Preclinical characterization of PC786, a potent antiviral inhibitor of respiratory syncytial virus replication

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Introduction

PC786 is a novel anti-respiratory syncytial virus (RSV) agent designed for inhalation treatment of RSV infection. In this study, the *in vitro* and *in vivo* profiles of PC786 were investigated against *RSV A and RSV B*.

Methods

The anti-viral activity of PC786 was assessed using *in vitro* cytopathic effect (CPE) assays in HEp-2 cells using RSV A2 (MOI of 1, 5 days), RSV B Washington (WST) (MOI of 0.2, 6 days) and the clinical isolates 1997/12-35, 2001/2-20 & 2000/3-4 (all RSV A from BEI Resources), and a minigenome-based reporter assay conducted in HEp-2 cells. Specificity was determined using *in vitro* CPE assays against PIV3, Measles, Influenza H1N1, Rhinovirus 16, HIV-1 and Herpes simplex virus 1, and inhibition of Hepatitis C replication was determined using a replicon assay (Southern Research Institute). *In vivo* efficacy of PC786 was also evaluated in mice (Pneumolabs) and cotton rats (Sigmovir biosystems Inc.) infected with RSV A. *In vivo*, BALB/c mice were infected intranasally with RSV A2 (2.8 x 10^5 PFU/mouse) and 4 days later the virus titre in lung homogenates was evaluated. PC786 suspension in 10% DMSO/90% isotonic saline was given intranasally or intratracheally on day -1, day 0 (1 hour before infection) and on days 1, 2, 3 after infection once daily. In cotton rats infected intranasally with RSV A Long (1.0 x 10^5 PFU/rat), PC786 was treated on day 0 (4 hours before infection) and once daily on on days 1, 2, 3 post-infection.

Results

Anti-viral profiles of PC786 and reference compounds

PC786 was found to be a potent inhibitor of CPE induced by RSV A2 and B WST strains in HEp-2 cells with IC_{50} values of 0.40nM and 1.3nM respectively (Table 1). The average IC_{50} value of PC786 on CPE induced by 3 clinical isolates (1997/12-35, 2001/2-20 & 2000/3-4) was 0.34nM, which is approximately x1940 fold more potent than the known anti-RSV nucleotide inhibitor ALS-8112 (659nM) (Table 1). PC786 did not inhibit CPE induced by other respiratory viruses such as PIV3, influenza A and rhinovirus.

Table 1: IC_{50} values of PC786 and known antiviral compounds against respiratory virus assays

Compound		PC786	AZ comp. 11 J*	GS-5806	AL-8112	Ribavirin
Virus strain	Cell line	IC_{50} (nM)	IC_{50} (nM)	IC_{50} (nM)	IC_{50} (nM)	IC_{50} (nM)
RSV A2*1	HEp-2	0.40	1.2	0.12	160	19500
RSV A long*1	HEp-2	1.5	4.6	0.33	11700	ND
RSV B WST*1	HEp-2	1.3	266	0.0021	86	542
RSVA 1997/12-35*1	HEp-2	0.35	ND	0.056	693	ND
RSVA 2001/2-20*1	HEp-2	0.53	ND	0.10	768	ND
RSVA 2000/3-4*1	HEp-2	0.14	ND	0.072	515	ND
PIV3*2	LLC-MK2 7.1	>14000	ND	ND	650	ND
Measles virus*2	MRC5	>14000	ND	ND	>340000	ND
Influenza H1N1*2	A549	>14000	ND	ND	>340000	ND
Hepatitis C virus*3	Huh-7, GT1b replicon	320	ND	ND	>340000	ND
Rhinovirus 16*2	HeLa	>14000	ND	ND	224000	ND
HIV-1*2	CEM-SS	>14000	ND	ND	170	ND
Herpes simplex virus 1*2	Vero	>14000	ND	ND	44000	ND

CPE resazurin detection in 384well format, PC786 and GS-5806 were treated simultaneously with virus infection, and ALS-8112 was treated 24hrs before infection (*1)
CPE detection by MTS (CellTitre 96®, Promega) assay in 96 well format. All compounds treated 2 hours prior to virus infection (*2)

Viral replication detected by replicon assay in 96 well format. All compounds treated 2 hours prior to virus infection (*3)

IC_{50} IC_{90} = concentration required for 50% or 90% inhibition; ND: not done

Minigenome analysis

PC786 also produced potent and concentration-dependent inhibition of the expression of luciferase driven by a minigenome construct comprising of the L, M2-1, N and P proteins of RSV A2 (IC_{50} value: 0.48 nM), and was 20- and 115-fold more potent than the known RSV L-protein inhibitors AZ27 (Astra Zeneca) and Compound D (Boeringer Ingelheim), respectively, and over 430-fold more potent than ALS-8112. This is direct evidence that PC786 inhibits virus gene transcription/replication by the RSV-A2 derived ribonucleoprotein (RNP) complex (Fig. 1 and Table 2).

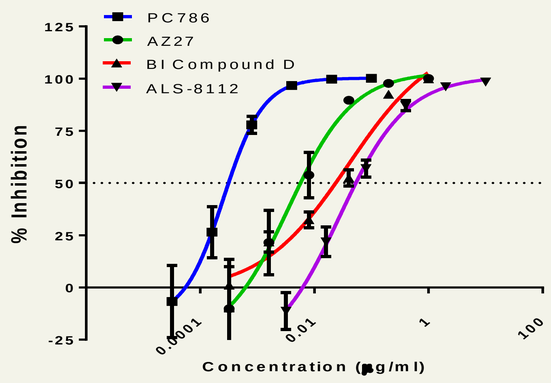

Figure 1. Inhibition of luciferase produced by RSV minigenome.

