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ORIGINAL ARTICLE

# Comparison of the efficacies of amantadine treatment of swine-origin influenza virus A H1N1 and seasonal influenza H1N1 and H3N2 in Japan (2008–2009)

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**Abstract** Amantadine is not thought to be effective for the treatment of swine-origin influenza virus (S-OIV) based on an analysis of genetic sequences of the M2 protein. However, the actual clinical efficacy of amantadine has not been well documented. Here, we were able to compare the efficacies of amantadine and neuraminidase inhibitors. Subjects consisted of 428 patients, including 144 with seasonal influenza (flu) identified between 2008 and 2009, and 284 with S-OIV identified between July 1 and November 30, 2009. Diagnosis of flu was established using a rapid diagnostic kit obtained commercially in Japan. Body temperature sheets were obtained from 95% of the S-OIV patients. Times required to recover normal body temperature were compared among subjects using different antiviral drugs. Genetic abnormalities in the M2 protein were also investigated in 66 randomly selected subjects from within the patient pool. Overall, the average hours required to recover normal body temperature in S-OIV patients treated with amantadine (160 cases), with oseltamivir (59 cases), or with zanamivir (65 cases) were $33.9 \pm 20.7$, $31.7 \pm 16.0$, or $36.3 \pm 21.6$, respectively. These differences were not statistically significant. The N31S abnormality was found in all 14 samples taken from the H3N2 patients and in all of the 23 samples taken from in S-OIV patients. However, this abnormality was not found in any of the 30 samples taken from seasonal H1N1 patients. Amantadine was found to be equally effective in treating S-OIV patients as neuraminidase inhibitors. The genetic abnormality resulting in S31N amino acid conversion identified in some of the H3N2 and S-OIV patients is thought to alter the function of M2 protein only mildly.

**Keywords** Swine-origin influenza virus (S-OIV) · Amantadine · Oseltamivir · Zanamivir · M2 protein · Amantadine resistance

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## Introduction

Patients infected with H1N1 swine-origin influenza virus A (S-OIV) were first reported in Mexico in April, 2009 [1], and were subsequently found in the USA [2, 3], Canada, Europe, Korea, and Japan by the middle of May. Fortunately, the clinical courses of patients with S-IOV infection have generally been mild, and resemble that of a patient with seasonal influenza (flu) [4]. Currently, there are over 10 million patients in Japan infected with S-OIV as of November 30. Amantadine has not been found to be effective for the treatment of S-OIV in the USA, and the use

of the drug is generally not recommended [5]. However, amantadine has been administered for flu A at the Keigu Clinic for the past 8 years with success. In the present study, we investigated the efficacy of amantadine against S-OIV during the period between July 1 and November 30, when seasonal flu is not a confounding factor, and compared the results to those of patients receiving oseltamivir/zanamivir. In addition, we randomly chose sputum samples collected from selected flu A patients between 2008 and 2009, and analyzed the genetic sequences of the influenza A virus to determine the location of a putative common abnormality in the M2 protein gene.

## Materials and methods

### Sample collection

Samples were collected from patients attending 4 participating clinics, and prior consent was obtained according to established guidelines. Subjects consisted of 428 patients, among which 144 were seasonal influenza (flu) cases identified between 2008 and 2009, and 284 were S-OIV patients identified between July 1 and November 30, 2009. Regarding the seasonal cases, 114 had flu A and 30 had flu B, as identified between November 1, 2008 and April 30, 2009.

### Determination of influenza

Commercially available point-of-care testing (POCT) kits in Japan were used on patients with a continuing fever of 37.5°C or greater for 6 h and clinical symptoms including headache, fatigue, muscle pain, and/or joint pains. Diagnosis of S-OIV was also made based on a positive POCT result.

### Anti-influenza drugs

The dosage of amantadine administered in this study was set at 4 mg/kg body weight for a duration of 4 days, with the maximum dosage being limited to 200 mg/day. Oseltamivir was used at 3–4 mg/kg for 5 days, and zanamivir was used at 4 blisters (1 sheet/day) for 5 days on selected flu A and flu B patients. Treatment generally started within 1 day following the onset of fever. During anti-influenza drug administration, antibiotics and NSAID were not given. Drug efficacy was judged as follows: excellent, less than 24 h required to recover to normal body temperature; good, less than 48 h to recover; poor, greater than 48 h required. Fever records gathered over a 7-day period were obtained from 62% of the seasonal flu and 95% of the S-OIV patients.

### Genetic analysis of M2 protein in influenza A

Analysis of the influenza virus M2 gene was performed on sputum samples obtained randomly from patients infected with the H1N1 virus, H3N2 virus, and S-OIV.

### Virus isolation

Viruses present in sputa were prepared for culture by suspending them in culture medium containing appropriate amounts of penicillin, streptomycin, gentamycin, and fungizone. Next, MDCK (Madin–Darby canine kidney) cells were inoculated with virus particles adjusted to a concentration of 500,000 particles/mL Eagle's MEM, and incubated for 4–5 days. Upon confirmation of a confluent monolayer of cells, the supernatant was discarded, maintenance medium containing trypsin was added, and the cells were incubated further at 35°C. When ++ level cytopathic effects were observed, hemagglutinin activity was tested. Subsequently, both hemagglutinin and neuraminidase activities were measured on the fifth day.

### PCR determination

Primers were acquired from the Japanese National Institute of Infectious Diseases. RNA was extracted by the AGPC (acid guanidium phenol chloroform) method using a commercial preparation (BiotecXL Lab). The actual reverse transcription and PCR reactions were carried out using commercially available kits.

### Statistical tests

Differences in recovery time among the various drug treatment groups were analyzed by one-way analysis of variance (ANOVA), with further Bonferroni analyses. Other comparisons among groups were conducted using the chi-square test.

## Results

### Fluctuations in patient numbers between December 2008 and November 2009 at the Keigu Clinic

One hundred forty-four seasonal flu patients were followed from December to April 30 at the Keigu Clinic. The peak of flu A occurred in early January. On the other hand, a peak period was not found for flu B, with 30 patients being identified over a period of several weeks (Fig. 1). Regarding S-OIV infection, 125 patients were followed from September 1 to November 30. A small peak was observed at