S-033188, a novel inhibitor of influenza virus cap-dependent endonuclease, leads to rapid and profound viral load reduction in immunocompromised mice model

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Introduction

Novel anti-influenza drugs that offer significant improvement over current therapy are urgently needed, because epidemic and pandemic influenza remain major public health concerns. S-033447, an active form of orally available prodrug S-033188, is a novel small molecule inhibitor of cap-dependent endonuclease (CEN) [1] of influenza A and B virus. CEN is an enzyme, located in the N-terminal domain of PA subunit of the influenza viral RNA polymerase complex, that is specific to influenza virus and essential for viral transcription and replication. Therefore, S-033188 represents a novel drug against a promising anti-influenza target. 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 [2].

Immunocompromised state seen in patient with a chronic disease leads to high risk for severe influenza [3]. Here, the effect of S-033188 on immunocompromised mice infected with influenza A (H1N1) virus and PA amino acid substitution in viruses derived from their lungs were examined.

Study Objective

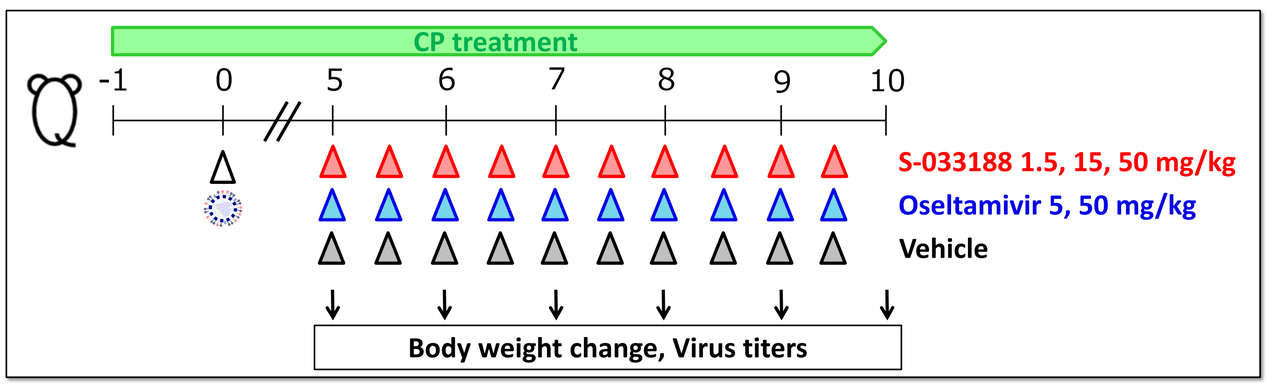
(1) To examine the efficacy of S-033188 in immunocompromised mice infected with influenza A virus (H1N1) and compare its inhibitory effect with oseltamivir phosphate

(2) To examine whether viruses derived from lung homogenates of S-033188-treated immunocompromised mice exhibit PA amino acid substitution

Material and Method

Immunocompromised mice model: Female BALB/c mice were subcutaneously treated with 0.2 mg/mouse of cyclophosphamide (CP) from 1 day before to 9 days after the inoculation of A/Puerto Rico/8/34 (H1N1) virus for the induction of immunocompromised state and prolongation of the period of virus infection. CP-treated mice were intranasally inoculated with 100 50% tissue culture infectious dose ($TCID_{50}$)/mouse of virus suspension and then orally given, from 5 days post-infection, 1.5, 15 or 50 mg/kg of S-033188 (BID, for 5 days) or 5 mg/kg, clinically equivalent dose, or 50 mg/kg of oseltamivir phosphate (BID, for 5 days). For 5 days after the first administration, body weight change of mice was assessed and lung homogenates were prepared. Viral titer in the lung homogenates were quantified by standard $TCID_{50}$ method.

Sequence analysis of PA region: Sequence analysis of the PA gene of A/Puerto Rico/8/34 virus was performed by Sanger sequencing method. Sample RNA derived from the vehicle-treated group (sampling on 5 days post-infection [dpi]), the S-033188-treated groups (sampling on 6, 8, and 10 dpi), and the parent virus (A/Puerto Rico/8/34 virus) were analyzed.