Table 2: IC_{50} values

Compound	IC_{50} (nM)
PC786	0.48
ALS-8112	207
Compound D	55
AZ27	10

Forced mutation

RSV A2 virus was repeatedly passaged in HEp-2 cells with increasing concentrations of PC786. After 6 passages (at 0.001-3.125µg/ml) we obtained an escape mutant exhibiting a >650 fold IC_{50} shift in the RSV A2 (MOI of 1, 5 days) induced CPE assay when compared with the vehicle virus (Fig.2). When the complete RSV genome was sequenced the PC786 escape mutant showed two amino acid substitutions with high frequency; Tyrosine 1461 to Histidine (Y1631H) on the RSV L-gene, and also Valine 153 to Alanine (V153A) on the RSV M-gene.

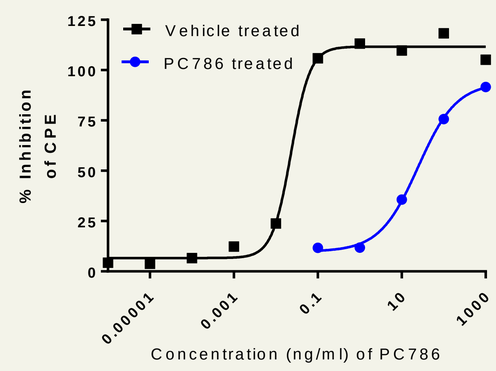

Figure 2. Inhibition effects of PC786 on CPE induced by RSV A2 obtained after repeated vehicle treatment and escalating concentrations of PC786 (6 passage) in HEp-2 cells.

In vivo study

In vivo, PC786 (2mg/ml) completely inhibited viral load in the lungs of BALB/c mice dosed by either intratracheal (20µL) or intranasal (40µL) routes (Table 3). In cotton rats, PC786 dose-dependently inhibited RSV titre in lung homogenates and significantly inhibited RSV virus titre at 3.3 and 10 mg/ml (50µL intranasally) (Table 4). PC786 also showed a dose-dependent inhibition of RSV NS-1 gene transcripts and of RANTES transcripts in lung (Fig.3).

Table 3: Effects of intratracheally or intranasally administered PC786 on RSV titre (PFU) in the lungs of RSV-infected mice.

Treatment	Dose (mg/mL)	N	Virus titre (log, PFU/lung) Geometric mean ± SD	Median	Interquartile range
Intratracheally					
Vehicle		10	3.1 ± 0.36	3.1	2.7 – 3.4
PC786	2	9	<0.33 (LOQ)	<0.33(LOQ)	N/A
Intranasally					
Vehicle		8	3.0 ± 0.54	3.2	2.8 – 3.4
PC786	0.2	8	2.4 ± 0.59	2.5	1.9 – 3.0
	2	8	<0.33 (LOQ)	<0.33 (LOQ)	N/A

Table 4: Effects of intranasally administered PC786 on RSV titre (PFU) in the lungs of RSV-infected cotton rats.

Treatment	Dose (mg/mL)	N	Virus titre (log, PFU/lung) Geometric mean ± SD	Median	Interquartile range
Intranasally					
Vehicle		6	5.0 ± 0.19	5.1	4.8 – 5.2
PC786	0.33	6	4.7 ± 0.15	4.7	4.5 – 4.8
	1.0	6	4.5 ± 0.20	4.5	4.4 – 4.8
	3.3	6	<2.3 (LOQ)	<2.3 (LOQ)	-
	10	6	<2.3 (LOQ)	<2.3 (LOQ)	-

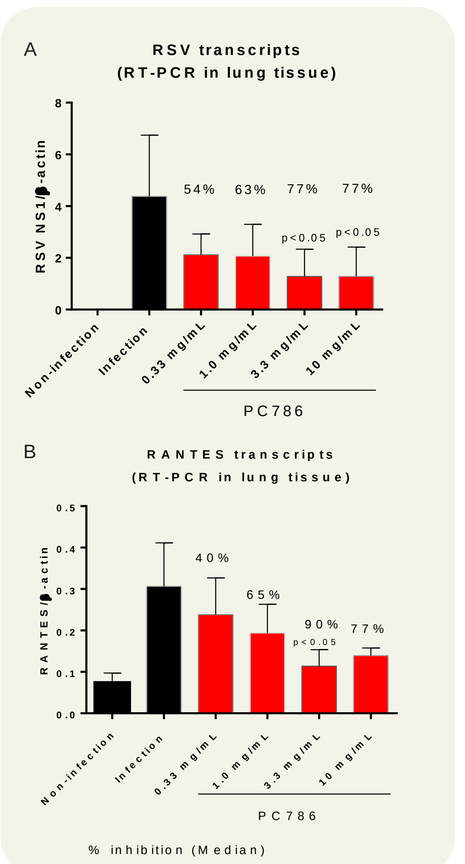

Figure 3. Inhibition of RSV NS-1 (A) and RANTES (B) gene induction measured by RT-PCR in cotton rat lung.

Conclusion

In this study, PC786 was shown to be a potent *RSV A* and *B* inhibitor via inhibition of RNP activity *in vitro*. We also found beneficial effects of PC786 in RSV infected mice and cotton rats by intranasal treatment. PC786 therefore has the potential to be a novel therapy for the treatment of *RSV* infections in humans.