Inhibitory effect of S-033188/S-033447, a novel inhibitor of influenza virus Cap-dependent endonuclease, against highly pathogenic avian influenza virus A/H5N1

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Background: 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 transcription and replication. Here, in vitro and in vivo efficacy against highly pathogenic avian influenza virus A/Hong Kong/483/97 (H5N1) strain was evaluated.

Material/methods: For in vitro virus yield reduction assay, Madin-Darby canine kidney (MDCK) cells in 96-well plate were infected with A/Hong Kong/483/97 strain at 100 tissue culture infectious dose 50 (TCID50)/well and incubated at 35°C in a CO2 incubator for 24 hours. Virus titer in the culture fluid was determined in MDCK cells and EC50 was calculated. For in vivo study, female BALB/c mice were intranasally inoculated with A/Hong Kong/483/97 strain at 75 TCID50/mouse. Immediately after the infection, mice were orally treated with S-033188 (0.5, 5, or 50 mg/kg) twice a day for 1 day, vehicle (0.5 w/v% methylcellulose) or oseltamivir phosphate (5 or 50 mg/kg) 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 then monitored through a 14-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:** S-033447 (mean EC$_{90}$ values: 1.64 nM) demonstrated more potent antiviral activity against A/Hong Kong/483/97 strain than oseltamivir acid (mean EC$_{90}$ values: 11.16 nM) in virus yield reduction assay. S-033188 (5 or 50 mg/kg, BID for 1 day) completely eliminated mortality and exhibited significant survival time compared to vehicle or oseltamivir (5 mg/kg, BID for 5 days: clinically equivalent dose) in mice infected with A/Hong Kong/483/97 strain (Fig.1). Consistent with the survival data, S-033188 treatment also led to significant reduction of viral titer and prevention of body weight loss compared to vehicle or oseltamivir treatment.

**Conclusions:** S-033188/S-033447 exhibited potent *in vitro* and *in vivo* activity against A/Hong Kong/483/97 (H5N1) strain compared to oseltamivir.

**Fig.1 Protective effect of S-033188 on mortality in mice infected with A/Hong Kong/483/97 (H5N1) strain**

![Graph showing survival rates](image-url)