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Efficacy of a single intravenous administration of laninamivir (an active metabolite of laninamivir octanoate) in an influenza virus infection mouse model

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ABSTRACT

Laninamivir, a potent neuraminidase (NA) inhibitor, is an active metabolite of laninamivir octanoate (code name: CS-8958) which is a long acting NA inhibitor and is commercially available under the brand name Inavir in Japan to complete the treatment of influenza by a single inhalation. It is supposed that the long acting character is provided by the long retention of laninamivir in the respiratory tract after intranasal administration of laninamivir octanoate in mice and with stable binding of laninamivir to NA of various influenza viruses such as N1, N2 subtypes and NA of B virus. Peramivir, another NA inhibitor, is also approved in Japan as a single intravenous infusion. In spite of the quick disappearance of peramivir from the blood after injection, the reason treatment can be completed by a single administration is thought to be that peramivir showed stable binding to NA with N9 subtype. Therefore, the stable binder, laninamivir is possibly effective by a single intravenous administration in the mouse model infected with influenza viruses. A single intravenous administration of laninamivir and peramivir at 30 mg/kg significantly prolonged mice survival at a comparable level in the mouse lethal model infected with the A/PR/8/34 (H1N1) virus. Also, a single intravenous administration of laninamivir and peramivir significantly suppressed virus proliferation in the lungs of mice infected with influenza B virus. Thus, laninamivir may be effective by a single intravenous infusion in treating influenza, the same as peramivir.

1. Introduction

Influenza is a serious respiratory illness which can be debilitating and causes complications that lead to hospitalization and death, especially in elderly individuals. This respiratory disease is caused by influenza A and B viruses, which are pathogens that are highly contagious for humans. Influenza A viruses are classified into subtypes on the basis of the antigenicities of hemagglutinin (HA) and neuraminidase (NA) molecules. To date, 16 HA subtypes (H1–H16) and 9 NA subtypes (N1–N9) have been reported. Seasonal influenza or influenza epidemics are caused by influenza A virus H1N1 and H3N2 and influenza B virus (Wright and Webster, 2001); and every year the global burden of influenza epidemics was believed to be 3.5 million cases of severe illness and 300,000–500,000 deaths (Fiore et al., 2007), before the new pandemic in 2009.

Two countermeasures, vaccinations and treatment with antivirals, are available to control human influenza. Although vaccinations play an important role in influenza prophylaxis, they are an insufficient tool both for prophylaxis and against a pandemic virus. Therefore, antivirals are an important tool that may be used to mitigate influenza pandemics. Currently, two types of anti-influenza virus drugs are available: M2 ion channel blockers (adamantane) (Davies et al., 1964) and NA inhibitors. However, adamantane-resistant viruses readily emerge and are already prevalent worldwide among the seasonal influenza viruses such as the H1N1, the H1N1pdm09 and the H3N2 subtypes (Bright et al., 2005, 2006a,b CDC, 2009).

The NA inhibitors were discovered by a structure based drug design with the advent of the N2, N9 and influenza B NA structures (Colman et al., 1983; Varghese et al., 1983; Baker et al., 1987; Burmeister et al., 1992; von Itzstein et al., 1993, 1994; Von Itzstein, 2007). Two NA inhibitors, zanamivir and oseltamivir, are used worldwide for treatment of influenza. In addition, two more inhibitors, peramivir and laninamivir octanoate (code name CS-8958) have been used since 2010 in Japan. Zanamivir (inhalant, Relenza®) and oseltamivir (oral drug, Tamiflu®) are required twice daily for 5 days for treatment; on the other hand, for peramivir (injection

Abbreviations: MDCK, Madin–Darby canine kidney; FBS, fetal bovine serum; PBS, phosphate buffered saline; BSA, bovine serum albumin; pfu, plaque forming unit; MLD_{50}, 50% mouse lethal dose; hpi, hours postinfection; dpi, days postinfection; MRT, mean residence time; half-life, $t_{1/2}$; AUC, area under curve.

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drug, Rapiacta®) and laninamivir octanoate (inhalant, Inavir®), a single administration of the drug is sufficient for treatment.

Oral drugs and inhaled drugs have a limitation in clinical use for treatment of serious and/or complicated influenza patients. Administration of enterically administered oseltamivir can be challenging in the ICU where patients may have difficulty tolerating oral administration of capsules or where intestinal ileus may cause malabsorption (Ariano et al., 2010). Use of inhalants such as zanamivir and laninamivir octanoate are not suitable for patients on mechanical ventilation (Writing Committee of the WHO, 2010). As part of the emergency public health response, the Food and Drug Administration issued an Emergency Use Authorization (EUA) for the experimental intravenous peramivir in 2009. In these situations, an intravenous drug is quite essential for the treatment of serious and/or complicated influenza patients and, in fact, clinical studies of injection of oseltamivir and zanamivir are ongoing (Wathen et al., 2012, http://clinicaltrials.gov/ct2/show/NCT01231620?term=zanamivir&rank=1, US NIH last accessed April 9, 2013).

Under the EUA, peramivir was distributed by the Centers for Disease Control and Prevention (CDC) for treatment of hospitalized pediatric and adult patients with evidence of H1N1pdm09 virus infection and who (1) were not responding to antiviral therapy, or (2) the clinician deemed that enteral or inhaled delivery of NAIs was not dependable or feasible (http://www.fda.gov/Drugs/DrugSafety/PostmarketDrugSafetyInformationforPatientsandProviders/ucm187980.htm, US FDA, last accessed April 9, 2013). Also, intravenous peramivir showed a significant shorter length of hospital stay for severe influenza patients infected with the H1N1pdm09 virus (Louie et al., 2012).

