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TP-434 is Metabolically Stable and Has Low Potential for Drug-Drug Interactions

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Abstract

Background: TP-434 is a broad-spectrum fluorocycline for treatment of serious hospital infections caused by tetracycline-susceptible and tetracycline–resistant bacteria.

Methods: Metabolite profiles after incubation of 10 µM TP-434 with hepatocytes from rats, dogs, monkeys, and humans for 4 hours were determined by mass spectrometry and selective ion monitoring analysis. The potential for TP-434 to inhibit the activity of human hepatic microsomal enzymes ± pre-incubation was determined using CYP-selective substrates and LC/MS/MS detection. The blood:plasma (B:P) distribution ratio of 0.5, 1, and 10 µM TP-434 in pooled human blood in the presence of EDTA, heparin, and sodium citrate was determined. The apparent passive permeability (Papp A→B) and potential transport (Papp B→A) of 1 µM TP-434 in MDCK cell cultures over-expressing Multi-Drug Resistance 1 gene (MDR1) was determined by adding TP-434 to apical (A) or basolateral (B) sides of the cultures, incubation at 37ºC, and measurement of concentrations at 1 & 2 hours by LC/MS/MS.

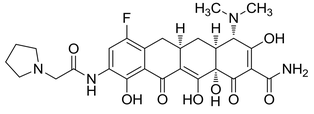
Results: The metabolic stability of TP-434 was high, with 103.2%, 95%, 94.0%, and 85.3% remaining after 4 hours incubation with rat, dog, monkey, and human cells, respectively, suggesting that hepatic metabolism of TP-434 is negligible. Inhibition (IC50 values) against relevant human microsomal CYP isozymes directly or in a time-dependent fashion, was ≥85 µM, suggesting that CYP-mediated drug-drug interactions are unlikely. The B:P ratio ranged from 1.3 ± 0.1 to 0.9 ± 0.1, demonstrating that TP-434 is not preferentially distributed to red blood cells (RBCs). Permeability in MDCK MDR1+ cultures was low, with mean Papp A→B of 0.44 x 10-6 cm/sec. The mean efflux Papp B→A was 1.57 x 10-6 cm/sec.

Conclusions: 1) The metabolic stability and lack of CYP inhibition are consistent with the low potential of TP-434 for drug-drug interactions. 2) TP-434 is not sequestered in RBCs. 3) TP-434 is a poor substrate for MDR1-mediated transport and has low potential for distribution into brain.

Introduction

The need for new antibacterial agents capable of treating multidrug-resistant gram-positive and gram-negative bacterial infections is well recognized. TP-434 is a novel, synthetic tetracycline indicated for the parenteral treatment of serious hospital infections, with activity against isolates containing tetracycline-specific efflux and ribosomal protection mechanisms (posters F1-2155, F1-2157-F1-2160). TP-434 is not subject to mechanisms conferring specific resistance to other classes of antibiotics. The pharmacokinetics, safety and tolerability of TP-434 have been evaluated in Phase 1 single- and multiple-ascending dose trials (A1-027, A1-028). Prior to Phase 1 trials, studies to characterize the disposition of TP-434 and to better understand the potential for this novel structure to interact with important drug metabolizing enzymes and transporters were done, including human plasma protein binding, the blood:plasma (B:P) distribution ratio, metabolic stability in human and animal hepatocytes, human microsomal CYP inhibition, and permeability and efflux potential.

TP-434



Results

PLASMA PROTEIN BINDING

The unbound (free) fraction of 1 µM (~0.5 µg/mL) TP-434 was 11.5 ± 0.4% in human plasma by ultrafiltration. However, when the unbound fraction was measured by equilibrium dialysis in plasma collected with heparin, concentration-dependent protein binding was observed. This observation has been reported for tigecycline (Muralidharan, G., et al. 2005. Antimicrob. Agents Chemother. **49**: 220–229).

