

# Therapeutic Effect of Intravenous Peramivir (BCX-1812) Treatment in Immunocompromised Mice Infected with Influenza A Virus

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## Abstract

### Background:

Peramivir (Fig.1) is a potent and selective inhibitor of influenza virus NA that is structurally different from oseltamivir. Therapeutic effect of intravenous dosing of peramivir was evaluated using influenza virus-infected immunosuppressed mice.

### Methods:

The immunosuppression in mice was induced by cyclophosphamide (CP). CP-treated mice were infected with influenza virus type A and the morbidity, viral titer and cytokines in lung was determined. The therapeutic effect of peramivir investigated using the virus-infected immunosuppressed mice and compared with that of oseltamivir.

### Results:

The immunosuppression in CP-treated mice was confirmed by the lower 50% mouse lethal dose (MLD<sub>50</sub>) of influenza virus than that of untreated mice. Delayed clearance of virus in lung and suppressed production of IL-6 and IFN-gamma also indicated immunosuppression in mice. Single treatment with peramivir was only weakly effective in the CP-treated mice infected with influenza virus, with the maximum survival rate (40%) seen in mice receiving 100 mg/kg of the compound. When peramivir was treated repeatedly for 5 days, the effect of peramivir was significantly enhanced, with the survival rate (80%) seen in mice receiving 10 mg/kg/day. The 50% of effective dose (ED<sub>50</sub>) of repetitive dosing of peramivir on virus-infected mice with CP treatment was 6.8 mg/kg/day. This therapeutic effect was more potent than that of oseltamivir phosphate (ED<sub>50</sub> = 78.6 mg/kg/day).

### Conclusions:

CP treatment resulted in increased mouse mortality, sustained virus high titer and marked suppression of cytokine expression in influenza virus-infected mice. Therapeutic benefit of peramivir after repeat intravenous dosing was shown in the immunosuppressed mice infected with influenza virus. Efficacy was better with repeat dosing of i.v. peramivir compared to repeat oral dosing of oseltamivir phosphate.

## Introduction

- Influenza virus infection results in substantial morbidity and mortality in hospitalized patients whose immune system is compromised.
- At present, studies of the efficacy of approved neuraminidase (NA) inhibitors such as oseltamivir and zanamivir in immunocompromised hosts are limited.
- Peramivir is a new neuraminidase inhibitor for intravenous administration that was first introduced in clinical practice for adult patients in Japan. The US Food and Drug Administration issued an emergency use authorization for peramivir exclusively for severe pandemic A (H1N1) influenza virus infection.
- In order to predict the efficacy of peramivir in immunocompromised patients, therapeutic effect of peramivir was evaluated using influenza virus-infected immunosuppressed mice and compared with that of oseltamivir.

## Materials and Methods

### Virus, cells and compounds

Laboratory strains of influenza virus type A, A/WS/33 (H1N1), was obtained from the American Type Culture Collection (Manassas, VA, USA). The MDCK cells were obtained from the American Type Culture Collection and maintained in Eagle's minimum essential medium (MEM) containing 5% fetal bovine serum. Peramivir, oseltamivir phosphate were used.

### Cyclophosphamide (CP)-treatment and viral infection

The immunosuppression in mice (BALB/c, female, 6-week-old) was induced by intraperitoneal injection of CP on days -1, 3 and 7 post-virus inoculation (Fig.2). CP-treated mice were inoculated intranasally with A/WS/33 (H1N1) virus under anesthesia. CP-treated mouse mortality was determined by survival number at 21 days post-virus inoculation.

### Determination of lung viral titers

On designated period after virus inoculation, three mice from CP-treated group and untreated control group were sacrificed and the lung was removed and homogenized in PBS. Supernatants of the homogenate were collected by centrifugation and filtration and were assayed in MDCK cells in 96-well plates. The appearance of cytopathic effect on the cell were scored visually and the final titer was determined. Virus titer was expressed as the 50% of tissue culture infectious dose (TCID<sub>50</sub>).

### Cytokine quantification

Each cytokine in homogenized lung fluid were measured with Cytometric Beads Array (CBA system; BD Bioscience) kit by standard procedure. Cytokine quantification was performed using FCAP Array Software (BD Bioscience).

