

# Evaluation of effects of peramivir against seasonal influenza B virus infection in cynomolgus macaques

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## Abstract

### Background:

Peramivir (Fig.1) is a novel potent inhibitor of influenza neuraminidase (NA). Therapeutic effect of peramivir and oseltamivir phosphate was investigated using virus-infected cynomolgus macaques model.

### Methods:

Inhibitory activity on NA activity was measured using MUNANA as a substrate. The therapeutic effects of peramivir were investigated using the virus-infected cynomolgus macaques model and compared with that of oseltamivir phosphate. Virus titers and levels of inflammatory cytokines/chemokines in nasal swab fluid were determined with MDCK cells and CBA Flex set (BD Biosciences), respectively. Body temperature was monitored by a telemetry probe implanted in the peritoneal cavities of each macaque.

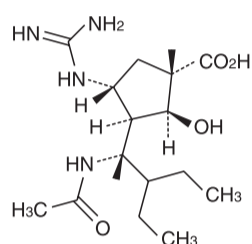
### Results:

Virus titers and levels of IL-6 production in nasal swab fluid of macaques treated with peramivir were significantly lower than those of the vehicle-treated group. On the other hand, there was no significant effect of oseltamivir on virus titers, whereas reduction of IL-6 production was observed compared with the vehicle-treated groups. Body temperature of macaques treated with peramivir was substantially lower than those of macaques in the vehicle-treated, whereas suppression of fever was not apparent in macaques treated with oseltamivir phosphate.

### Conclusions:

We established the cynomolgus macaque model of influenza B virus infection and demonstrated that peramivir had beneficial effects on viral titers and symptoms of cynomolgus macaques with influenza B virus infection, which is superior to oseltamivir phosphate.

Fig.1 Chemical structure of peramivir



## Introduction

- Peramivir is a novel, potent and selective inhibitor of influenza neuraminidase (NA) and showed strong inhibition to various influenza A and B viruses including recently isolated viruses.
- Regarding influenza B viruses, NA inhibition of peramivir was more potent than that of oseltamivir carboxylic acid and zanamivir (Yoshida et al ICAAC2009).
- Cynomolgus macaque, which are evolutionally related to humans, may be more predictive of the disease course of the influenza infection and immune system.
- Therefore, we established the cynomolgus macaque model of influenza B virus infection and investigated the therapeutic efficacy of peramivir against seasonal influenza B virus infection in cynomolgus macaques.

## Materials and Methods

### Animal

Three- to five-year-old female, influenza B virus seronegative, cynomolgus macaques were used with permission of the Shiga University of Medical Science Animal Experiment Committee and Biosafety Committee. A telemetry probe (TA10CTA-D70, Data Sciences International) was implanted in the peritoneal cavity of each macaque to monitor body temperature every 15 min.

### Viruses and cells

Clinical isolates of influenza virus were obtained from Sendai Medical Center (Sendai, Japan). Animals were inoculated with  $2 \times 10^5$  TCID<sub>50</sub> of B/SendaiH/1051/2007 into nasal cavities under anesthesia. Nasal swab 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>.

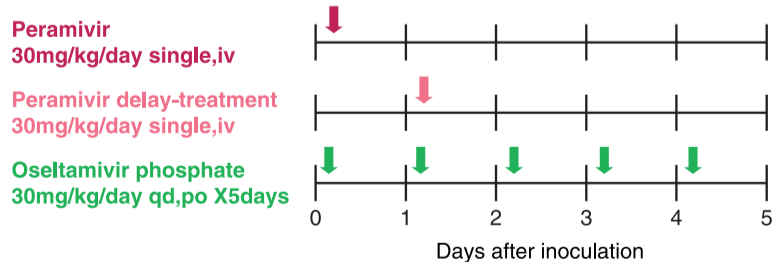
### Therapeutic effects in cynomolgus macaques