PLASMA PROTEIN BINDING BY EQUILIBRIUM ANALYSIS

Dosing Conc. (µg/mL)	% bound to human plasma	% free
0.1	38.3	61.7
0.25	56.1	43.9
1	59.4	40.6
2.5	65.5	34.5

BLOOD:PLASMA DISTRIBUTION RATIO

The B:P in human blood at concentrations of 0.5, 1, and 10 µM was similar in the presence of EDTA or citrate, but more consistently gave a ratio of ~1 when the B:P ratio was determined with heparin.

BLOOD:PLASMA RATIO: THE IMPACT OF DIFFERENT ANTI-COAGULANTS

TP-434 Conc.	Plasma Extraction with		
	Heparin	Citrate	EDTA
0.5 µM	1.3 ± 0.1	1.9 ± 0.2	no sample
1	1.1 ± 0.2	2.0 ± 0.1	2.6 ± 0.0
10	0.9 ± 0.1	2.8 ± 0.1	2.9 ± 0.1

METABOLIC STABILITY IN CRYOPRESERVED HEPATOCYTE SUSPENSIONS

TP-434 was stable when incubated for up to 4 hr with metabolically-competent hepatocytes from rats, dogs, monkeys, and humans, with less than 15% of added TP-434 metabolized.

Percent TP-434 Remaining After 4 Hours at 37°C			
Rat Hepatocytes	Dog Hepatocytes	Monkey Hepatocytes	Human Hepatocytes
103.2%	95%	94.0%	85.3%

DIRECT AND METABOLISM-DEPENDENT INHIBITION OF HUMAN CYP ACTIVITY

TP-434 did not inhibit the major human CYP enzymes *in vitro*, either directly or after pre-incubation, with IC50 values greater than 85.6 µM, the highest concentration tested. Direct inhibition of CYP2C8, 45%, was noted at 85.6 µM; however this value greatly exceeds the expected clinically-relevant concentrations of TP-434, ca. 5.3 µM.

Enzyme	CYP reaction	Direct inhibition		Time-dependent inhibition		
		Zero-minute preincubation		30-minute preincubation		
		IC_{50} (µM)	Maximum inhibition at 85.6 µM (%)	IC_{50} (µM)	Maximum inhibition at 85.6 µM (%)	Potential for time-dependent inhibition
CYP1A2	Phenacetin *O*-dealkylation	> 85.6	7.1	> 85.6	0.0	Little or no
CYP2B6	Efavirenz 8-hydroxylation	> 85.6	18	> 85.6	13	Little or no
CYP2C8	Amodiaquine *N*-dealkylation	> 85.6	45	> 85.6	46	Little or no
CYP2C9	Diclofenac 4´-hydroxylation	> 85.6	NA	> 85.6	1.6	Little or no
CYP2C19	*S*-Mephenytoin 4´-hydroxylation	> 85.6	5.3	> 85.6	11	Little or no
CYP2D6	Dextromethorphan *O*-demethylation	> 85.6	4.7	> 85.6	13	Little or no
CYP3A4/5	Testosterone 6β-hydroxylation	> 85.6	5.3	> 85.6	8.6	Little or no
CYP3A4/5	Midazolam 1´-hydroxylation	> 85.6	16	> 85.6	26	Little or no

PERMEABILITY AND P-gp EFFLUX POTENTIAL IN MDR1-MDCK CELLS

The mean A→B Papp for TP-434 was low, 0.44 (10^{-6} cm/sec) and efflux was modest, with a mean B→A Papp of 1.57 (10^{-6} cm/sec). For comparison the mean B→A Papp for the prototypical efflux substrate digoxin was 34.9 (10^{-6} cm/sec). These data demonstrate that TP-434 is unlikely to penetrate the CNS or interact strongly with P-gp.

Methods

PLASMA PROTEIN BINDING AND B:P RATIO

The *in vitro* protein binding of 1 µM TP-434 in plasma collected with EDTA from humans was determined in triplicate using ultrafiltration and LC-MS/MS analysis. TP-434 was added to 1.0 mL of plasma which was then incubated for 10 minutes at 37ºC prior to loading into the ultrafiltration reservoir. The samples were centrifuged at 37ºC at 1800 rpm for 12 minutes and approximately 0.10-0.12 mL of filtrate was collected. Aliquots of the filtrate and plasma were analyzed by HPLC-MS/MS and the peak area ratios of TP-434 were used to calculate the percent unbound for each sample. Plasma protein binding was also determined over a concentration range of 0.1-2.5 µg/mL using human plasma collected with heparin and equilibrium dialysis (Teflon microdialysis chambers) of test compound in phosphate-buffered saline against phosphate-buffered saline at 37°C for 22 hours.

