

Therapeutic Effect of Peramivir (BCX-1812) after Single Intravenous Treatment in Mice Infected with Influenza A Virus with H274Y mutated NA

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Abstract

Background: Peramivir (Rapiacta®) is an influenza neuraminidase (NA) inhibitor approved for sale in January 2010 in Japan. In this study, we evaluated peramivir's efficacy against A/WSN/33 (H1N1) bearing 274th amino acid mutation from histidine to tyrosine in NA (H274Y NA).

Methods: Inhibitory effect on NA activity was measured using a fluorescence assay. Inhibitory effect on virus replication was measured by virus reduction assay using RPMI2650 cells derived from human nasal septum. The therapeutic effect of peramivir was investigated using the virus-infected mouse lethal model and the survival rate was compared with that of oseltamivir phosphate.

Results: The concentration of peramivir required to 50% inhibition of NA activity (IC₅₀) was 0.61 nM for the WT and 18.2 nM for the H274Y NA. On the other hand, IC₅₀ of oseltamivir carboxylate was 1.13 nM for the WT and 151.6 nM for the H274Y NA. In the virus reduction assay, the concentration of peramivir required to 90% inhibition of the virus titer in culture supernatant (EC₉₀) was 0.58 nM for the WT virus and 75 nM for the H274Y NA virus. In the case of oseltamivir carboxylate, EC₉₀ was 4.0 nM for the WT virus and more than 1000 nM for the H274Y NA virus. Therapeutic efficacy of peramivir was evaluated in parallel with oseltamivir phosphate in mice infected with H274 NA virus. Peramivir administered as a single intravenous dose improved survival rate in a dose-dependent manner, 80% survival at 30 mg/kg and 100% survival at 90 mg/kg. In contrast, twice-daily administration of oseltamivir phosphate at 10 mg/kg for 5 days (50 mg/kg as a total dose) showed 0% survival.

Conclusions: These data demonstrate that single intravenous treatment of peramivir has strong *in vivo* therapeutic efficacy against H274Y NA virus, despite decreased *in vitro* inhibitory activity of peramivir when the H274Y mutation was introduced in NA.

Materials and Methods

NA inhibition

NA activity was measured with MUNANA as a substrate at a final concentration of 10 mM. The reactions were carried out at 37 °C for 30min in 33 mM MES buffer supplemented with 4 mM CaCl₂ and 120 mM NaCl. The IC₅₀ value reflects the concentration of drug that reduces the NA activity by 50%.

Viral growth inhibition

RPMI2650 cells, derived from human nasal septum, were infected with viruses at a multiplicity of 0.001. After 72 h of the cultivation, we harvested fluids from cultures and then assessed to determine the virus yield,

Viral infection in mice

Viruses of 3000 TCID₅₀ were inoculated intranasally into female BALB/c mice. The design of experiments is shown in Fig. 2. The infected mice were sacrificed at 1-5 days postinfection, and the lungs were harvested and homogenized. Virus titers were measured by TCID₅₀.

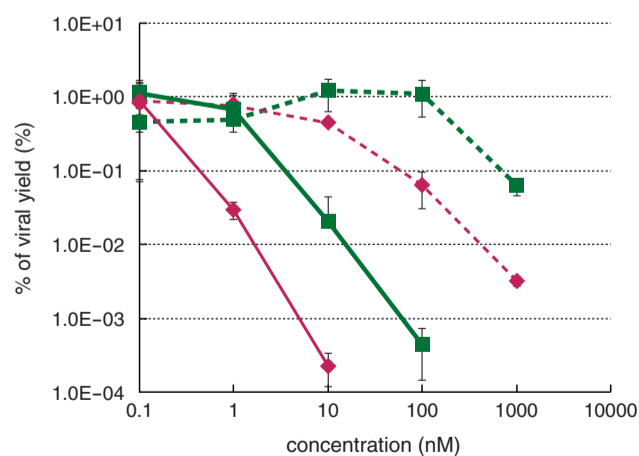
Results

Table 1 Inhibitory activities against the NA of A/WSN/33 carrying H274Y substitution

Virus	Mean IC ₅₀ ± SD (nM)	
	Peramivir	Oseltamivir carboxylate
A/WSN//33	0.61 ± 0.05	1.13 ± 0.04
A/WSN/33 H274Y	18.2 ± 0.8	151.6 ± 6.3

• The IC₅₀ value of peramivir for the H274Y NA was 30 times greater than that for the wild-type NA.