Peramivir (30mg/kg/day) was administered as a single intravenously immediately or 24hrs after virus inoculation and oseltamivir phosphate (30mg/kg/day) was administered orally once daily for 5 days after virus inoculation (n=3). Vehicle (0.5% methylcellulose)-treated macaques (n=3) were included in the same treatment schedule with repeated dosing of oseltamivir phosphate (Fig.2). Virus titers and levels of inflammatory cytokines/chemokines production in nasal swab from infected macaques were measured using MDCK cell and CBA Flex set, respectively. Results were calculated as the area under the curve (AUC) by trapezoidal rule. Difference of AUC between the vehicle and peramivir and between the vehicle and oseltamivir were analyzed by Dunnett's multiple comparison method. Body temperature were expressed by calculating the average of the highest and lowest temperatures during 1 day and the body temperature after the virus inoculation was compared with that before the virus inoculation.

### Hemagglutination inhibition (HI) assay

Serum were treated with receptor destroying enzyme, heat-inactivated at 56°C for 1h, mixed with 16 HA units of virus antigen at room temperature for 1h, and incubated with 0.5% chicken red blood cells.

Fig.2 Experimental design



## Results

Table.1 Inhibition of influenza B/SendaiH/1051/2007 neuraminidase with peramivir, oseltamivir carboxylic acid and zanamivir

Strain	IC50(nM)		
	Peramivir	Oseltamivir carboxylic acid	Zanamivir
B/SendaiH/1051/2007	4.26 ± 0.17	13.09 ± 0.50	10.93 ± 0.98

- Peramivir showed potent inhibitory activity against NA of B/SendaiH/1051/2007.
- Oseltamivir carboxylic acid and zanamivir showed lower inhibitory activity than peramivir.

Fig.3 Inhibitory effect on viral replication activity in cynomolgus macaques

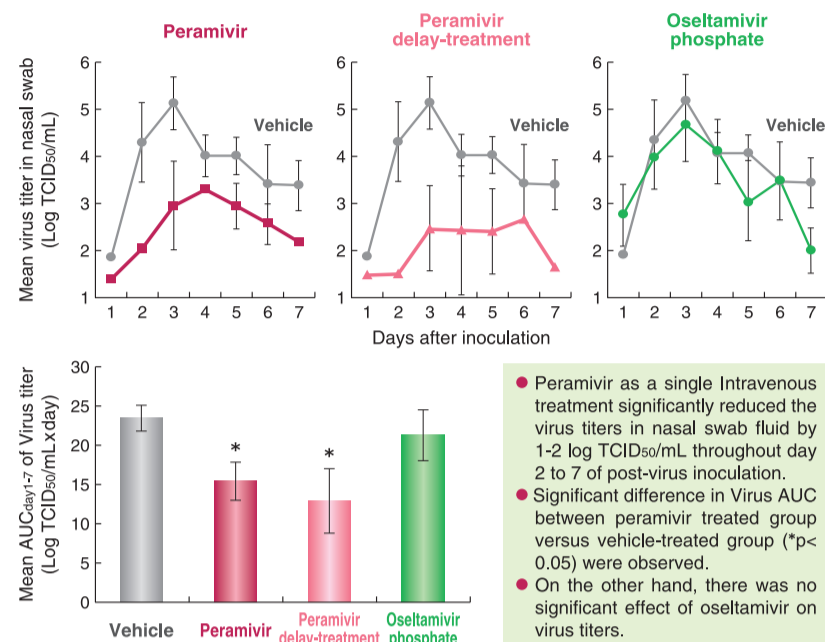
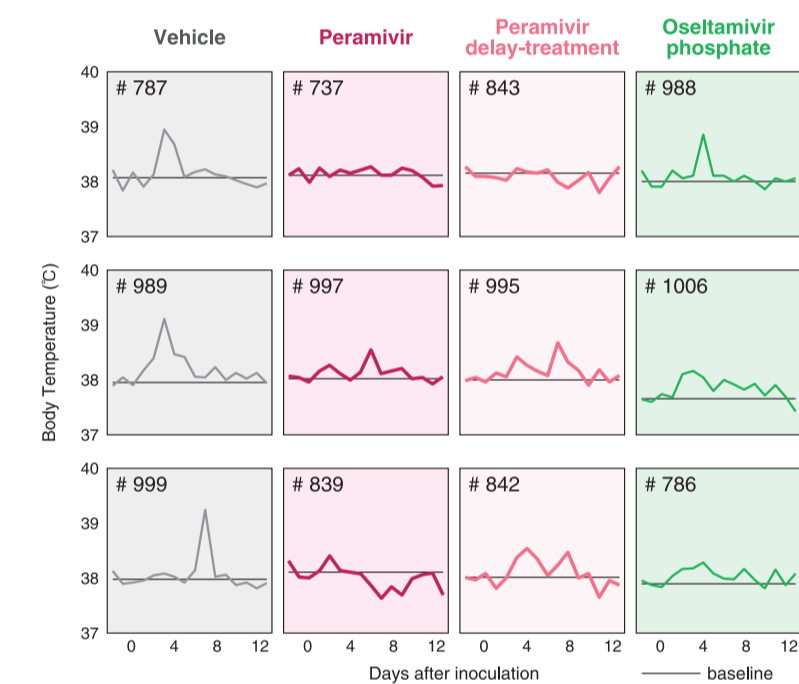
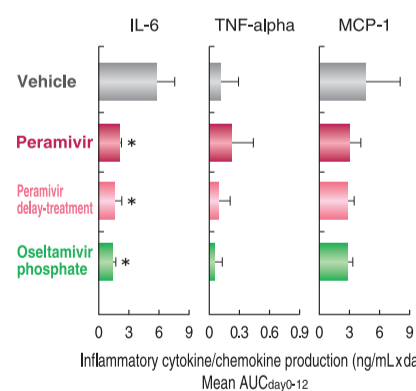


