Lower Clinical Effectiveness of Oseltamivir against Influenza B Contrasted with Influenza A Infection in Children

Norio Sugaya, Keiko Mitamura, Masahiko Yamazaki, Daisuke Tamura, Masataka Ichikawa, Kazuhiro Kimura, Chiharu Kawakami, Maki Kiso, Mutsumi Ito, Shuji Hatakeyama, and Yoshihiro Kawaoka

Department of Pediatrics, Keiyu Hospital, Yokohama City Institute of Health, Yokohama, Department of Pediatrics, Eiju General Hospital, Division of Virology, Department of Microbiology and Immunology, International Center for Infectious Diseases, Institute of Medical Science, Department of Infectious Diseases, Graduate School of Medicine, University of Tokyo, Zama Children’s Clinic, Zama, Department of Pediatrics, Ishihara Kyodo Hospital, Ishihara, and Core Research for Evolutional Science and Technology (CREST), Japan Science and Technology Agency, Saitama, Japan; and Department of Pathobiological Sciences, School of Veterinary Medicine, University of Wisconsin–Madison, Wisconsin

(See the editorial commentary by Wright on page 203)

Background. Recently, many Japanese physicians have claimed that oseltamivir is less effective in children with influenza B virus infection. This study assesses the effectiveness of oseltamivir against influenza A (H3N2) and influenza B in children on the basis of the duration of febrile illness.

Methods. We used oseltamivir to treat 127 children with influenza A (H3N2; mean age, 6.97 years [range, 1–15 years]) and 362 children with influenza B (mean age, 5.16 years [range, 1–15 years]) in outpatient clinics. The duration of fever after the start of oseltamivir therapy was compared in the influenza A group and the influenza B group.

Results. The mean duration of fever after the start of oseltamivir therapy was significantly greater in the influenza B group than in the influenza A (H3N2) group (2.18 days vs. 1.31 days, respectively; P<.001). The difference was marked in young children (1–5 years old; 2.37 days for the influenza B group vs. 1.42 days for the influenza A group) but was not significant among older children (11–15 years old). The 50% inhibitory concentration of oseltamivir against influenza B virus was 75.4 ± 41.7 nmol/L and was substantially higher than that for type A (H3N2) virus (0.3 ± 0.1 nmol/L). Only 3 (1.6%) of 192 influenza B viruses were resistant to oseltamivir.

Conclusions. Oseltamivir is much less effective against influenza B virus infection in young children, probably because of the low sensitivity of influenza B viruses to oseltamivir. The effectiveness of oseltamivir against influenza B is influenced by age and host immunity. A few oseltamivir-resistant influenza B strains were isolated before the start of oseltamivir therapy.

Dramatic advances in the diagnosis and treatment of influenza in Japan have been made in recent years [1]. Most patients with an influenza-like illness are now tested with rapid diagnostic tests and, if the results are positive, they are treated with the neuraminidase inhibitor oseltamivir. The rapid influenza diagnosis kit was introduced in Japan in time for the 1999–2000 epidemic; it was estimated that ∼17 million people (14% of Japan’s population) may have been tested with it during the 2004–2005 influenza season [2]. The diagnostic kits used in Japan are capable of detecting influenza A or B separately; thus, Japanese clinicians prescribe oseltamivir with the knowledge of whether their patients have been infected with the influenza A or B virus.

