifylline AUC when pentoxifylline was administered as a parent drug.

124.14. These results could be explained by the fact that insufficient data were collected during the initial distribution phase following (+)-(S)-M1 administration.

In humans lisofylline exibited linear disposition after intravenous administration at doses 1–3 mg/kg,[17] whereas pentoxifylline and its metabolite M1 (racemic) demonstrated dose-dependent pharmacokinetics following oral doses of 100 to 400 mg of pentoxifylline in solution.[18] The results of the preliminary analyses performed in the present study (data not shown) indicated that the 50 mg/kg dose used is insufficient to reveal the presence of nonlinear kinetics of pentoxifylline disposition in mice.

The observed difference between the conversion clearance of pentoxifylline to (−)-(*R*)-M1 in comparison to that of pentoxifylline to (+)-(*S*)-M1 in vivo is in accord with the results of in vitro studies using human hemolysed erythrocytes.[19] The rate of formation of (+)-(*S*)-M1 was calculated to be 15 times higher than that of (−)-(*R*)-M1, whereas in the present study the ratio of the conversion clearance for the transformation of pentoxifylline to (+)-(*S*)-M1 was 16-fold greater than that for (−)-(*R*)-M1. In turn, it has been assessed that in human erythrocytes the formation of pentoxifylline from (+)-(*S*)-M1 was 4-fold faster than from (−)-(*R*)-M1. In vivo this process was also in favour of (+)-(*S*)-M1 and the ratio of the appropriate conversion clearances was even higher and reached the value of 7. In human liver microsomes these differences were less profound as reflected by the ratio of corresponding clearances that did not exceed 2.[11]

Our results showed that reversible metabolism observed in serum plays a modest role in the disposition of pentoxifylline and (−)-(*R*)-M1. When fitting pentoxifylline and (−)-(*R*)-M1 concentration versus time data separately to a one-compartment pharmacokinetic model, the values of pharmacokinetic parameters were slightly underestimated only in the case of pentoxifylline. The increased exposure to both compounds caused by the occurrence of reversible metabolism does not seem to have any therapeutic significance as both compounds are very rapidly eliminated from the body, thus their accumulation is not likely to take place. However, it should be borne in mind that the metabolite-to-parent ratio in serum does not reflect this ratio in all tissues. In several tissues there are distinct differences between exposure to (−)-(*R*)-M1 and pentoxifylline and their metabolites, as expressed by the respective AUC values.

The transformation of pentoxifylline to metabolite M1 occurs in liver, but this organ is not believed to be the main site of pentoxifylline metabolism as its clearance several times exceeds liver blood flow.[20] It has been shown that most pentoxifylline reduction takes place in erythrocytes by enzymes of the carbonyl reductase type.[19,21] In mice, substantial amounts of carbonyl reductase can be found in the Clara cells, which are known to be sites of pulmonary metabolism.[22] This is presumably the reason for the very high lung levels of (−)-(*R*)-M1 determined after intravenous administration of pentoxifylline in the present study. In addition, the reaction catalysed by pulmonary carbonyl reductases seems to be enantioselective as lungs exposure to (−)-(*R*)-M1 optical antipode was approximately four times lower. In turn, high concentrations of (+)-(*S*)-M1 in liver tissue observed after pentoxifylline administration provide evidence that this enantiomer is the major product of pentoxifylline reduction in this organ. The latter observation supports the results of the in vitro studies demonstrating that in human liver cytosol pentoxifylline is exclusively reduced to (+)-(*S*)-M1, while in microsomes this process is 85% stereoselective in favor of the formation of this enantiomer.[11]

The mouse is a common animal model for studying sepsis and septic shock.[23] The results of our study indicated that after intravenous administration (−)-(*R*)-M1 reaches considerably higher levels than pentoxifylline in murine organs when given as parent drugs. This observation may be important from the therapeutic point of view as in vitro studies indicated that immunomodulatory effects of both compounds were dose-dependent.[24]

CONCLUSIONS

In conclusion, reversible metabolism plays a modest role in the disposition of pentoxifylline and (−)-(*R*)-M1. Our results demonstrate that pentoxifylline and (−)-(*R*)-M1 exhibit different tissue distribution and metabolite profiles in mice. Due to the fact that (−)-(*R*)-M1 attained higher concentrations in target organs in comparison with pentoxifylline, it seems that the latter compound has less favorable pharmacokinetic properties. High levels of (−)-(*R*)-M1 observed after pentoxifylline administration in certain tissues such as liver or lungs suggest that pentoxifylline may constitute an effective prodrug for (−)-(*R*)-M1 in these organs.

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