Inhibitory Effect of S-033188, a novel inhibitor of influenza virus cap-dependent endonuclease, against avian influenza A/H7N9 virus *in vitro* and *in vivo*.

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Introduction

Human infection with avian influenza A (H7N9) virus first emerged in China in 2013 and 2016/2017 season saw the largest epidemic to date [1]. Although neuraminidase inhibitors (NAI) are used to treat influenza A (H7N9) infection, NAI resistant viruses have been reported in some patients [2]. Therefore, novel anti-influenza drugs that improve over current therapy are urgently needed. S-033447, an active form of orally available prodrug S-033188, is a novel small molecule inhibitor of cap-dependent endonuclease that is essential for influenza virus gene transcription and replication. A Phase 3, Multicenter, Randomized, Double-blind Study of a Single Dose of S-033188 Compared with Placebo or Oseltamivir 75 mg Twice Daily for 5 Days in Otherwise Healthy Patients with Influenza was completed in 2017 [3]. Here, *in vitro* and *in vivo* efficacy against avian influenza virus A/Anhui/1/2013 (H7N9) strain was evaluated.

Study Objective

- To investigate the cell culture antiviral activity of S-033447 against influenza A (H7N9) and its NAI-resistant mutant virus.
- To investigate the efficacy of S-033188 in mice infected with influenza A (H7N9).

Material and Method

***In vitro* study:** Madin-Darby canine kidney (MDCK) cells seeded on 96-well plates were inoculated with A/Anhui/1/2013 (H7N9) or its NA/R292K mutant strain at 100 tissue culture infectious dose 50 ($TCID_{50}$) /well. After incubation in 5% CO_2 at 35°C for 1 hour, the cells were washed and incubated in 5% CO_2 at 35°C for 24 hours with S-033447 or oseltamivir acid. Virus titer in the culture supernatants was determined in MDCK cells and EC_{90} was calculated.

***In vivo* study:** Female BALB/c mice were intranasally inoculated with A/Anhui/1/2013 strain at 4.0 x 10^5 $TCID_{50}$/mouse. Immediately after the infection, mice were orally treated with S-033188 (0.5, 5, or 50 mg/kg/shot) twice a day (12 hours interval between each dosing) for 1 or 5 day(s), vehicle (0.5 w/v% methylcellulose) or oseltamivir phosphate (OTV, 5 (clinically-equivalent dose [4]) or 50 mg/kg/shot) twice a day for 5 days. Viral titer in the lung 1, 3, or 5 day(s) after the infection was determined in MDCK cells. Survival time and body weight change were monitored through a 28-day period after the infection. Mice were euthanized and regarded as dead if their body weights were lower than 70% of the initial body weights according to humane endpoints.

Results

***In vitro* study:** The mean EC_{90} values of S-033447 against A/Anhui/1/2013 and its NA/R292K mutant strain was 0.80 and 1.12 nM, respectively. By contrast, the mean EC_{90} values of oseltamivir acid against A/Anhui/1/2013 and its NA/R292K mutant strain was 15.41 and 142389.79 nM, respectively. The fold change values of S-033447 and oseltamivir acid for this mutant virus were 1.39 and 9239.94, respectively.

***In vivo* study:** All mice survived by 5-day dosing of S-033188 while vehicle-treated mice died within 7 days after infection. One-day dosing of S-033188 was also strongly effective: 90% (0.5 mg/kg) or 100% (5 or 50 mg/kg) of mice survived, respectively (data not shown). The survival time of S-033188-treated groups was compared with that of OTV-treated groups. All groups treated with S-033188 significantly prolonged the survival time as compared with the group treated with OTV 5 mg/kg (survival rate: 30%). Five-day dosing of S-033188 suppressed body weight loss due to virus infection. In contrast, OTV had little or weak effect on body weight loss compared to S-033188. Virus titers for the overall time in all groups treated with S-033188 were significantly lower than those in vehicle-treated group. Notably, virus titers of almost all of mice, 5-day treatment with S-033188 at dose of 50 mg/kg, showed LLOQ. The virus titers in S-033188-treated groups were compared with those in OTV-treated groups. There was significantly less virus titers for the overall time in S-033188-treated groups than those in OTV 5 or 50 mg/kg-treated group.

Conclusion

- S-033447 was a potent inhibitor against replication of A/Anhui/1/2013 (H7N9) and its NA/R292K mutant strain compared with oseltamivir acid.
- S-033447 exhibited no potency shift against NAI-resistant strains (NA/R292K).
- S-033188 (5 or 50 mg/kg BID) completely improved mortality accompanied by suppression of body weight loss and virus titers in the lung compared to those of oseltamivir phosphate.