It has been reported that a single intravenous injection of peramivir showed efficacy similar to repeated oral administrations (twice daily for 5 days) of oseltamivir for influenza patients in the clinical study (Kohno et al., 2011). In spite of the quick disappearance of peramivir from the blood after intravenous injection (MRT = about 3 h, (http://www.info.pmda.go.jp/go/pack/6250405A1032_1_01/, JP PMDA, last accessed April 9, 2013), the reason to treatment can be completed by a single administration is thought to be that peramivir showed stable binding to NA with N9 subtype (Bantia et al., 2006).

Laninamivir octanoate (code name: CS-8958) shows an effective anti-influenza activity by a single intranasal administration in the mouse and ferret models infected with various influenza viruses (Yamashita et al., 2009; Kubo et al., 2010; Kiso et al., 2010). The reasons for the long actions are accounted for by the long retention of the active metabolite, laninamivir, in animal respiratory tracts after intranasal administration (Koyama et al., 2009) and the stable binding ability of laninamivir to NA of various influenza viruses (Kiso et al., 2010; Yamashita, 2010; Yamashita et al., 2010). In these previous reports, laninamivir is also indicated to be a strong binder to NAs of H1N1, H1N1pdm09, H3N2 and type B viruses. The binding stability of laninamivir is similar to peramivir and stronger than zanamivir for H1N1, H1N1pdm09; similar to peramivir and zanamivir for H3N2; however, it is stronger than both for type B virus. Therefore, the stable binder laninamivir is possibly effective by a single intravenous administration in the mouse model infected with influenza viruses. In this report, to evaluate the *in vivo* efficacy of intravenous laninamivir, we compare the efficacy by intravenous administration of laninamivir to peramivir and zanamivir in the mouse infection model with influenza viruses.

2. Materials and methods

2.1. Viruses and cells

The influenza viruses A/Puerto Rico/8/34 (A/PR/8/34, H1N1) and B/Malaysia/2506/2004 were provided by the National Institute of Infectious Diseases, Japan. MDCK cells were obtained from the American Type Culture Collection (ATCC CCL-34) and purchased from DS Pharma Biomedical Co., Ltd. (Japan); maintained in minimum essential medium containing 10% FBS, 50 units/ml penicillin and 50 μg/ml streptomycin. The cells were cultured in 5% CO_2 at 37 °C.

2.2. Compounds

Laninamivir octanoate, laninamivir and zanamivir were synthesized by Daiichi Sankyo Co., Ltd. Commercially available Rapiacta (Shionogi Co. Ltd.) was used as peramivir.

2.3. Inhibitory activity to influenza virus NA

The NA activity was measured according to the method described elsewhere. (Kubo et al., 2010). Briefly, the virus solution and the test compound were mixed in a 32.5 mM 2-(*N*-morpholino)ethanesulfonic acid–NaOH buffer (pH 6.5) containing 4 mM $CaCl_2$ and the mixture was preincubated at 37 °C for 30 min. Then, 4-MU-NANA (final concentration of 100 μM) was added and the mixture was incubated for another 60 min at 37 °C. The generated fluorescence was measured with the excitation at 360 nm and the emission at 460 nm, using a CytoFluor series 4000 instrument (Applied Biosystems Japan, Ltd.). The 50% inhibitory concentration (IC_{50}) was calculated by linear regression analysis using SAS System Release version 8.2 (SAS Institute Inc.) software.

2.4. Animal experiments

Female BALB/c mice (5–6 weeks old, specific pathogen free; Charles River Laboratories Japan, Inc.) were kept in a controlled room throughout the experiments. The rearing conditions of the room were as follows: a temperature of 20–26 °C, relative humidity of 55 ± 10% and a 12-h lighting cycle. The mice were anesthetized with isoflurane (Abbott Japan Co., Ltd.) and were intranasally infected with A/PR/8/34 at 100 pfu (3.7 MLD_{50}) or B/Malaysia/2506/2004 at 1000 pfu. For a single administration, 10 or 30 mg/kg of laninamivir, zanamivir, peramivir or saline was intravenously administered once at 13 hpi. Laninamivir octanoate at 0.14 mg/kg was intranasally administered once as well. For repeated administration, 3.0 mg/kg of these compounds or saline was intravenously administered once daily from 13 hpi for 5 days. Mice survival was monitored until 20 dpi. The virus titers in the mice lungs at 2, 3 and 6 dpi were measured by a plaque assay described below. All the experimental procedures were performed in accordance with the guidelines of the Institutional Animal Care and Use Committee of Daiichi Sankyo Co., Ltd.

2.5. Plaque Assay

All the lungs excised from mice were homogenized in 1 ml of PBS containing 0.4% BSA, 50 units/ml penicillin and 50 μg/ml streptomycin (PBS-BSA/PS). After centrifuging the homogenates at 2200*g* for 5 min at 4 °C, the supernatants were diluted 10-fold serially with minimum essential medium containing 0.2% BSA, 50 units/ml penicillin and 50 μg/ml streptomycin. Confluently grown MDCK cells in 6-well plates were washed with PBS, and 200 μl of the each diluted sample was added in duplicate. After the cells were incubated at 37 °C under 5% CO_2 in an incubator for 1 h, they were washed with PBS. Then, 2.5 ml of modified eagle medium containing 0.2% of BSA, 25 mM of HEPES buffer, 0.01% of DEAE-Dextran, 1 μg/ml of trypsin, 0.001% of phenol red and 0.6% of agar was added to the wells. The plates were placed in the CO_2 incubator for 2 days. After removing the agar medium, 0.1% crystal violet cell in 19% methanol was added to the wells to fix