

Antiviral Efficacy of Peramivir (BCX-1812) against Highly Pathogenic Avian Influenza Viruses (H5N1) with or without the H274Y mutation in mice

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Background

Peramivir is a potent influenza virus neuraminidase (NA) inhibitor. A single intravenous injection of peramivir was shown to be effective in clinical studies, leading to marketing authorization in January 2010 in Japan (Rapiacta®). In this study, we evaluated the efficacy of peramivir against highly pathogenic A/Hong Kong/483/97 (HK483) influenza viruses with or without the NAH274Y mutation in a mouse model.

Methods

Viruses were generated from plasmids containing the full-length cDNA of HK483 influenza viruses. Mutations encoding substitutions at conserved NA residues (H274Y) were separately introduced into the NA plasmids by site-directed mutagenesis (HK483/H274Y). Recombinant viruses were produced in embryonated chicken eggs.

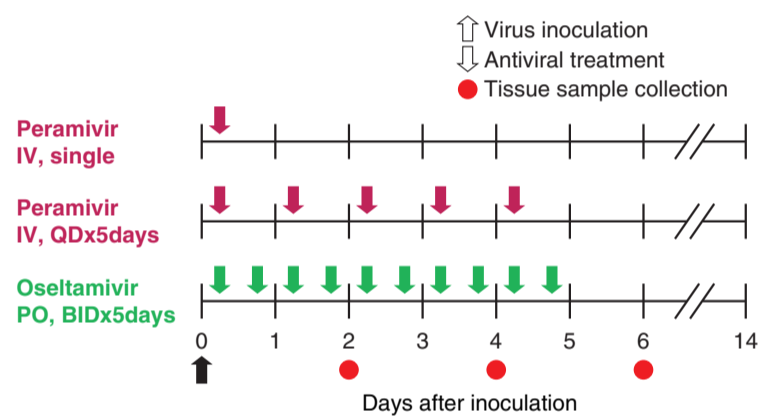
<in vitro antiviral assay>

Fetuin was used as a substrate, and the amount of free sialic acid liberated by viral NA was measured. The concentration achieving 50% inhibition of NA activity (IC₅₀) was calculated. The viruses released from the infected MDCK cells were titrated by TCID₅₀. The inhibitory effect was represented by the concentration achieving 90% protection versus influenza virus infection (EC₉₀) values.

<in vivo assay>

Viruses were inoculated to BALB/c mice (10MLD₅₀). Treatment with antiviral compound was started immediately after virus inoculation. Peramivir was administered intravenously as a single injection or once daily for 5 days injection, and oseltamivir phosphate was administered orally twice daily for 5 days. Antiviral efficacy was determined by the prevention of death at 14 days and by the virus reduction in lung at designated dates after virus inoculation.

Figure 1. Dosing and experimental design



Results

Table 1. IC₅₀ values of peramivir and oseltamivir carboxylic acid against neuraminidase of A/HK/483/97 WT or carrying NA H274Y

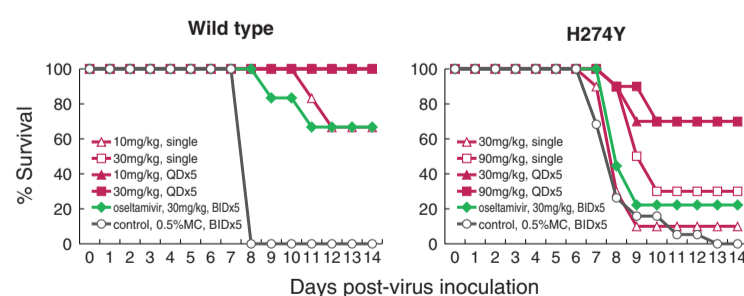
compound	Wild type IC ₅₀ (nM)		H274Y IC ₅₀ (nM)		Fold change
	mean	SD	mean	SD	
Peramivir	0.16	0.03	88.9	26.1	555
Oseltamivir	6.4	2.2	1004	155	158

Table 2. EC₉₀ values of peramivir and oseltamivir carboxylic acid against A/HK/483/97 WT or carrying NA H274Y viruses on replication

compound	Wild type EC ₉₀ (nM)		H274Y EC ₉₀ (nM)		Fold change
	mean	SD	mean	SD	
Peramivir	1.8	0.6	256	24.9	145
Oseltamivir	11.3	1.43	1289	114	114

