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Metabolic Stability of PTK 0796 (Omadacycline)

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ABSTRACT

PTK 0796 (PTK, omadacycline) is a novel aminomethylcycline now in Phase 3 clinical development. An in vitro assessment of the potential for metabolism and/or drug-drug interactions was undertaken.

Objectives. The in vitro stability and interaction of PTK with human cytochrome P450 isozymes was determined to assess the potential for *in vivo* modification or whether there was a significant potential for drug-drug interactions.

Methods. Metabolism assays were conducted using either pooled human liver microsome preparations, S9, liver cytosol, or recombinant flavin monooxygenases (FMO1, FMO3, FMO5) (BD Bioscience, Woburn, MA). Metabolism of 14C-PTK (5-50 µM) was determined with either NADPH or UDPGA or a combination of both co-factors. Binding of 14C-PTK to liver microsomes was determined by ultracentrifugation. The metabolism of 14C-PTK by human hepatocytes (Celsis, Baltimore, MD) was tested at 2.5 µM and 12.5 µM with 2 x 10^6 cells/ml at 37°C for 2-24hrs. PTK and metabolites were detected by HPLC with radio-detection. CYP450 induction was determined in primary human hepatocytes (1 x 10^5 cells) incubated with 1-100 µM PTK and substrate probe for 24 and 48hrs. Inhibition of CYP450 isozymes was determined using pooled human microsomes (BD Biosciences, Bedford, MA) with PTK (1-50 µM) and probe concentrations approximating the Km of each probe. Time-dependent inhibition was determined by preincubating microsomes with 1-50 µM . Probe metabolism was determined by LC-MS.

Results. There was no detectable metabolism of omadacycline by human microsomes, hepatocytes, S9 or cytosol, FMO1, FMO3, or FMO5. PTK did not induce activities of CYP 1A2, 2B6, 2C8, 2C9, 2C19, or 3A. There was no or minimal (less than 40% of maximal positive control response) induction of mRNA for CYP 1A1, 1A2, 1B1, 2B6, 2C8, 2C9, 2C19, 2J2, 3A4, or 3A5. There was no significant inhibition of CYP 1A2, 2A6, 2B6, 2C8, 2C9, 2C19, 2D6, 2E1, or 3A4/5 activity by PTK without pre-incubation nor time-dependent inhibition of CYP 1A2, 2C9, 2D6, or 3A4/5. There was no significant binding to human microsomes.

Conclusions. In vitro studies indicate that PTK 0796 (omadacycline) is unlikely to undergo significant metabolism in humans. Further, there was no induction or inhibition of CYP enzymes indicating little potential for drug-drug interactions based on these mechanisms.

Key words. PTK 0796, omadacycline, metabolism

INTRODUCTION

PTK 0796 (omadacycline) is a novel aminomethycycline and the first member of this class of tetracyclines to enter clinical development. PTK 0796 is a broad spectrum antibacterial agent being developed as a once daily intravenous and oral therapy for the treatment of community acquired skin and skin structure infections, community-acquired bacterial pneumonia, and urinary tract infections.
Studies of the potential for PTK 0796 to undergo metabolic modification and/or induce or inhibit drug metabolizing enzymes were undertaken to assess the potential of PTK to interact with concomitant medications.

METHODS

Metabolism assays were conducted using either pooled human liver microsome preparations, S9, liver cytosol, or recombinant flavin monooxygenases (FMO1, FMO3, FMO5) (BD Bioscience, Woburn, MA). Metabolism of [14C]PTK 0796 (5-50 µM) was determined with either NADPH or UDPGA or a combination of both co-factors. The non-specific binding of [14C]PTK 0796 to liver microsomes was determined by ultracentrifugation. The metabolism of [14C]PTK 0796 by human hepatocytes (Celsis, Baltimore, MD) was tested at 2.5 µM and 12.5 µM with 2 x 10^6 cells/ml at 37°C for 2-24hrs. [14C]PTK 0796 and metabolites were detected by HPLC with radio-detection. CYP450 induction was determined in primary human hepatocytes (1 x 10^5 cells) incubated with 1-100 µM PTK 0796 and substrate probe for 24 and 48hrs. Inhibition of CYP450 enzymes was determined using pooled human microsomes (BD Biosciences, Bedford, MA) with PTK 0796 (1-50 µM) and probe concentrations approximating the Km of each probe. Time-dependent inhibition was determined by preincubating microsomes with 1-50 µM PTK 0796 up to 20 minutes prior to assay. Probe metabolism was determined by LC-MS.