Japan currently has the highest amount of neuraminidase inhibitor use in the world. More than 70%–80% of the total oseltamivir prescribed throughout the world every year is used in Japan [2]. Oseltamivir therapy is now routine in Japan, and almost all children with influenza are treated with oseltamivir, regardless of their underlying condition.
A major mixed epidemic caused by the influenza A/Fujian/411/2002 (H3N2)–like and influenza B/Shanghai/361/2002 (Yamagata lineage)–like strains occurred in Japan during the 2004–2005 influenza season [3]. Approximately 55% of the influenza viruses isolated were influenza B/Shanghai–like stains, making it the largest influenza B epidemic to occur to date after the introduction of oseltamivir in Japan during the 2000–2001 season. During the epidemic, many Japanese physicians claimed that children with influenza B often had a persistently high fever despite receiving oseltamivir therapy within 48 h of the onset of illness and, thus, that influenza B viruses may have become resistant to oseltamivir. This was a clinically serious problem, because in children, influenza B is as severe an illness as influenza A and is a frequent cause of pediatric hospitalizations during the winter influenza season [4, 5]. Moreover, it was reported that oseltamivir might be less effective against influenza B, namely in adult patients [6].

We have compared the effectiveness of oseltamivir against influenza A (H3N2) and influenza B on the basis of the duration of the febrile period, because high and prolonged fever is one of the most important symptoms of influenza virus infection in children [7], and because it can be objectively assessed. We also determined the IC50 for B viruses that were isolated.

PATIENTS AND METHODS

Patients. We used oseltamivir to treat 127 children with influenza A (H3N2) (mean age, 6.97 ± 3.55 years [range, 1–15 years]) and 362 children with influenza B (mean age, 5.16 ± 3.04 years [range, 1–15 years]) in the outpatient clinics of the pediatric departments of our hospitals. All patients presented with a temperature >38.0°C within 48 h after the onset of a febrile illness. They were tested with rapid diagnostic tests (in most cases, Espline Influenza A&B-N; Fujirebio), and were diagnosed with influenza A or B before the start of oseltamivir therapy. Diagnoses were confirmed by virus isolation; 103 influenza A (H3N2) and 321 influenza B viruses were isolated.

In approximately three-fourths of the patients who were observed, oseltamivir was prescribed in weight-based unit doses, taken twice daily for 5 days (for patients who weighed <15 kg, 60 mg/day; patients 15–23 kg, 90 mg/day; patients 23–40 kg, 120 mg/day; and patients >40 kg, 150 mg/day). The remaining patients were treated with oseltamivir, 4 mg per kg per day for 5 days.

Parents were instructed to take their children’s temperatures twice daily—in the morning and in the evening. These temperature values were recorded on a fever record sheet that we prepared, which was returned to us on the day of the final visit. When a patient’s temperature decreased to <37.5°C and remained there for 2 more measurements, we considered the temperature to have returned to normal. If a patient’s temperature decreased to <37.5°C once and remained <37.5°C for the next 2 measurements (~24 h), we considered the patient to have a biphasic fever pattern.

Because we had no cases for comparison of influenza A that was not treated with oseltamivir for the total febrile period (because oseltamivir is now routine therapy for influenza in Japan), we analyzed the clinical course of patients who had influenza A (H3N2) during the 2001–2002 epidemic who were not treated with antiviral agents [8]. Their diagnosis was confirmed by virus isolation. The control subjects for influenza B were those who did not receive oseltamivir during the 2004–2005 epidemic, because their rapid test results were negative at the first visit (influenza B viruses were isolated from these patients after the first visit, however).

METHODS

Virus isolation and titration. Clinical specimens (throat swabs or nasal swabs) collected during the 2004–2005 influenza season that were shown to be virus-positive by rapid diagnostic kits were stored at −80°C until virus isolation. Madin-Darby canine kidney (MDCK) cells (for type A viruses) and MDCK cells overexpressing the β-galactoside α2,6-sialyltransferase I (ST6Gal I) gene were maintained at 37°C in minimal essential medium (MEM) containing 5% newborn calf serum and antibiotics under a 5% CO2 atmosphere and used for viral isolation and plaque assay. MDCK cells overexpressing the ST6Gal I gene support clinical isolates of human influenza viruses better than MDCK cells do [9].

