

# Efficacy of a single intravenous injection of peramivir (BCX-1812) compared to oral oseltamivir against seasonal influenza B virus infection in ferrets

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## Abstract

### Background

Influenza B virus causes indistinguishable clinical symptoms from those caused by influenza A virus. In addition, it has been reported that influenza B virus was isolated more frequently and for a longer period after antiviral therapy than was influenza A virus. Therefore, further evaluation of efficacy of antiviral drugs against influenza B virus is needed. In this study, we investigated the therapeutic efficacy of an intravenous neuraminidase inhibitor peramivir (Fig.1), compared to oral oseltamivir against seasonal influenza B virus infection in the ferret model.

### Materials and Methods

Ferrets were inoculated with 300 TCID<sub>50</sub> of B/Kadoma/1/2005 into nasal cavities. Treatment with antiviral compounds was started 24hrs after virus inoculation. Peramivir (30 and 60 mg/kg) was administered as a single intravenous infusion and oseltamivir phosphate (30 and 60 mg/kg) was administered orally twice a day for 3days. Nasal wash fluid was collected daily for 4 days after virus inoculation. Body temperature was recorded by data loggers implanted into their peritoneal cavities.

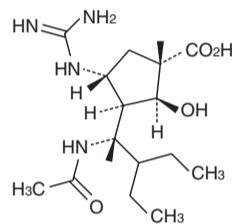
### Results

Therapeutic effect of peramivir (30 and 60 mg/kg) and oseltamivir phosphate (60 mg/kg) on virus titer in nasal wash fluids were statistically significant at 48 hrs after virus inoculation compared with the untreated control group ( $p < 0.001$ ,  $0.01$  and  $0.05$ , respectively). Change in body temperature of ferrets treated with peramivir (60 mg/kg) was significantly less than that of ferrets in the untreated control group ( $0.65$  °C vs  $1.65$  °C,  $p < 0.05$ ), whereas suppression of fever was not apparent in ferrets treated with oseltamivir phosphate (60 mg/kg) ( $1.35$  °C vs  $1.65$  °C,  $p = 0.81$ ).

### Conclusions

These results demonstrated that peramivir had beneficial effects on viral titers and symptoms of ferrets with influenza B virus infection. The effects observed with peramivir treatment seem to be superior to those observed with oseltamivir phosphate treatment. Therefore, peramivir could be an alternative to oseltamivir phosphate to treat patients with acute influenza B virus infection.

Fig.1 Chemical structure of peramivir



## Materials and Methods

### Animal

Nine- to eleven-month-old female ferrets (Japan SLC Inc., Shizuoka, Japan) were used. A data logger (DS1921H-F5, Maxim Integrated Products, Inc., Sunnyvale, CA, USA) was implanted in the peritoneal cavity of each ferret to monitor body temperature every 15 min.

### Viruses and cells

Clinical isolate of influenza virus were obtained from Osaka Prefectural Institute of Public Health (Osaka, Japan). Animals were inoculated with 300 TCID<sub>50</sub> of B/Kadoma/1/2005 into nasal cavities under anesthesia. Nasal wash fluid was collected daily and stored at  $-80$  °C until use. MDCK cells were obtained from the American Type Culture Collection and maintained in Eagle's minimum essential medium (MEM) containing 10 % fetal bovine serum.

### NA inhibition assay

Whole viruses inactivated by NP-40 were used as a source of NA activity. MUNANA was used as a substrate and the fluorometric intensity of 4-methylumbelliferon released from MUNANA was measured, determined % inhibition and calculated IC<sub>50</sub>.

### Pharmacokinetic analysis

Ferrets (three per group) were given peramivir (30 mg/kg) intravenously or oseltamivir phosphate (30 mg/kg) orally. Blood samples were collected at time intervals from 0.083 h to 24 h after dosing and centrifuged to obtain plasma. The concentration of peramivir and oseltamivir carboxylate in plasma was determined by liquid chromatography-tandem mass spectrometry (LC/MS/MS). PK parameters were calculated and modeling performed using WinNonlin software ver. 4.0 (Pharsight Corp., Mountain View, CA).