RESULTS

PTK 0796 was not nonspecifically bound to the human liver microsome fraction over a range of 0.05 to 2.0 mg microsomal protein/ml and at either 4.8 or 48 µM PTK 0796. >90% PTK 0796 was recovered unbound after 10 minutes of incubation at 37°C.

There was no detectable metabolism of PTK 0796 by human liver microsomes or hepatocytes as shown in Table 1 and 2.

Table 1. Recovery of PTK0796 after 30 minute incubation with Human Liver Microsomes

Cofactor	% Recovery of PTK796
None	92.09
NADPH	91.99
UDPGA	91.82
NADPH + UDPGA	91.24

Table 2. Stability of PTK 0796 in the presence of Human Hepatocytes

Incubation time, hours	% Recovery of PTK 0796
2	93.2
4	87.4
8	87.8
24	86.4

Similar results were observed following incubations with human liver subcellular fractions (i.e. S9 and cytosolic fraction) recombinant human flavin mono-oxygenase (FMO) enzymes FMO1, FMO3, FMO5.

As shown in Table 3, PTK 0796 did not inhibit the activity of the P450 isozymes tested when added simultaneously with substrate in the reaction.

Table 3. Lack of Inhibition of cytochrome P450 isozyme activity by PTK 0796

CYP450 Isozyme	Probe Reaction	PTK 0796 IC50 (µM)*
CYP 1A2	Phenacetin O-deethylation	>100
CYP 2A6	Coumarin 7-hydroxylation	>100
CYP2B6	Bupropion hydroxylation	>100
CYP2C8	Amodiaquine N-deethylation	>100
CYP2C9	Diclofenac 4'-hydroxylation	>100
CYP2C19	S-mephenytoin 4'-hydroxylation	>100
CYP2D6	Bufuralol 1'-hydroxylation	>100
CYP2E1	Chlorzoxazone 6-hydroxylation	>100
CYP3A4/5	Midazolam 1'-hydroxylation	>100
CYP3A4/5	Testosterone 6β-hydroxylation	>100

*PTK 0796 concentrations tested: 0.5, 1.0, 5.0, 10, 25, 50, 100 µM

In addition, PTK 0796 did not exhibit time-dependent inhibition when preincubated with liver microsomes prior to initiation of the enzyme assay (Table 4).

Table 4. Absence of time dependent Inhibition by PTK 0796[a]

Human CYP	Probe Reaction	Test Compound[b]	K_I (µM)	k_{inact} (min^{-1})
CYP1A2	Phenacetin O-deethylation	Furafylline	0.827 ±0.155	0.0916 ±0.00573
		PTK 0796	No inhibition	No inhibition
CYP2C9	Diclofenac 4'hydroxylation	Tienilic acid	0.421 ±0.0685	0.0724 ± 0.00330
		PTK 0796	No inhibition	No inhibition
CYP2D6	Bufuralol 1'-hydroxylation	Paroxetine	0.562 ± 0.175	0.0686 ± 0.00641
		PTK 0796	No inhibition	No inhibition
CYP3A4/5	Midazolam 1'hydroxylation	Troleandomycin	0.234 ± 0.0496	0.0763 ± 0.00364
		PTK 0796	No inhibition	No inhibition

a. Preincubation for 0 to 20 minutes prior to addition of NADPH
b. Test compound concentrations: PTK 0796 0.05, 0.1, 0.25, 0.5, 1.25, 2.5 µM; furafylline, tienilic acid, and paroxetine 0.005, 0.01, 0.025, 0.05, 0.125, 0.25 µM; troleandomycin .01, 0.02, 0.05, 0.1, 0.25, 0.5 µM

PTK 0796 does not exhibit significant induction of CYP450 activity in cultured hepatocytes as shown in Table 5.