Clinical samples possibly containing influenza viruses were incubated with the MDCK cells overexpressing the ST6Gal I gene for 3 days at 33°C in the presence of tosyl phenylalanine chloromethyl ketone–treated trypsin (0.5 μg/mL) in MEM containing 7.5% bovine serum albumin. They were then cultured in infection medium (7.5% bovine serum albumin, 0.5 μg/mL of trypsin, and 1% agarose in MEM). Viral subtypes were determined by conventional hemagglutinin and neuraminidase inhibition assays.

Sialidase sensitivity to oseltamivir. The sialidase sensitivity of influenza B viruses to oseltamivir was evaluated with a sialidase inhibition assay, as described previously [10]. Briefly, 2′-(4-methylumbelliferyl)-α-D-α-acetyleneuraminic acid (MUNANA; Sigma) at a final concentration of 0.1 mmol/L was used as a substrate. After mixing 10 μL of the virus dilution (pre-determined to contain sialidase activity in the range of 800–1200 fluorescence units in this assay) and 10 μL of the NA inhibitor (0.01 nmol/L to 10 μmol/L) in a calcium and 2-[N-morpholino]ethanesulfonic acid buffer (33 mmol/L MES, 4 mmol/L CaCl2, pH 6.0) and incubating at 37°C for 30 min, 30 μL of the substrate was added. The mixture was further incubated at 37°C for 60 min, and the reaction was stopped by adding 150 μL of 0.1 mol/L NaOH in 80% ethanol (pH,
Informed consent was obtained from children or guardian, and it was recorded in each chart. Informed consent was obtained orally from each child’s parent or guardian, and it was recorded in each chart. Informed consent was also obtained from children >7 years of age who were able to understand the concepts and procedures of the protocol. This study was conducted with the approval of the ethics committees of our hospitals.

RESULTS

Total febrile period. There was a significant difference in the mean duration of fever period between the study patients with influenza A (H3N2) who were treated with oseltamivir and the control subjects (2.19 ± 0.97 days vs. 4.44 ± 1.13 days; P < .001). Control subjects with influenza A who were not treated with oseltamivir were those observed during the 2001–2002 epidemic (n = 9; mean age, 2.60 ± 1.40 years).

There was also a significant difference in the mean duration of the febrile period between the patients treated with oseltamivir and the control subjects with influenza B (2.98 ± 1.47 days vs. 5.55 ± 2.51 days; P < .001). The control subjects with influenza B were those who did not receive oseltamivir during the 2004–2005 epidemic (n = 11; mean age, 3.19 ± 1.64 years).

Duration of fever after the start of oseltamivir therapy. The mean duration of fever after the start of oseltamivir therapy in patients with influenza A (H3N2) was 1.31 ± 0.76 days, and was significantly longer in patients with influenza B (2.18 ± 1.39 days; P < .001). There was a small difference in mean age between the influenza A (H3N2) group and the influenza B group (6.97 vs. 5.16 years; P < .001).

The body temperature of 90.6% of patients with influenza A (H3N2) decreased to a normal level within 2 days after the start of oseltamivir therapy (table 1), as opposed to only 62.2% of the patients with influenza B (P < .001). Many patients with influenza B had a prolonged febrile illness that lasted 3–7 days after the start of oseltamivir. Biphasic fever was observed in 20.4% of the patients with influenza B who were treated with oseltamivir, but in only 3.1% of the patients with influenza A (H3N2) who were treated with oseltamivir (P < .001).

Effectiveness of oseltamivir according to age. Table 2 shows patient age and the duration of fever after the start of oseltamivir therapy. The duration of fever in the patients with influenza B was significantly longer in young children (age range, 1–5 years; mean duration of fever, 2.37 days) than in older children (age range, 6–10 years [mean duration of fever, 1.97 days; P = .013]) and 11–15 years (mean duration of fever, 1.54 days, P = .006). The difference in duration of fever between patients with influenza B who were 6–10 years old and those who were 11–15 years old was not significant (P = .14). By contrast, there were no significant differences in fever duration between age groups of patients with influenza A (H3N2).