Results

- The virus titer in the lung was maintained over 4 $log_{10}TCID_{50}$/mL from 5 to 10 days post-infection in CP-treated mice, whereas it decreased to 2 $log_{10}TCID_{50}$/mL by 9 days post-infection in CP-untreated mice, indicating a prolonged duration of virus infection by CP treatment (Figure 2).
- Body weight loss in oseltamivir phosphate-treated groups was slightly but not significantly suppressed, whereas that in S-033188-treated groups was significantly suppressed (Figure 1).
- In the S-033188 1.5, 15, and 50 mg/kg-treated groups, the virus titer in the lung decreased in a dose-dependent manner at 24 hours after the first dose, and then reached the lower limit of quantification (1.5 $log_{10}TCID_{50}$/mL) rapidly in a dose-dependent manner. The S-033188 1.5, 15, and 50 mg/kg-treated groups maintained a significantly lower virus titer in the lung than the vehicle-treated group and the oseltamivir phosphate 5 and 50 mg/kg-treated groups (Figure 2).
- No amino acid substitution in PA region was observed in all sequenced samples (vehicle-treated group [sampling on 5 dpi] and S-033188-treated groups [sampling on 6, 8, and 10 dpi] [5 mice / group]) compared to the parent virus (Table 1).

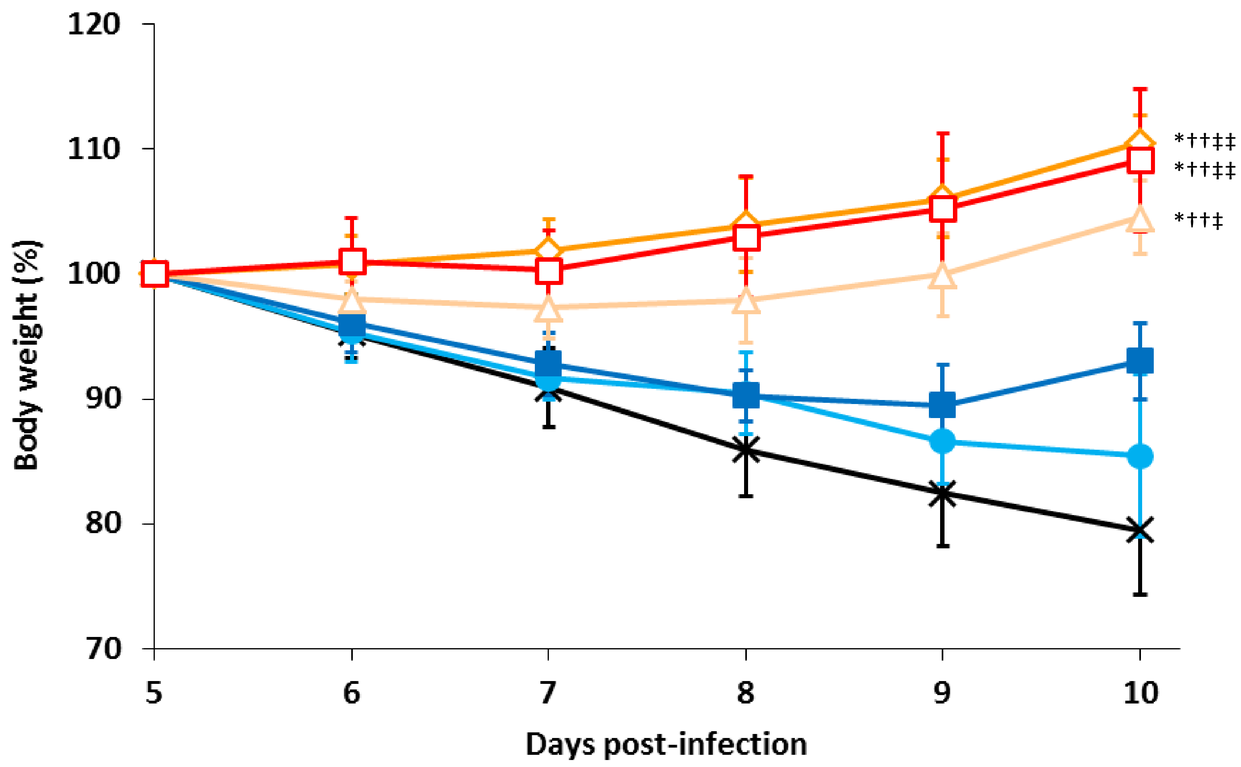
Conclusion

- Five days dosing of S-033188 significantly suppressed body weight loss compared to that of oseltamivir phosphate in immunocompromised mice model.
- Five days dosing of S-033188 suppressed virus replication more strongly and persistently than that of oseltamivir phosphate in immunocompromised mice model without emergence of PA amino acid substitution.
- S-033188 can be expected to inhibit virus replication more strongly and clear the virus from the body more rapidly than oseltamivir phosphate in an immunocompromised high-risk group.