Fig.4 Body temperature change of infected cynomolgus macaques



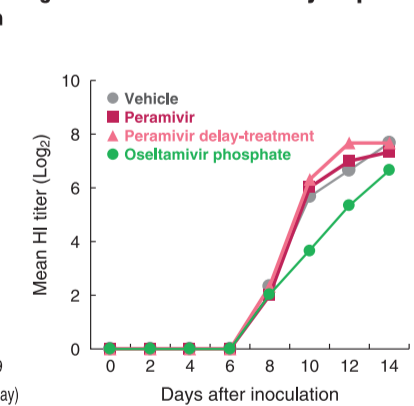
- Higher body temperature than that before the virus challenge was observed for days 1 to 4 after inoculation in vehicle-treated group.
- Body temperature of macaques treated with peramivir was substantially lower than those of macaques in the vehicle-treated group
- On the other hand, suppression of fever was not apparent in macaques treated with oseltamivir.

Fig.5 Levels of inflammatory cytokine/chemokine production



- Level of IL-6 production in nasal swab fluid of macaques treated with peramivir and oseltamivir were significantly lower than those of vehicle-treated group (\*p<0.05).
- On the other hand, there was no significant effect of peramivir and oseltamivir on TNF-alpha and MCP-1 production compared with those in vehicle-treated group.

Fig.6 Serum anti-HA antibody response



- HI activities against B/SendaiH/1051/2007 were observed in serum of all macaques 8 days after inoculation.
- Both of peramivir and oseltamivir did not inhibit the development of serum anti-HA antibodies to B/SendaiH/1051/2007 in macaques.

## Conclusions

- Peramivir as single intravenous treatment had beneficial effects on viral titers and symptoms of cynomolgus macaques with influenza B virus infection.
- The beneficial effects observed with peramivir treatment seem to be superior than that observed with oseltamivir phosphate treatment.

## Acknowledgement

- We thank Dr. Hidekazu Nishimura of Sendai Medical Center (Sendai, Japan) for providing clinical isolates of influenza virus for this study.