Among children 1–5 years old and 6–10 years old, there were significant differences in the duration of fever between the patients with influenza A (H3N2) and the patients with influenza B (1.42 days vs. 2.37 days [P < .001] and 1.23 days vs. 1.97 days [P < .001], respectively) (table 2). By contrast, the difference in fever duration among children 11–15 years old between patients with influenza A (H3N2) and patients with influenza B was not significant (P = .54).

Although the duration of fever after the start of oseltamivir was longer in the influenza B group than in the influenza A (H3N2) group, the difference in children aged 11–15 years was not significant. Lower effectiveness of oseltamivir against influenza B was observed in younger children, especially in the 1–5-year-old age group.

Effectiveness of oseltamivir and history of vaccination. The influenza A/Fujian/411/2002–like strain (the representative strain being influenza A/Wyoming/3/2003, a component of the 2004–2005 vaccine) was the most frequently isolated influenza A (H3N2) virus strain during the first half of the 2004–2005 season. A history of vaccination was confirmed in 102 of the 127 patients with influenza A (H3N2). Fifty-four patients had received influenza vaccine before the epidemic, and 48 had not. There was no significant difference in the duration of fever between patients with influenza A (H3N2) who had been vaccinated and those who had not (1.36 ± 0.70 days vs. 1.36 ± 0.90 days), and there were no significant differences between each age group.

Table 1. Duration of fever after the start of oseltamivir therapy.

<table>
<thead>
<tr>
<th>Days</th>
<th>Influenza A (H3N2)</th>
<th>Influenza B</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>75 (59.1)</td>
<td>118 (32.6)</td>
</tr>
<tr>
<td>2</td>
<td>40 (31.5)</td>
<td>107 (29.6)</td>
</tr>
<tr>
<td>3</td>
<td>9 (7.1)</td>
<td>56 (15.5)</td>
</tr>
<tr>
<td>4</td>
<td>3 (2.4)</td>
<td>48 (13.3)</td>
</tr>
<tr>
<td>5</td>
<td>0 (0)</td>
<td>23 (6.4)</td>
</tr>
<tr>
<td>6</td>
<td>0 (0)</td>
<td>9 (2.5)</td>
</tr>
<tr>
<td>7</td>
<td>0 (0)</td>
<td>1 (0.3)</td>
</tr>
</tbody>
</table>

NOTE. Data are no. (%) of patients.

*Oseltamivir against Influenza B • CID 2007;44 (15 January) • 199*
of vaccination was confirmed in 333 of the 362 patients with influenza B; 139 had received influenza vaccine before the epidemic, and 194 had not. There was no significant difference in the duration of fever after the start of oseltamivir between the vaccinated group and the unvaccinated group (2.14 ± 1.36 days vs 2.25 ± 1.42 days; P = .47). However, in the 1–5-year-old age group, fever duration was significantly shorter in the vaccinated group than in the unvaccinated group (2.14 ± 1.33 vs 2.56 ± 1.54 days; P = .03). Among the older patients (age, 6–15 years), there was no significant difference between the vaccinated group and the unvaccinated group (P = .30). These results show that, in young children (age, 1–5 years) vaccination was synergistically effective with oseltamivir in reducing fever duration in influenza B.

**Virus shedding.** Figure 1 shows changes in virus shedding in the throat after the start of oseltamivir therapy in 18 patients with influenza A (H3N2) (mean age, 5.89 years) and 39 patients with influenza B (mean age, 3.94 years). All patients were treated with a weight-based unit dose of oseltamivir, administered twice daily for 5 days. Before oseltamivir therapy (day 0), mean virus infectivity was similar in the influenza A (H3N2) group and influenza B group (3.53 ± 0.74 [n = 18] and 4.00 ± 1.18 [n = 39], respectively; virus infectivity titers are expressed as log10 plaque-forming units/mL). After the start of oseltamivir therapy, virus titers in the specimens from the patients with influenza A (H3N2) decreased much faster than in the specimens from patients influenza B. Two days after the start of oseltamivir therapy, there was a significant difference in the mean virus titers between influenza A (H3N2) group and influenza B group (0.61 ± 0.91 [n = 14] and 2.84 ± 1.51 [n = 27], respectively; P < .001).