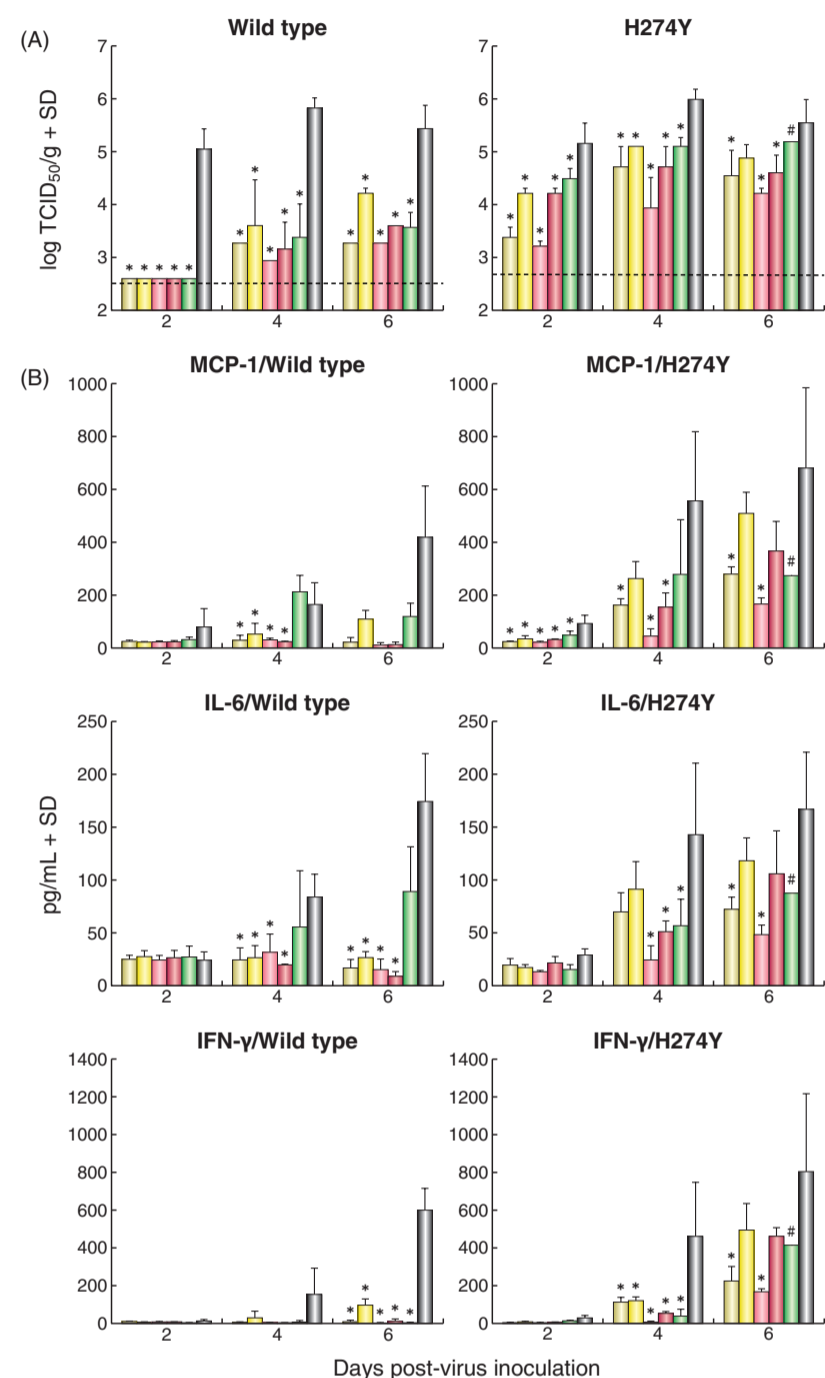
Introduction of the H274Y NA mutation reduced the susceptibility to peramivir and oseltamivir in vitro.

Figure 2. Therapeutic effects of peramivir and oseltamivir on survival in mice infected with A/Hong Kong/483/97 WT or carrying NA H274Y mutation



Peramivir significantly prevented death of mice inoculated with lethal doses of HK483 virus. Moreover, peramivir at a dose of 30 and 90 mg/kg once daily for 5 days significantly prevented the death of mice inoculated with lethal doses of HK483/H274Y (70% survived) compared to untreated control (100% mortality). Conversely, survival rate by dosing of oseltamivir phosphate at 30mg/kg (twice daily for 5 days) was 70% and 20% after lethal doses of HK483 and HK483/H274Y, respectively.

Figure 3. Detection of viral load (A) and proinflammatory chemokine, cytokines (B) in lung.

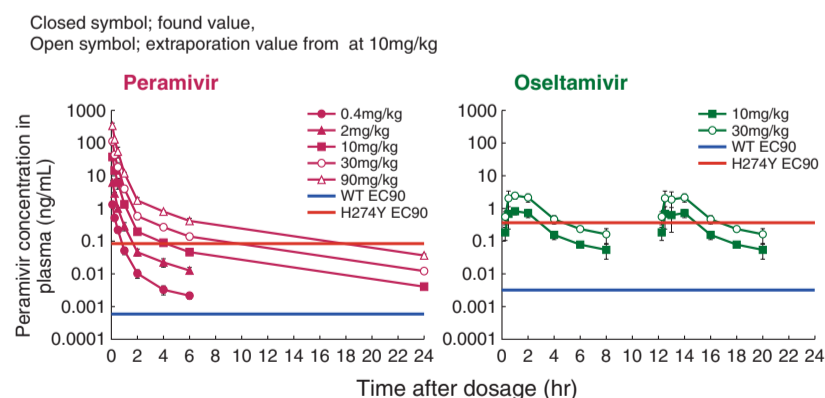


* Dunnett's multiple comparison; p<0.05

Legend for Figure 3: Peramivir, single 10(30) mg/kg; Peramivir, QDx5days 10(30) mg/kg; Oseltamivir, BIDx5days 10(30) mg/kg; Peramivir, single 30(90) mg/kg; Peramivir, QDx5days 30(90) mg/kg; Control, 0.5%MC BIDx5days; (): administered to rgH274Y-infected mice

Peramivir significantly reduced the virus titers and cytokine/chemokine productions in the lungs compared to the control group. These results correlated well with the survival rate of HK483-infected mice.

Figure 4. Simulated analysis from pharmacokinetic study against wild type and NA H274Y of A/Hong Kong/483/97 virus.



The peak plasma concentrations achieved with intravenous peramivir are nearly two orders of magnitude higher than those achieved with standard doses of oral oseltamivir. High plasma level therapy by intravenous administration provides optimal bioavailability, which may result in faster viral clearance.

Conclusions

These data demonstrate that peramivir has a strong inhibitory activity against highly pathogenic avian influenza viruses with or without the H274Y mutation.