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Lack of Interaction of PTK 0796 (Omadacycline) with Human Transporter

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ABSTRACT

PTK 0796 (PTK) is a novel aminomethylcycline in Phase 3 development as an IV and oral therapy for bacterial skin infections and community-acquired pneumonia.

Objective The *in vitro* interaction of PTK with human drug transporter proteins was determined to establish the potential for drug-drug interactions based on this mechanism of drug uptake and efflux.

Methods Transport of 14C-PTK by human organic ion transporters was determined in HEK293 cells stably expressing hOAT1, hOAT3, or hOCT2 compared to the parental host cell line HEK-Flp-In. Transport of PTK by organic anion transport polypeptide transporters OATP1B1 and OATP1B3 was assessed similarly in HEK cells stably expressing these transporters. Transport of 14C-PTK by P-glycoprotein (P-gp) was determined in Caco-2 cells. Induction of P-gp and multidrug resistance-associated protein-2 (MRP2) was determined in human hepatocytes by mRNA levels. Inhibition of human Breast Cancer Resistance Protein (BRCP), P-gp, and MRP2 was determined in cell lines T8, T0.3, and MDCKII cell lines.

Results At 25μM14C-PTK, there was no difference in intracellular concentrations with or without hOAT1 or hOAT3. Probenecid did not reduce the accumulation of PTK (8μM) whereas there was a significant effect on the probe substrate. Uptake of PTK into cells was rapid, reaching more than 100 pmol/mg protein within 5min independent of transporters. PTK appears to be a substrate for P-gp, with a Km for efflux of approximately 81.5μM and a Jmax of 1140 pmole·hr^{-1}·cm^{-1}. PTK did not inhibit the function of hOAT3 and at 25μM inhibited hOAT1 by only 30%. Intracellular accumulation of PTK was not affected by hOATP. PTK (100μM) reduced transport of probes for hOATP1B1 and hOATP1B3 by ±10.1%.PTK did not induce P-gp or MRP-2 mRNA. PTK did not inhibit the activity of BRCP, P-gp or MRP-2 up to 50μM.

Conclusion The potential for drug-drug interactions based on PTK interactions with drug transporters appears to be minimal. PTK does not interact with the transporters tested except P-gp. It is a substrate for P-gp (Km=81.5μM) but is unlikely to act as an inhibitor (no inhibition up to 50μM) nor an inducer of P-gp.

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 with a spectrum of antibacterial activity suitable 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 interact with drug transporters were undertaken to assess the potential of PTK to interact with concomitant medications.

METHODS

Transporter substrate and inhibition assays of human organic anion transporter 1 and 3 (hOAT1, hOAT3), human organic anion transport polypeptide transporter 1B1 and 1B3 (hOATP1B1, hOATP1B3), and human organic cation transporter 2 (hOCT2) utilized stably transfected HEK293 (HEK Flp-In) cells. Substrate studies of P-glycoprotein (P-gp), multidrug resistance-associated protein 2 (MRP2), and breast cancer resistance protein (BCRP) utilized Caco-2 cells. The kinetics of P-gp efflux of PTK 0796 was determined in the presence and absence of GF120918. For inhibition of BCRP, a topotecan-selected cell line (T8) with increased expression was licensed from The Netherlands Cancer Institute, Amsterdam, Netherlands. For P-gp inhibition, paclitaxel-selected cell line MDA435 T0.3 cells with increased expression of P-gp protein and mRNA were obtained from Novartis Research Oncology, East Hanover, NJ. For MRP2, MDCKII cells were stably transfected with human MRP2. All assays included appropriate substrate and inhibitor control compounds. Induction of P-gp and MRP2 mRNA utilized fresh primary hepatocytes obtained from 2 donors were purchased from BD Biosciences/Gentest, Woburn, MA.

RESULTS

The uptake of PTK 0796 into mammalian cells was determined using [^{14}C]PTK 0796. Uptake into control cells appeared to be linear up to 15 min and therefore the effect of transport protein inhibitors was examined following 4 min incubations with control and transporter-expressing cells. As shown in table 1, the intracellular accumulation of PTK 0796 was independent of transporter activity (Table 1).