**IC50 of oseltamivir carboxylate.** We tested 192 influenza B viruses that were isolated from the patients described above prior to the initiation of oseltamivir treatment for sensitivity to oseltamivir carboxylate, the active form of oseltamivir phosphate. The mean IC50 of oseltamivir by the sialidase inhibition assay was 75.4 ± 41.7 nmol/L, which is ∼250-fold less susceptible to oseltamivir as influenza A (H3N2) viruses (0.3 ± 0.1 nmol/L) [10]. Three of 192 viruses had IC50 values >200 nmol/L and, thus, may have been resistant to oseltamivir. If so, only 3 (1.6%) of the 192 patients who were tested had been infected by oseltamivir-resistant viruses; a detailed analysis of these oseltamivir-resistant viruses will be reported elsewhere.

Because we suspected that the lower clinical effectiveness of oseltamivir in patients with influenza B reflected decreased sensitivity of the influenza B viruses that were isolated in 2004–2005, we determined the IC50 of earlier influenza B epidemic strains. However, there were no significant differences between the sensitivity to oseltamivir of influenza B isolates in the 2004–2005 epidemic and the earlier influenza B strains. Similarly, there was no significant difference between the Yamagata and the Victoria lineages (figure 2).

![Figure 1](https://academic.oup.com/cid/article-abstract/44/2/197/329747)

**Figure 1.** Changes in virus infectivity after the start of oseltamivir therapy. The results were mean viral titers (± SD) of samples obtained from the throat, before the start of oseltamivir therapy (day 0) and after the start of oseltamivir therapy (days 1–5).

| Table 2. Duration of fever after the start of oseltamivir therapy, by age group |
|-----------------------------|-----------------|-----------------|-----------------|-----------------|
| Type of influenza, patient characteristic | 1–5 years | 6–10 years | 11–15 years | All ages |
| Influenza A (H3N2) | | | | |
| No. of patients | 44 | 60 | 23 | 127 |
| Age, years | 2.95 ± 1.42 | 7.93 ± 1.29 | 12.41 ± 1.32 | 6.97 ± 3.35 |
| Duration of fever after the start of oseltamivir, days | 1.42 ± 0.78 | 1.23 ± 0.82 | 1.30 ± 0.54 | 1.31 ± 0.76 |
| Influenza B | | | | |
| No. of patients | 218 | 116 | 28 | 362 |
| Age, years | 3.21 ± 1.37 | 7.08 ± 1.14 | 12.41 ± 1.31 | 5.16 ± 3.04 |
| Duration of fever after the start of oseltamivir, days | 2.37 ± 1.46 | 1.97 ± 1.28 | 1.54 ± 1.81 | 2.18 ± 1.39 |

**NOTE.** Data are mean ± SD, unless otherwise indicated.
DISCUSSION

Although oseltamivir has been thought to be equally effective against influenza A and B virus infections [11–14], the results of this study show that it is clinically much less effective, on the basis of duration of febrile illness after the start of oseltamivir therapy, against influenza B infection in children than it is against influenza A (H3N2) infection in children. Analyses performed according to age groups demonstrated lower effectiveness in younger children (age, 1–10 years), especially in children aged 1–5 years. There was no significant difference observed in the effectiveness of oseltamivir between patients with influenza A (H3N2) and patients with influenza B in older children (age, 11–15 years).

Lower effectiveness of oseltamivir against influenza B in young children was demonstrated not only by clinical data, but also by virus infectivity data (figure 1). Oseltamivir was not effective against influenza B viral shedding. Our data indicate that young patients with influenza B may be fully infectious until 3 days after the start of oseltamivir therapy.