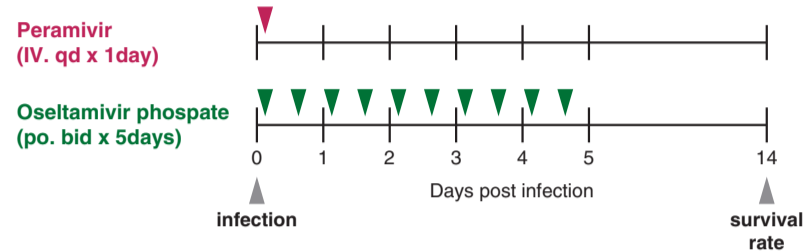
Fig.1 Inhibitory activities against the growth of A/WSN/33 carrying H274Y substitution in the NA



• Inhibitory activity of peramivir against the growth of A/WSN/33 carrying H274Y substitution in the NA was decrease almost 100 fold.

Fig.2 Therapeutic effect in mice infected with A/WSN/33 carrying H274Y substitution in the NA

A) Experimental design



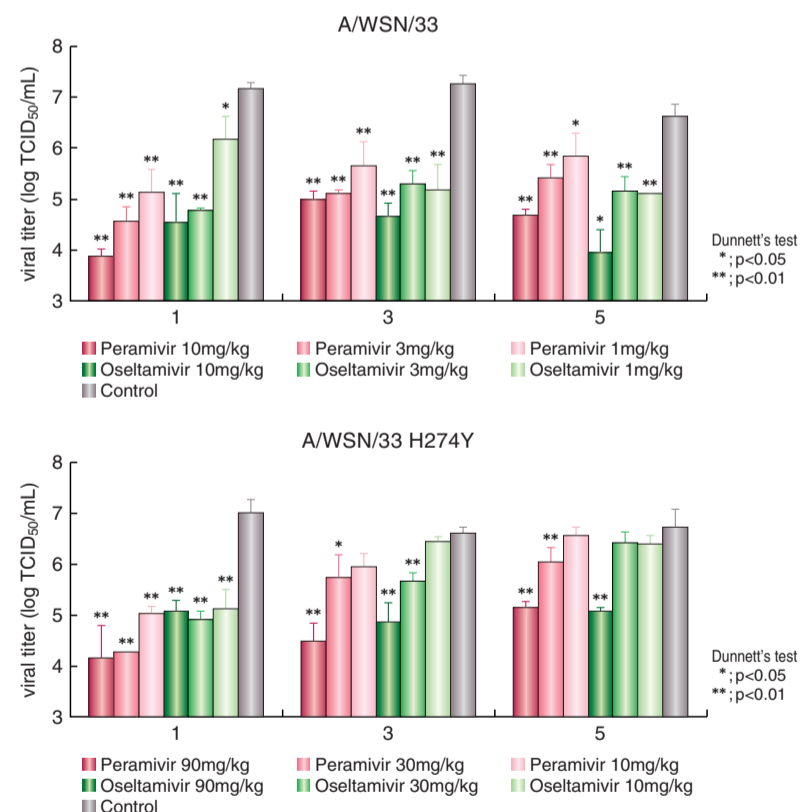
B) Mice survival rate

Virus	Treatment	Dose (mg/kg/day)	Survival/total	Survival rate (%)	ED ₅₀ (mg/kg/day)
A/WSN/33	Peramivir (iv. qd x 1day)	1	1/5	20	1.0
		3	5/5	100	
		10	5/5	100	
	Oseltamivir phosphate (po. bid x 5days)	1	4/5	80	0.4
		3	5/5	100	
10		5/5	100		
	control	0	0/5	0	-
A/WSN/33 H274Y	Peramivir (iv. qd x 1day)	10	0/5	0	28.5
		30	4/5	80	
		90*	5/5	100	
	Oseltamivir phosphate (po. bid x 5days)	10	0/5	0	29.5
		30	3/5	60	
90		5/5	100		
	control	0	0/5	0	-

*clinical dose

• Therapeutic effect of single dosing of peramivir was the same level as that of repeat dosing of oseltamivir phosphate in mice infected with A/WSN/33 carrying H274Y in the NA.

Fig. 3 Virus titers in lungs



• Therapeutic treatments of peramivir (90 mg/kg) against mice infected with A/WSN/33 carrying H274Y in the NA reduced the virus titers in lungs by 2 log TCID₅₀/mL compared to that of control groups throughout day 1 to 5 of post-virus inoculation.

Conclusions

• Single intravenous treatment of peramivir has strong therapeutic efficacy against the mice infected with the virus carrying H274Y substitution in the NA *in vivo*, despite the decreased inhibitory activity *in vitro*.

• The efficacy of single intravenous treatment of 90 mg/kg peramivir to mice, equivalent to the 600 mg clinical dose to human was better than that of repeat oral treatment of daily 10 mg/kg oseltamivir phosphate to mice, equivalent to the daily 150 mg clinical dose.