### Antiviral study

Peramivir was administered intravenously single or once daily for 5 days and oseltamivir phosphate was administered orally twice daily for 5 days. Starting time of administration was 48 hr after virus inoculation (Fig.2). As parameter for anti-viral evaluation, prevention of death was observed through 21 days after virus inoculation.

Fig.1 Chemical structure of peramivir

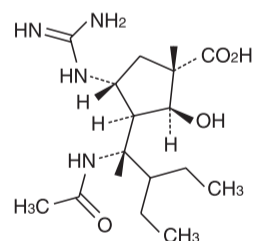
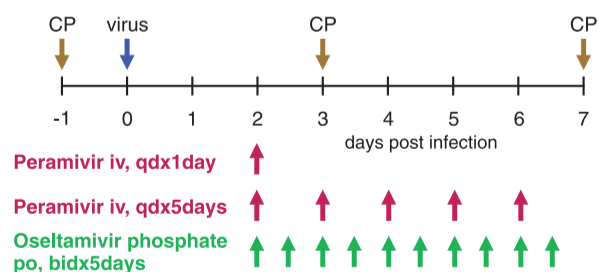
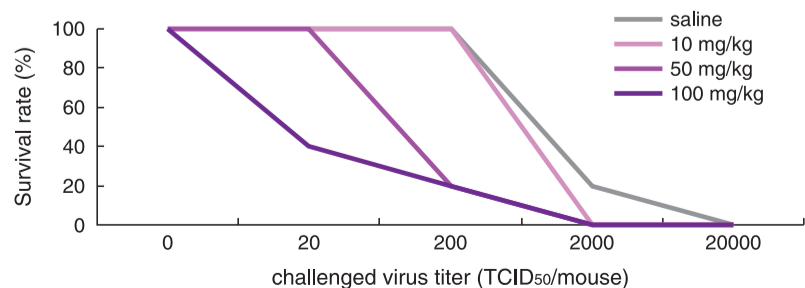


Fig.2 Experimental design



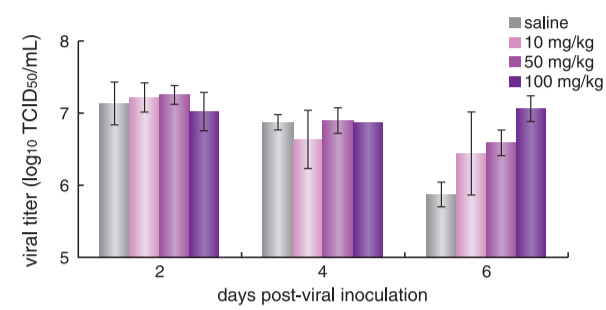
## Results

Fig.3 Mortality of CP-treated mice after virus infection



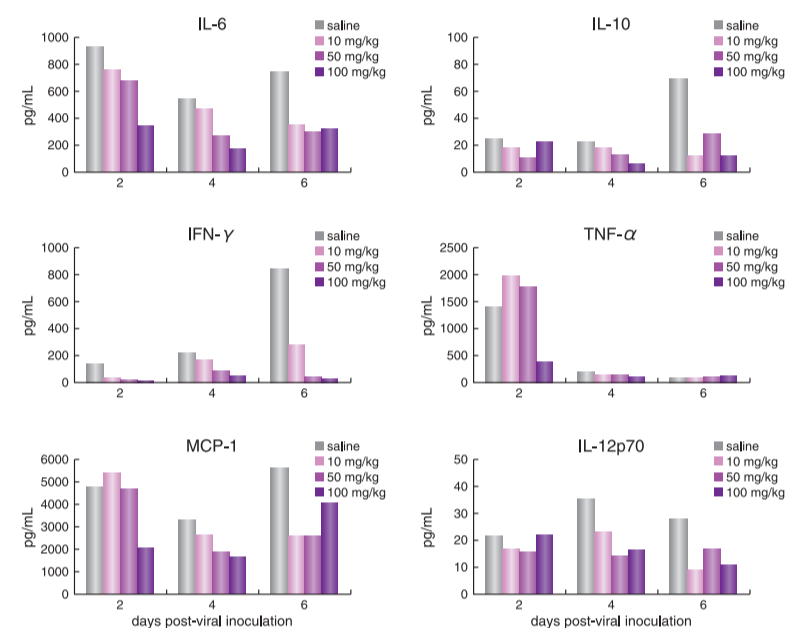
• Mortality of A/WS/33-infected mice was increased by 50 and 100 mg/kg of CP treatment compared with untreated (only saline-treated).