Reference

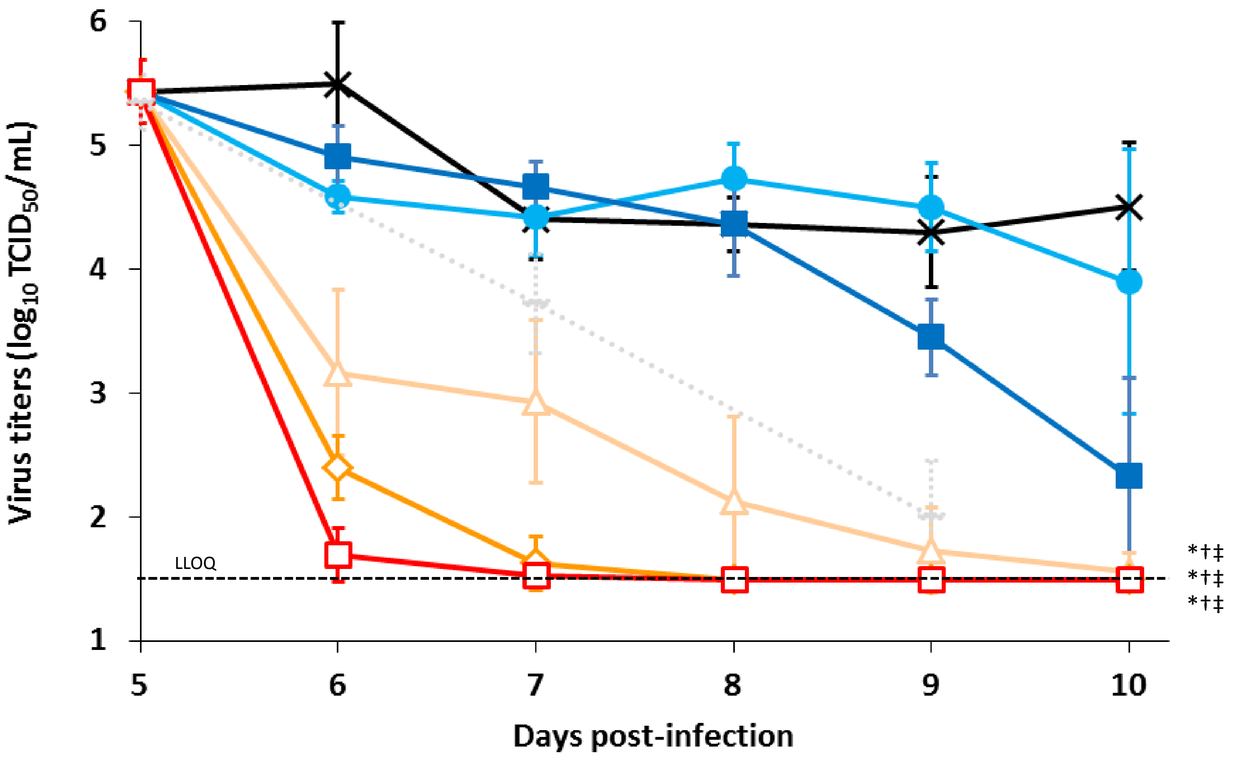
[1] Plotch SJ, Bouloy M, Ulmanen I, et al. Cell 1981; 23: 847-58.
[2] Study Protocol Number: 1601T0831.
[3] Centers for Disease Control and Prevention. MMWR Recomm Rep 2011; 60:1–24.

Figure 1. Effect of S-033188 on body weight loss of A/Puerto Rico/8/34 virus-infected immunocompromised mice



Data represent as relative value, with the body weight of each mouse on 5 dpi being 100%. * P<0.0001 vs vehicle, † P<0.0005 vs oseltamivir 5 mg/kg, †† P<0.0001 vs oseltamivir 5 mg/kg (the mixed model repeated measures and the fixed sequence procedure), ‡ P<0.005 vs oseltamivir 50 mg/kg, ‡‡ P<0.0001 vs oseltamivir 50 mg/kg (the mixed model repeated measures).

Figure 2. Reduction of viral titer by treatment with S-033188 or oseltamivir phosphate in A/Puerto Rico/8/34 virus-infected immunocompromised mice



* P<0.0001 vs vehicle, † P<0.0001 vs oseltamivir 5 mg/kg (the linear model with heteroscedastic errors and the fixed sequence procedure), ‡ P<0.0001 vs oseltamivir 50 mg/kg (the linear model with heteroscedastic errors).

Table 1. Sequence analysis of the PA gene of A/Puerto Rico/8/34 virus derived from lung homogenates of S-033188-treated immunocompromised mice

Dpi	Vehicle	S-033188		
		1.5 mg/kg	15 mg/kg	50 mg/kg
5	-	ND	ND	ND
6	ND	-	-	-
8	ND	-	-	-
10	ND	-	-	-

-, no amino acid substitution was observed in 5 mice, compared to the parent virus (A/Puerto Rico/8/34 virus); ND, not done

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