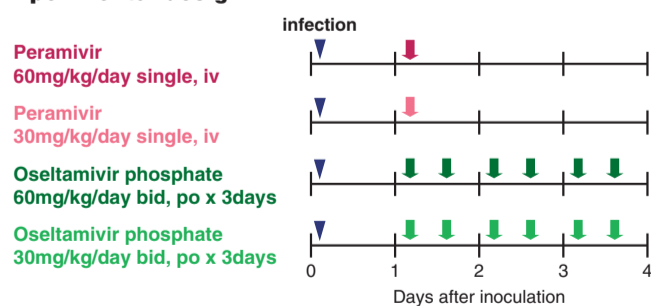
### Therapeutic effects in ferrets

Peramivir (30 or 60mg/kg/day) was administered as a single intravenously 24hrs after virus inoculation and oseltamivir phosphate (30 or 60 mg/kg/day) was administered orally once daily for 3 days after virus inoculation (n=4). Vehicle (0.5% methylcellulose)-treated ferret (n=6) were included in the same treatment schedule with repeated dosing of oseltamivir phosphate (Fig.2). Virus titers in nasal cavities from infected ferrets were measured using MDCK cell. Inflammatory cells in nasal washes were counted microscopically in a hemocytometer. The protein concentration in cell-free nasal washes was measured by using a protein reagent (Wako Pure Chemicals, Osaka, Japan). Body temperature was expressed by calculating the average temperatures during nighttime (10 pm – 8 am) for avoiding the effect of anesthesia and was compared with that before virus inoculation.

### Statistical analysis

Comparison of the virus titers, protein concentration in nasal wash fluids and body temperature were carried out by the Dunnett's multiple comparison method. The efficacy of peramivir was compared with that of oseltamivir by using Student's t-test. P values below 0.05 were considered as statistically significant (\*;  $p < 0.05$ , \*\*;  $p < 0.01$ ).

Fig.2 Experimental design



## Results

Table.1 Sensitivity of influenza B/Kadoma/1/2005 virus to NA inhibitors

Strain	Mean IC <sub>50</sub> and IC <sub>90</sub> ± SD (nM)	
	Peramivir	Oseltamivir carboxylate
B/Kadoma/1/2005	1.78 ± 0.08, 5.93 ± 0.51	16.1 ± 0.73, 5.34 ± 4.60

Fig.3 Pharmacokinetics of peramivir and oseltamivir carboxylate in ferrets

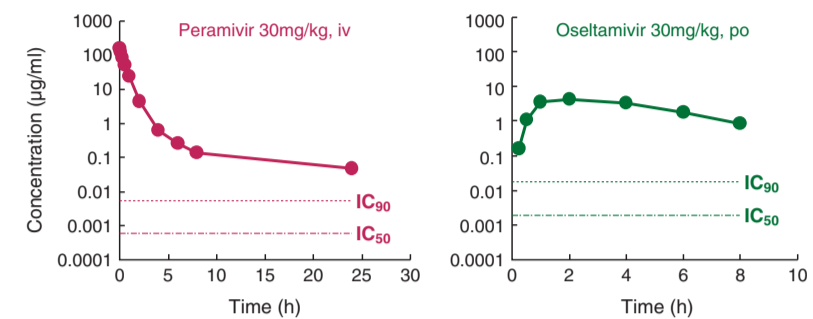


Table.2 Summary of pharmacokinetic parameters

Drug	Route	Dose (mg/kg)	Mean ± SD for indicated parameter <sup>a</sup>		
			C <sub>max</sub> (µg/mL) <sup>b</sup>	T <sub>max</sub> (hr)	AUC <sub>0-∞</sub> (µg · hr/mL) <sup>c</sup>
Peramivir	Intravenous	30	194 ± 56	NA <sup>d</sup>	89.1 ± 21.9
Oseltamivir carboxylate	Oral	30	4.38 ± 0.68	2.67 ± 1.15	23.7 ± 2.7

