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S-033447/S-033188, a Novel Small Molecule Inhibitor of Cap-dependent Endonuclease of Influenza A and B Virus: *In Vitro* Antiviral Activity against Clinical Strains

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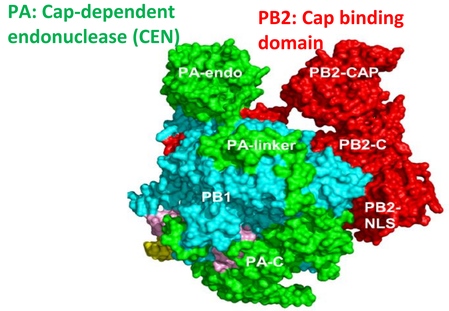
no conflicts of interest to declare

Introduction

S-033447, the active form of orally available prodrug S-033188, is a novel small molecule inhibitor of cap-dependent endonuclease (CEN) [Reference 1]. CEN is an enzyme that is specific to influenza virus and essential for viral transcription and replication (figure 1). Therefore, S-033447/S-033188 represents a novel drug against a promising anti-influenza target. A randomized, double-blind, placebo-controlled, phase 2 study of S-033188 in otherwise healthy adult patients with influenza was completed in 2016 [Reference 2]. To predict clinical efficacy, *in vitro* antiviral activity of S-033447 against clinically isolated influenza A and B virus in Japan from 2006 to 2014, including the neuraminidase (NA) inhibitor-resistant virus with H274Y substitution in NA (NA/H274Y), was evaluated by standard plaque reduction assay and yield reduction assay in Madin-Darby canine kidney (MDCK) cells.

Figure 1:Structure of RNA polymerase (PB1, PB2 and PA) complex [Reference 3]



Methods

In plaque reduction assay using 21 strains, MDCK cells seeded in 12-well plates were infected with virus at approximately 50 plaque forming unit (PFU)/well. After 1 hour incubation, the cells were washed and overlaid with agar medium containing S-033447 or favipiravir. After 3 days incubation at 33°C in a CO_2 incubator, the cells were fixed and plaque number was counted under a microscopy. The concentration achieving 50% inhibition of plaque formation (EC_{50}) was calculated.

In virus yield reduction assay using 6 strains, MDCK cells seeded in 96-well plate were infected with virus at 100 tissue culture infectious dose 50 ($TCID_{50}$)/well. After 1 hour incubation, the cells were washed and incubated with S-033447 or favipiravir at 37°C in a CO_2 incubator for 24 to 30 hours. Virus titer ($TCID_{50}$/mL) in the culture fluid of each well was determined in MDCK cells and the concentration achieving 90% reduction of virus titer (EC_{90}) was calculated. The mean and standard deviation (SD) values were calculated with three independent experiments.

Figure 2:Clinically isolated influenza A and B virus in Japan (geographical distribution)

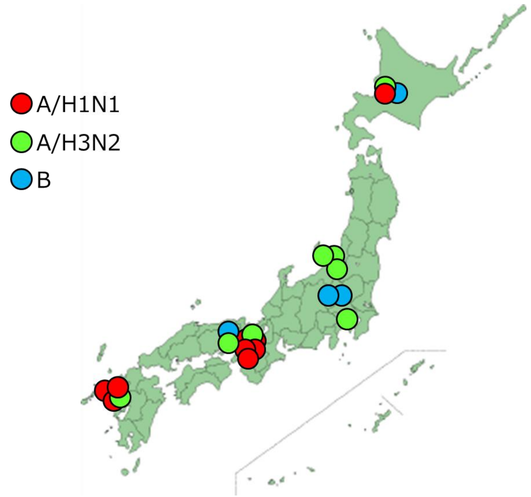
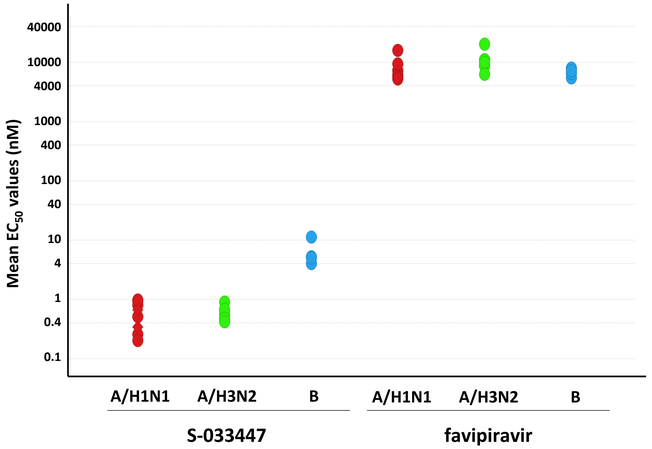