Table 1. Accumulation of PTK796 in control or transporter expressing HEK293 cells in the absence or presence of inhibitors*

Transporter	Substrate Concentration (μM)	Inhibitor Concentration (μM)	Accumulation (pmol/mg protein)	
			HEK-Flp-In	Transfected Cells
hOAT1	PTK 0796 (25)	None	403 ± 5.2	395 ± 23
		Probenecid (100)	424 ± 16	398 ± 25
hOAT3	PTK 0796 (25)	None	403 ± 5.2	442 ± 42
		Probenecid (100)	424 ± 16	425 ± 25
hOAT1B1	PTK 0796 (39.8)	None	402.5 ± 5.2	407 ± 39.8
		Rifamycin (100)	401.8 ± 37.1	357.7 ± 43.1
hOAT1B3	PTK 0796 (39.8)	None	103.0 ± 10.6	137.1 ± 18.1
		Rifamycin (25)	102.4 ± 8.0	140.4 ± 13.5
hOCT2	PTK 0796 (10)	None	24.0 ± 3.0	24.6 ± 2.1
		Decynium (20)	23.4 ± 2.2	23.8 ± 1.7

*model substrates para-aminohippurate (hOAT1), estrone-3-sulfate (hOAT3), estradiol-17β-D-glucuronide (hOATP1B1), cholecystokinin (hOATP1B3) and metformin (hOCT2) performed as expected with and without inhibitors. Uptake of [14C]PTK 0796 was determined after 4 minutes of incubation at 37°C.

Similarly, PTK 0796 was not a substrate for human BCRP or MRP2 but appears to be a substrate for P-gp (Table 2).

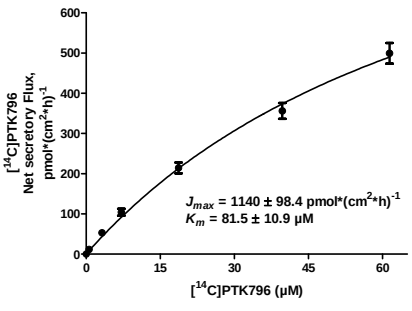
Table 2. [^{14}C]PTK 0796 transport rates across Caco-2 cell monolayers and the effect of transport protein inhibitors*

PTK 0796 Concentration (μM)	Inhibitor Concentration (μM)	Transporter	Papp x 10^{-5} Ap to Bl (cm/min)	Papp x 10^{-5} Bl to Ap (cm/min)
2.0	None	-	2.73 ± 0.43	35.7 ± 3.7
	LY335979 (1.0)	P-gp	7.66 ± 0.56	10.8 ± 1.2
	MK571 (10)	MRP2	3.97 ± 0.76	35.1 ± 1.7
	K0143 (1.0)	BCRP	3.71 ± 0.99	36.9 ± 2.3
12	None	-	2.10 ± 0.14	26.6 ± 2.4
	LY335979 (1.0)	P-gp	5.80 ± 0.22	6.99 ± 0.26

*Uptake experiments conducted over 120 minutes at 37 °C.

The flux of PTK 0796 in the absence (Flux (Bl to AP)$_0$) compared to the flux in the presence of GF120918 (Flux (Bl to Ap)$_i$) were compared to determine the net secretory flux and affinity (Figure 1).

Figure 1. Kinetics of P-gp Flux of PTK 0796 across Caco-2 cells in the Bl to Ap direction



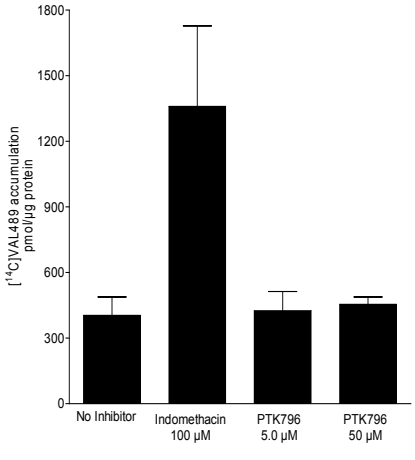
None of the transporters were significantly inhibited by PTK 0796 although hOAT1 did exhibit moderate inhibition (20-32%) at higher PTK 0796 concentrations (Table 3). In addition, PTK 0796 did not inhibit MRP2-mediated [^{14}C]valsartan ([^{14}C]VAL489) efflux from MDCKII/MRP2cells (Figure 2).