Fig.4 Viral titer in CP-treated mice lung after virus infection



• Virus titer in lung was decreased gradually in normal (saline-treated) mice. However, in the case of CP-treated mice, high titer in lung was sustained from +2 to +6 days after virus inoculation.

Fig.5 Cytokine expression in CP-treated mice lung after virus infection



• Cytokine expression in CP-treated mice, especially IL-6 and IFN-g, was suppressed dose proportionally.

Fig.6 Therapeutic effect of peramivir and oseltamivir phosphate on immunosuppressed mice with A/WS/33 virus

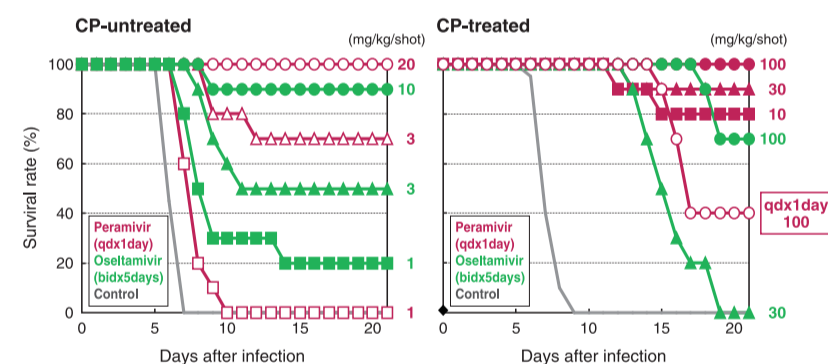


Table.1 Therapeutic effect of peramivir and oseltamivir phosphate on the survival rate in A/WS/33-infected mice

CP dose (mg/kg/shot)	Test and reference substances	Dose (mg/kg/day)	Survived / total per group	Survival <sup>a</sup> rate (%)	ED <sub>50</sub> (mg/kg/day)
0	Peramivir (iv, qd x 1 days)	1	0/10	0	2.4
		3	7/10	70*	
		10	10/10	100*	
	Oseltamivir phosphate (po, bid x 5 days)	1	2/10	20	
	3	5/10	50*	2.8	
	10	9/10	90*	-	
	Control; 0.5% MC (po, bid x 5 days)	0	0/20	0	-
50	Peramivir (iv, qd x 1 days)	10	0/10	0	> 100
		30	0/10	0	
		100	4/10	40*	
	Peramivir (iv, qd x 5 days)	1	0/10	0	
	3	1/9	11		
	10	8/10	80*, & #		
	30	9/10	90*, & #		
	100	10/10	100*, &	78.6	
	Oseltamivir phosphate (po, bid x 5 days)	10	0/10	0	-
		30	0/10	0	-
		100	7/10	70*	-
	Control; 0.5% MC (po, bid x 5 days)	0	0/20	0	-

a: on day 21 after virus inoculation  
\* p < 0.05 compared to 0.5%MC-treated control  
& p < 0.05 compared to peramivir trihydrate (iv, single) at the same dose  
# p < 0.05 compared to oseltamivir phosphate (po, bidx5 days) at the same dose

- Therapeutic effect of single dosing of peramivir was the same level as that of repetitive dosing of oseltamivir phosphate in virus-infected mice without CP treatment.
- However, therapeutic effect of peramivir by single dosing and oseltamivir phosphate by repetitive dosing was weakened by the CP treatment.
- Repetitive dosing of peramivir in the virus-infected mice with CP treatment was more effective than that of single dosing of peramivir trihydrate or repetitive dosing of oseltamivir phosphate.

## Conclusions

- CP treatment resulted in increased mouse mortality, sustained virus high titer and marked suppression of cytokine expression in influenza virus-infected mice.
- Therapeutic effect of peramivir after repetitive intravenous dosing was shown in the immunosuppressed influenza virus-infected mice. Efficacy was better with repeat dosing of i.v. peramivir compared to repeat oral dosing of oseltamivir phosphate.