Figure 3: EC_{50} (nM) values of S-033447 and favipiravir against clinically isolated influenza A and B viruses in plaque reduction assay



The mean EC_{50} values were calculated from 3 independent experiments.
●Wild type, ◆H274Y substitution in the neuraminidase

Table 1:Clinically isolated influenza A and B virus from 2006 to 2014 (temporal distribution)

Year	A/H1N1	A/H3N2	B
2006	A/Kadoma/3/2006		
2009	A/Osaka/129/2009 A/Osaka/180/2009[a]		
2010	A/Nagasaki/10N073/2011 A/Kyoto/10K124/2011[a] A/Kyoto/10K118/2011		
2011		A/Hyogo/10K051/2011 A/Niigata/10F017/2011	B/Kyoto/10K131/2011 (Victoria lineage)
2012		A/Niigata/11F027/2012 A/Tokyo/11IM003/2012	B/Hokkaido/11H011/2012 (Victoria lineage)
2013		A/Hokkaido/12H048/2013 A/Niigata/12F392/2013	B/Gunma/12G045/2013 (Yamagata lineage)
2014	A/Hokkaido/13H020/2014 A/Nagasaki/13N019/2014 A/Nagasaki/13N059/2014[a]	A/Kyoto/13SK042/2014 A/Nagasaki/13N033/2014	B/Gunma/13G004/2014 (Yamagata lineage)

[a] H274Y substitution in the neuraminidase

Table 2: EC_{90} (nM) values of S-033447 and favipiravir against clinically isolated influenza A and B viruses in yield reduction assay

	S-033447			Favipiravir		
Strain	Mean	±	SD	Mean	±	SD
A/Kadoma/3/2006 (H1N1)	0.88	±	0.49	3417.76	±	1810.86
A/Osaka/129/2009 (H1N1)	0.86	±	0.08	4183.69	±	6.29
A/Osaka/180/2009[a] (H1N1)	0.95	±	0.41	3945.39	±	1216.98
A/Hokkaido/12H048/2013 (H3N2)	0.63	±	0.25	3335.60	±	1102.14
A/Niigata/12F392/2013 (H3N2)	0.87	±	0.52	1898.14	±	806.10
B/Hokkaido/11H011/2012	6.48	±	0.97	1735.19	±	252.28
B/Gunma/12G045/2013	6.10	±	0.33	2585.87	±	1825.91

The mean and SD were calculated from 3 independent experiments.
[a] H274Y substitution in the neuraminidase

Results

In this study, we tested clinically isolated influenza virus A and B strains that were collected from hospitals in Japan between 2006 and 2014. The geographical and temporal distribution of the viral strains are shown in Figure 2 and Table 1. EC_{50} values of S-033447 in plaque reduction assay ranged from 0.20 to 0.99 nM for type A and from 4.01 to 11.26 nM for type B, which were typically at least three orders of magnitude lower than those of favipiravir (Figure 3). EC_{90} values of S-033447 in yield reduction assay ranged from 0.63 to 0.95 nM for type A and from 6.10 to 6.48 nM for type B, which were typically at least three orders of magnitude lower than that of favipiravir (Table 2). No potency shift was observed for S-033447 against virus with NA/H274Y substitution in both assays. These results indicated that S-033447 achieved potent inhibition of influenza viral replication at low nanomolar concentrations.

Conclusion

- S-033447 exhibited broad and potent antiviral activity against clinically isolated influenza A and B viruses isolated in Japan from 2006 to 2014 in both plaque reduction assay and virus yield reduction assay compared with favipiravir.
- S-033447 exhibited no potency shift against oseltamivir-resistant virus (NA/H274Y).

Reference

[1] OPTIONS IX, Poster No. P-185, 408, 418, 610

[2] OPTIONS IX, Oral presentation No.LBO-1

[3] Reich S, Guilligay D, Pflug A, et al. Structural insight into cap-snatching and RNA synthesis by influenza polymerase. Nature. 2014 Dec 18;516(7531):361-6.