Table 5. Change of enzyme activity in cultured primary human hepatocytes[a]

Enzyme	Positive control[b] with Maximum induction	Enzyme activity	% Max[c] (Range)
CYP1A2	BNF, 10 µM	Phenacetin O-deethylation	(0.187 – 10.2)
CYP2B6	PB, 1000 µM/ RIF, 5 µM	OH-bupropion formation	(-2.92 – -1.38)
CYP2C8	PB, 1000 µM/ RIF, 25 µM	*N*-desethylamodiaquine formation	(-11.4 – 14.5)
CYP2C9	RIF, 5/ 25 µM	4' OH-diclofenac formation	(-17 – 2.56)
CYP2C19	RIF, 5/ 25 µM	4' OH-*S*-mephenytoin formation	(0.624– 11.9)
CYP3A	PB, 1000 µM/ RIF, 5/50 µM	1' OH-midazolam formation	(-3.43 – -1.14)
UGT1A1	BNF, 10 µM	estradiol glucuronidation	(-54.1 – 48.5)

a. Hepatocytes from 3 donors. Incubation for 48 hours at 37°C. PTK 0796 at 100 µM, highest tested concentration
b. BNF β-napthoflavone; PB phenobarbitol; RIF rifampicin
c. %Max (range) is the range of % change of the 3 donor cultures.

Induction of CYP450 isozyme mRNA was determined in human liver hepatocytes from 2 donors. Minimal induction of some CYP450 mRNA was observed but was not to the degree of known inducers (Table 6).

Table 6. Induction of CYP450 mRNA by real-time PCR, from cultured primary human hepatocytes*

Enzyme	Mean Fold change in mRNA ± SD			
	Liver 1		Liver 2	
	PTK 0796[a]	Positive Control[b]	PTK 0796	Positive Control[b]
CYP1A1	2.97 ± 0.66	42.0 ± 11 (BNF, 10 µM)	2.00 ± 0.93	66.6 ± 5.1 (BNF, 10 µM)
CYP1A2	4.89 ± 1.2	15.4 ± 4.4 (BNF, 10 µM)	3.25 ± 1.5	22.4 ± 4.8 (BNF, 10 µM)
CYP1B1	0.933 ± 0.23	3.98 ± 1.0 (BNF, 10 µM)	0.636 ± 0.36	5.04 ± 1.0 (BNF, 10 µM)
CYP2B6	2.67 ± 0.98	17.6 ± 8.5 (RIF, 5 µM)	1.43 ± 0.83	17.0 ± 1.9 (PB, 1000 µM)
CYP2C8	2.99 ± 0.50	5.56 ± 1.3 (RIF, 25 µM)	3.97 ± 0.97	7.92 ± 1.1 (PB, 1000 µM)
CYP2C9	1.69 ± 0.97	2.03 ± 1.5 (RIF, 10 µM)	1.11 ± 0.35	2.29 ± 0.60 (PB, 1000 µM)
CYP2C19	1.98 ± 0.50	4.08 ± 2.2 (RIF, 10 µM)	1.50 ± 0.15	2.64 ± 0.91 (RIF, 5 µM)
CYP2J2	1.48 ± 0.32	2.87 ± 1.3 (RIF, 1 µM)	1.62 ± 0.34	0.987 ± 0.18 (RIF, 25 µM)
CYP3A4	1.60 ± 0.24	24.4 ± 9.7 (RIF, 25 µM)	0.821 ± 0.29	10.9 ± 4.4 (PB, 1000 µM)
CYP3A5	1.95 ± 0.85	1.86 ± 0.16 (PB, 1000 µM)	0.523 ± 0.17	0.551 ± 0.33 (RIF, 10 µM)
UGT1A1	1.77 ± 0.36	2.41 ± 0.40 (RIF, 10 µM)	1.81 ± 0.55	3.35 ± 1.2 (RIF, 10 µM)

*Cultures incubated at 37°C for 48 hours.

a. PTK 0796 tested at 100µM.
b. BNF β-napthoflavone; PB phenobarbitol; RIF rifampicin

CONCLUSIONS

- **There was no significant in vitro metabolism, inhibition, or induction of CYP450 isozymes by PTK 0796.**
- **Low level induction of mRNA over 48 hours in human hepatocytes was observed but generally to a much lower degree than observed with known inducers.**
- **There was no nonspecific binding of PTK 0796 to liver microsomes.**
- **The results suggest that PTK 0796 has low potential for clinically significant drug-drug interactions based on interactions with CYP450 enzymes and that PTK 0796 metabolism is unlikely to be a major contributor to systemic clearance.**