Reference

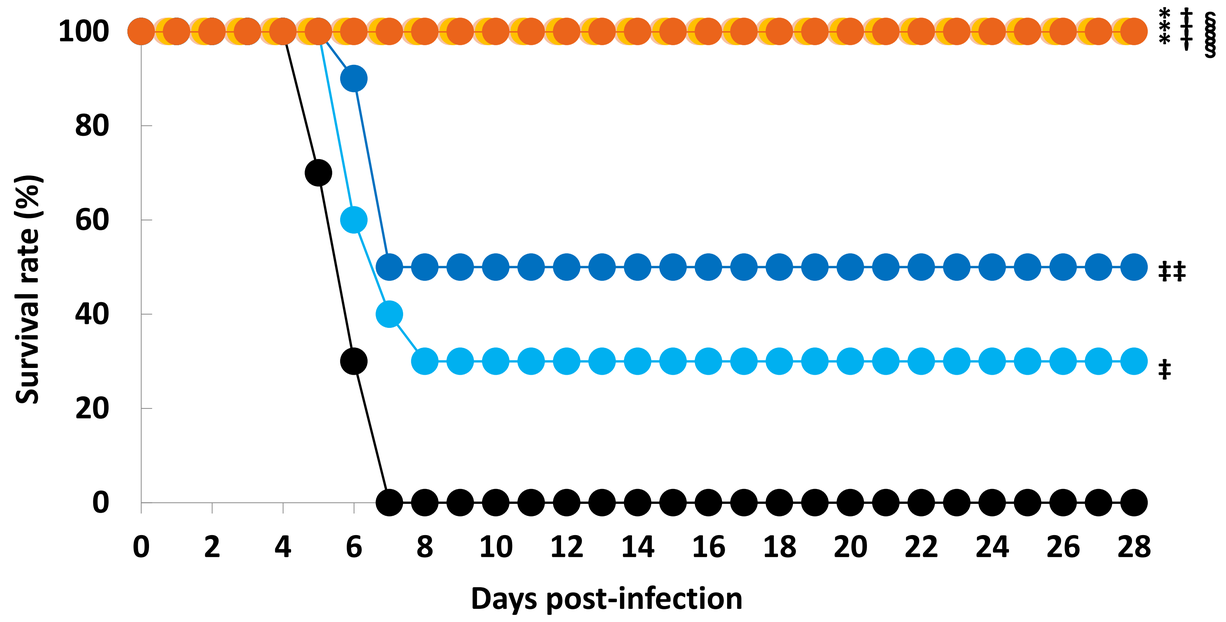
1. Zhang F et al., J Infect, 2017
2. Hai R et al., Nat Commun, 2013
3. Study Protocol Number: 1601T0831
4. Ward P et al., J Antimicrob Chemother, 2005

Table 1. EC_{90} (nM) values of S-033447 and Oseltamivir acid against A/Anhui/1/2013 and its NA/R292K mutant strain in virus yield reduction assay

	S-033447			Oseltamivir acid		
Strain	Mean	±	SD	Mean	±	SD
A/Anhui/1/2013 (H7N9)	0.80	±	0.36	15.41	±	11.56
A/Anhui/1/2013-NA/R292K[a] (H7N9)	1.12	±	0.53	142389.79	±	6601.02

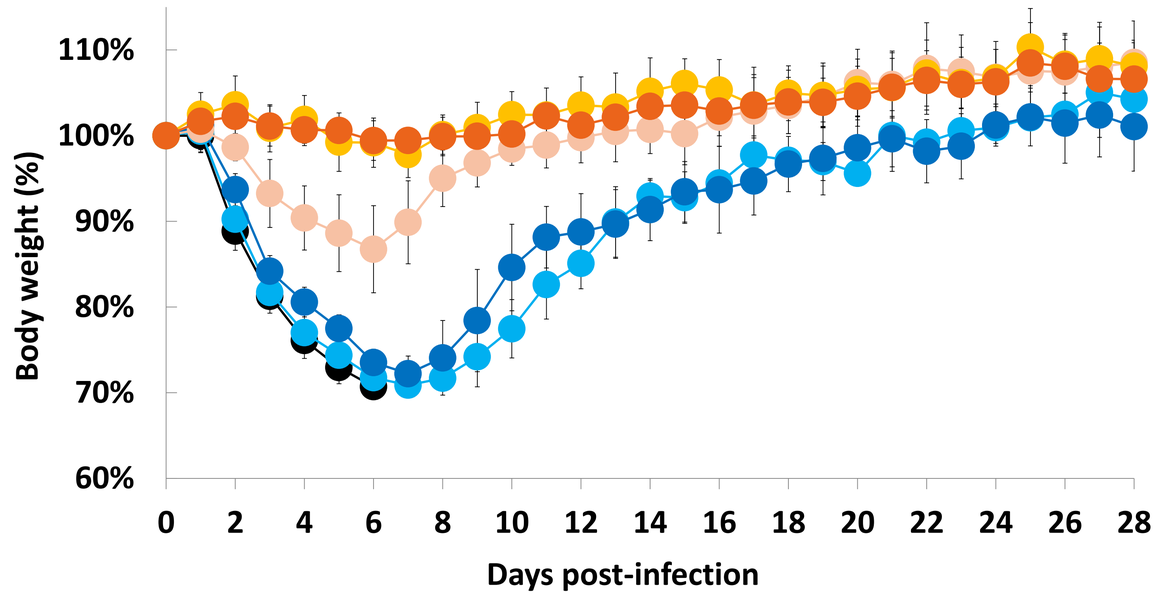
The mean and SD were calculated from 3 independent experiments.
[a] R292K substitution in the neuraminidase