<sup>a</sup>Means and SD of data from three animals are shown <sup>b</sup>C<sub>max</sub> was calculated as C<sub>0</sub> for intravenous dose <sup>c</sup>AUC<sub>0-∞</sub>: area under the concentration-time curve from 0 h to infinity <sup>d</sup>NA, not applicable

Fig.4 Virus titers in the nasal wash fluids

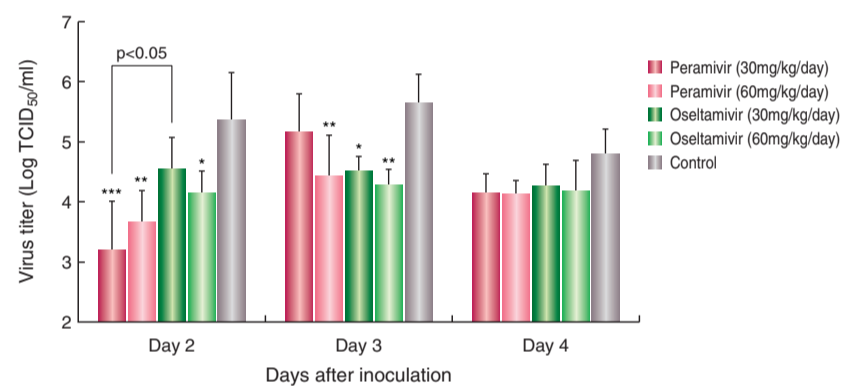


Fig.5 Body temperature changes in ferrets

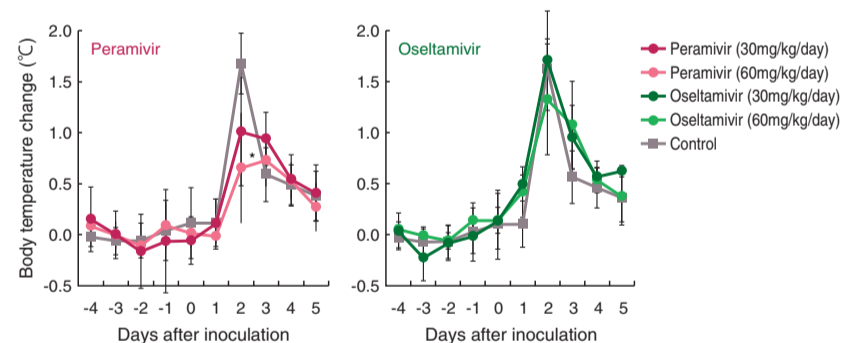
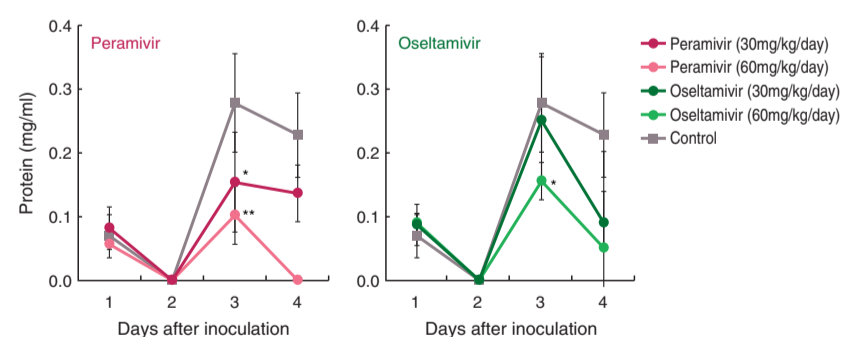


Fig.6 Protein concentration in the nasal wash fluids



## Conclusions

- This results suggested that viral propagation in an early phase after virus infection contributed to severity of symptoms including fever and that suppression of virus replication in the early phase during infection was crucial to ameliorate symptoms in the ferret model
- We demonstrated that peramivir injected once intravenously had beneficial effects on viral titers and symptoms of ferrets with influenza B virus infection. The effects observed with peramivir treatment seem to be superior to those observed with twice daily oseltamivir phosphate treatment in ferret. Therefore, single intravenous administration of peramivir could be an alternative to oseltamivir to treat patients with acute influenza B virus infection.