We suspected that the sensitivity (measured as IC$_{50}$) of influenza B viruses to oseltamivir changed and became much lower during the 2004–2005 influenza season. However, because there were no significant differences in mean IC$_{50}$ values between influenza B viruses isolated from 1994 to 2005 (figure 2), we could not attribute the observed lower clinical effectiveness to a reduced sensitivity (IC$_{50}$) of influenza B viruses to oseltamivir.

Although we identified several influenza B viruses among our isolates that showed high IC$_{50}$ values against oseltamivir, and although some resistant viruses may circulate in the community, the lower clinical effectiveness against influenza B virus infection described here is unlikely to be attributable to virus resistance to the drug, because only 1.6% of the patients who were examined in this study shed viruses with IC$_{50}$ values >200 nmol/L.

Comparison of the effectiveness of oseltamivir against influenza B between age groups revealed lower effectiveness in younger children and no significant difference in the effectiveness of oseltamivir against influenza A and the effectiveness of oseltamivir against influenza B in older children (age, 6–15 years). We observed a phenomenon in which the effectiveness of oseltamivir against influenza B increased with patient age (table 2). Therefore, we believe that the clinical effectiveness of oseltamivir against influenza B is related to patient age or to their immune status.

Recent influenza B epidemics have been caused by influenza viruses of the Yamagata and/or Victoria lineages. The antigenic characteristics of these 2 lineages are totally different, and there is no cross-reactivity between their hemagglutination inhibition antibodies [15]. A mixed influenza B epidemic caused by influenza B/Harbin/07/94 (Yamagata lineage)– and influenza B/Shandon/67/97 (Victoria lineage)–like strains occurred in 1998–1999 [16], and influenza B/Johannesburg/5/99 (Yamagata lineage)–like strain caused a minor epidemic in 2000–2001 [17]. After that time, there were no influenza B epidemics caused by influenza viruses in the Yamagata lineage until the 2004–2005 season. Therefore, when the large influenza B epidemic occurred in the 2004–2005 season that was caused by the influenza B/Shanghai/361/2002 (Yamagata lineage)–like strain [3], most young children 1–5 years old did not have immunity against the epidemic influenza B virus. Perhaps, then, oseltamivir is not fully effective against influenza B in children if they have not immunity against influenza B; this probably reflects the reduction in the sensitivity (IC$_{50}$) of influenza B viruses to oseltamivir, which are ~250-fold less susceptible to oseltamivir than influenza A (H3N2) viruses. One piece of supporting evidence is that, during the 2004–2005 season, vaccination was synergistically effective against influenza B virus infection for reducing fever duration after the start of oseltamivir in young children 1–5 years old.

Although the IC$_{50}$ of influenza B virus to oseltamivir has been reported to be higher than that of influenza A (H3N2) [18–23], the differences have not been large: 0.45 nmol/L versus 8.5 nmol/L [18] and 0.73 nmol/L versus 11.53 nmol/L [19] for influenza type A virus and influenza type B virus, respectively. However, although we followed the method of Gubareva et al. [24] and our IC$_{50}$ values for influenza A viruses were similar, for unknown reasons, our mean IC$_{50}$ value for influenza B viruses was higher than obtained by other investigators [18–23].

The limitations of this study should be recognized. It was an observational—not a randomized—study. Although parents were instructed to administer antipyretics (e.g., acetaminophen) only when their child’s temperature increased to 38.5$^\circ$C, the effect of antipyretic use on fever duration was unknown.

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Figure 2. IC$_{50}$ of oseltamivir for influenza B viruses. Horizontal bars, mean values.
In conclusion, the effectiveness of oseltamivir against influenza B virus infection is influenced by the age or the immunity of the host—like that caused by influenza vaccine [25]—probably because of the low sensitivity of influenza B viruses to oseltamivir. Thus, oseltamivir is much less effective against influenza B virus infection in young children. We should reconsider the use of oseltamivir against influenza B infection in children, especially in young children. Increased dosage of oseltamivir or use of zanamivir are probable options for influenza B virus infection in children.

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References