Figure 1. Effect of S-033188 on mortality due to infection with A/Anhui/1/2013 strain in mice



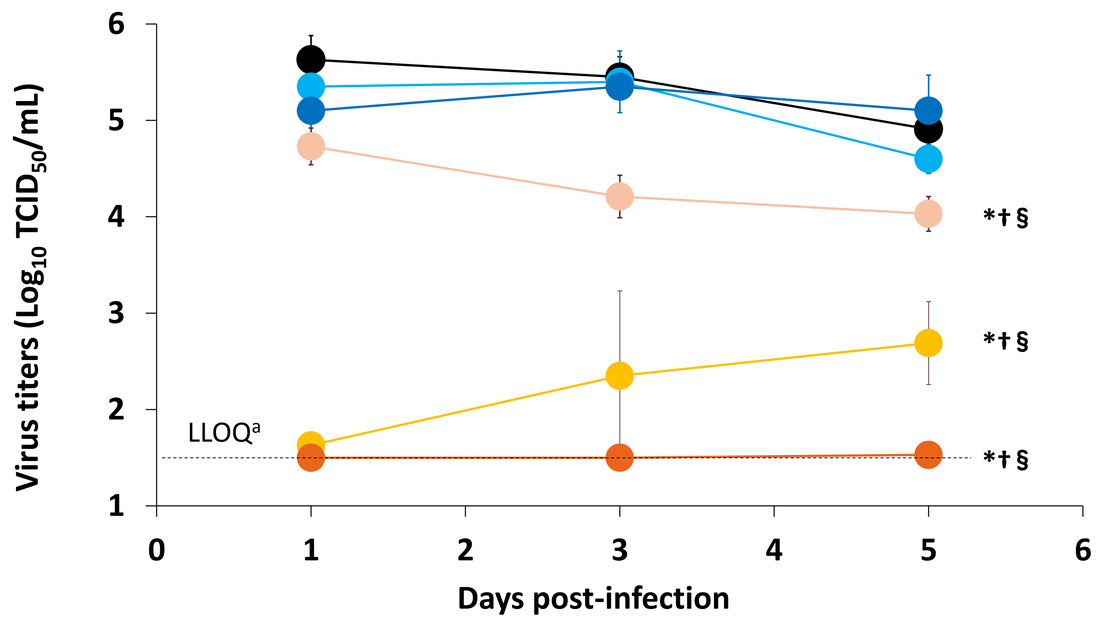
* P<0.0001 vs vehicle, † P<0.005 vs oseltamivir 5 mg/kg, ‡ P<0.05 vs vehicle, ‡‡ P<0.001 vs vehicle, § P<0.05 vs oseltamivir 50 mg/kg (The log-rank test and the fixed-sequence procedure)

Figure 2. Effect of S-033188 on body weight loss due to infection with A/Anhui/1/2013 strain in mice



Values are mean body weight change ± SD of the living mice at each day.

Figure 3: Effect of S-033188 on virus titers in lungs at 1, 3, and 5 days post-infection in A/Anhui/1/2013 strain-infected mice



[a] LLOQ: Lower limit of quantification (1.5 Log_{10} $TCID_{50}$/mL)
* P<0.0001 vs vehicle, † P<0.0001 vs oseltamivir 5 mg/kg, § P<0.0001 vs oseltamivir 50 mg/kg (The two-way analysis of variance followed by pairwise comparison and the fixed-sequence procedure)