Table 3. Inhibition of human transporter proteins by PTK 0796

Transporter	Substrate Concentration (μM)	Inhibitor Concentration (μM)	Substrate Accumulation ± SD (pmol/mg protein)		% Inhibition
			HEK-Flp-In	Tansfected Cells	
hOAT1	PAH (1.0)	None	6.4 ± 0.3	32.5 ± 1.2	-
		Probenecid (100)	6.4 ± 0.5	7.0 ± 0.5	98.0
		PTK 0796 (5.0)	5.9 ± 0.2	27.6 ± 2.6	16.7
		PTK 0796 (25)	6.0 ± 0.2	23.7 ± 1.9	32.1
hOAT3	E_2S (1.0)	None	3.0 ± 0.5	68.2 ± 3.1	-
		Probenecid (100)	3.2 ± 0.4	5.5 ± 0.3	96.5
		PTK 0796 (25)	3.1 ± 0.3	68.4 ± 0.6	0
hOATP1B1	$E_2$17βG (1.0)	None	0.78 ± 0.09	25.52 ± 0.67	
		Rifamycin (25)	0.82 ± 0.03	0.94 ± 0.09	99
		PTK 0796 (100)	0.72 ± 0.15	23.12 ± 1.40	9.7
hOATP1B3	CCK8 (44 nM)	None	0.198 ± 0.014	0.670 ± 0.044	-
		Rifamycin (25)	0.184 ± 0.004	0.173 ± 0.010	105
		PTK 0796 (100)	0.225 ± 0.006	0.629 ± 0.073	8.5
hOCT2	Metformin (16)	None	2.52 ± 0.22	126.0 ± 2.2	-
		Decynium22 (20)	1.43 ± 0.52	2.38 ± 0.52	99
		PTK 0796 (100)	1.90 ± 0.52	114.0 ± 2.8	9.2

*Cells incubated with substrate ± inhibitor for 5 min (hOAT) or 4 min (hOATP and hOCT) at 37°C.

Figure 2. Effect of PTK 0796 on MRP2 mediated efflux from MDCKII cells



PTK 0796 did not inhibit BCRP or P-gp activities in T8 or MDA cells, respectively (Table 4).

Table 4. Inhibition of BCRP and P-gp by PTK 0796*

Transporter	Substrate Concentration (μM)	Inhibitor Concentration (μM)	Fluorescence (Geometric mean)
BCRP	BDP (0.05)	None	23.3
		PTK 0796 (50)	24.9
P-gp	Rho123 (0.1)	None	9.56
		PTK 0796 (50)	7.04

*Inhibitors fumitremorgin C and cyclosporine A performed as expected. Transporter substrates: BDP (Bodipy FL prazosin), Rho123 (rhodamine 123). Cells incubated with substrate ± inhibitor for 60 minutes (BCRP) or 90 minutes (P-gp) at 37°C.

There was no increase of mRNA for either P-gp or MRP2 transporter genes indicating the PTK 0796 was not an inducer of either transporter after 48 hours of incubation at 37 °C.

Table 5. Induction of mRNA of P-gp and MRP2

mRNA	Treatment	Inducer Concentration (μM)	Mean Fold Change ± SD	
			Liver 1	Liver 2
P-gp	None	-	1.0	1.0
	PTK 0796	100	1.16 ± 0.34	1.31 ± 0.25
	Rifamycin	10	2.53 ± 0.37	3.13 ± 0.99
MRP2	None	-	1.0	1.0
	PTK 0796	100	0.759 ± 0.23	1.01 ± 0.63
	Rifamycin	10	1.58 ± 0.4	1.48 ± 0.38

CONCLUSION

- **PTK 0796 (omadacycline) rapidly accumulates within mammalian cells, consistent with its large volume of distribution and rapid distribution from plasma.**
- **PTK 0796 is not a substrate for any of the tested transporters tested except P-glycoprotein. The Km value for the association of PTK796 with P-gp transporter was 81.5 μM indicating a rather weak affinity interaction.**
- **PTK 0796 is a relatively weak inhibitor of only hOAT1 (30% at 25μM), with no evidence of significant inhibition of any other transporter tested.**
- **PTK 0796 does not induce increased transcription of either P-gp or MRP2 genes. Further, there appears to be no increase in activity of transporters in the presence of PTK 0976.**
- **There appears to be no significant interaction of PTK 0796 with the human drug transporters tested suggesting that drug-drug interactions based on transporter activity is unlikely.**