The blood:plasma distribution ratio (B:P) of 0.5, 1, and 10 µM TP-434 in pooled (n=3) human blood in the presence of EDTA, heparin, and sodium citrate was also determined in triplicate. TP-434 (0.5, 1, 10 µM) was added to pooled whole blood and incubated for 30 minutes at 37ºC. After centrifugation, TP-434 concentrations were measured by HPLC-MS/MS using appropriate standard curves and the B:P ratio calculated as the ratio of the concentration in both matrices. Pooled plasma (n=3) and drug-free pooled human blood (n=3) were purchased from Bioreclamation, Liverpool NY. Centrifree® ultrafiltration devices with Ultracel YM-T membranes (1 mL) were bought from Millipore Corp. Billerica MA.

METABOLIC STABILITY IN CRYOPRESERVED HEPATOCYTE SUSPENSIONS

The metabolic stability of 10 µM TP-434 was determined after incubation with cryopreserved hepatocytes from SD rats, beagle dogs, cynomologus monkeys and humans. Approximately 1.0 X 10^6 viable cells in William's E media were incubated in 24-well plates for 0, 0.5, 1, 2, 3, and 4 hr with TP-434 (in duplicate) at 37°C in an atmosphere of 95:5% (air:CO2). Control incubations with boiled hepatocytes were also completed. Reactions were quenched with equal volumes of acetonitrile containing 0.1% formate and centrifuged. Aliquots of the supernatants were injected onto an HPLC system (CN column) and analyzed by positive ion LC/MS and LC/MSn (Finnigan XP Deca ion trap) for metabolites. 7-ethoxycoumarin was incubated with hepatocytes as a positive control for phase I (oxidative) and phase II (conjugation) metabolic activities. The concentration and viability of hepatocytes were determined by Trypan Blue exclusion.

DIRECT AND METABOLISM-DEPENDENT INHIBITION OF HUMAN MICROSOMAL CYP ACTIVITY

The potential for TP-434 to inhibit the major human hepatic microsomal CYP activities CYP1A2, 2B6, 2C8, 2C9, 2C19, 2D6, and 3A4/5 with and without pre-incubation was determined with a pool (n=16) of microsomes and CYP-selective drug substrates. For direct inhibition, TP-434 (0, or 0.9 to 85.6 µM) was incubated with substrate prior to the initiation of the reaction by a NADPH-generating system. Also, TP-434 was pre-incubated with microsomes and an NADPH-generating system for 30 min prior to the addition of substrate, to determine the potential for metabolism-dependent inhibition. Reactions were terminated after 5 min by the addition of internal standards in acetonitrile, and the samples were centrifuged prior to HPLC-MS analysis. IC50 values for TP-434 and positive control inhibitors were calculated.

PERMEABILITY AND P-gp EFFLUX POTENTIAL IN MDR1-MDCK CELLS

The permeability of TP-434 and potential efflux by P-gp were determined after incubation of 1 µM TP-434 with confluent cell monolayers for 2 hr at 37°C. All donor chambers were preincubated for 15 min with dosing solution to decrease nonspecific binding. Concentrations of TP-434 were measured at 2 hr by HPLC-MS and the apparent permeability Papp was calculated in triplicate. Monolayer integrity was determined by TEER values and Lucifer Yellow Papp. Positive controls atenolol, propranolol, and digoxin (A-B and B-A) were also tested.

Conclusions

- **TP-434 exhibits concentration and anticoagulant-dependent protein binding in human plasma**
- **The metabolic stability and lack of CYP inhibition are consistent with the low potential of TP-434 for drug-drug interactions**
- **TP-434 is not sequestered in RBCs**
- **TP-434 is a poor substrate for MDR1-mediated transport and has low potential for distribution into brain**

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