Limited Inhibitory Effects of Oseltamivir and Zanamivir on Human Sialidases

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Received 11 March 2008/Returned for modification 30 May 2008/Accepted 31 July 2008

Oseltamivir (Tamiflu) and zanamivir (Relenza), two extensively used clinically effective anti-influenza drugs, are viral sialidase (also known as neuraminidase) inhibitors that prevent the release of progeny virions and thereby limit the spread of infection. Recently mortalities and neuropsychiatric events have been reported with the use of oseltamivir, especially in pediatric cases in Japan, suggesting that these drugs might also inhibit endogenous enzymes involved in sialic acid metabolism, including sialidase, sialyltransferase, and CMP-synthase, in addition to their inhibitory effects on the viral sialidase. The possible inhibition could account for some of the rare side effects of oseltamivir. However, there has been little direct evidence in regard to the sensitivities of animal sialidases to these drugs. Here, we examined whether these inhibitors might indeed affect the activities of human sialidases, which differ in primary structures and enzyme properties but possess similar structures similar to those of the viral enzymes. Using recombinant enzymes corresponding to the four human sialidases identified so far, we found that oseltamivir carboxylate scarcely affected the activities of any of the sialidases, even at 1 mM, while zanamivir significantly inhibited the human sialidases NEU3 and NEU2 in the micromolar range ($K_i = 3.7 \pm 0.48$ and $12.9 \pm 0.07 \mu M$, respectively), providing a contrast to the low nanomolar concentrations at which these drugs block the activity of the viral sialidases.

The continuing threat of an influenza pandemic is a serious worldwide concern. For the prevention of influenza, potent and selective anti-influenza drugs have been developed. The currently approved agents include inhibitors of the virus sialidase (8, 25). Viral sialidases are membrane components that destroy the sialic acid-containing receptors on the surfaces of infected cells and are thus involved in the release of newly budded virions from the host cell surface to begin a new round of infection. They may thus play key roles in the spread of the viral infection together with another viral surface glycoprotein, hemagglutinin, involved in the binding of the virus particles to receptors on the host cells. Studies of the crystal structures of some of the viral sialidases have facilitated the rational designing of sialidase inhibitors; the two potent inhibitors, oseltamivir (Tamiflu) and zanamivir (Relenza), are sialic acid analogues that interact with the active sites on the enzymes. Oseltamivir is a prodrug that is metabolized to its active form, oseltamivir carboxylate, after oral administration, while zanamivir is designed for delivery by inhalation. Recently mortalities and neuropsychiatric events have been reported with the use of oseltamivir, especially in pediatric cases in Japan (1, 2, 6, 9, 12). With the drug prescribed frequently for the treatment of influenza in Japan and its consumption accounting for more than 70% of that around the world, Tamiflu has been suspected to cause abnormal behavior and deaths in Japan, particularly in teenagers, although no statistically significant relationship has been established up to the present. Since both of the drugs are targeted against the viral sialidase, the question has arisen as to whether they may also affect the activities of the endogenous sialidases in humans. In fact, there have been a number of observations (5, 10, 18, 29) pointing to the inhibitory effects of oseltamivir on the endogenous sialidases in rats and mice; however, the question remains open, because direct validation of these findings remains limited to one recent report (11) of the effect of the drug against a recombinant sialidase. Under these circumstances, we were prompted to investigate the effects of these drugs on the activities of endogenous human sialidases.

Up to now, four types of human sialidases have been identified and characterized, designated NEU1, NEU2, NEU3, and NEU4 (15). They differ in their subcellular localization and enzymatic properties and in the chromosomal localization of the genes encoding them; the enzymes are expressed in a tissue-specific manner. The major subcellular localizations of NEU1, NEU2, and NEU3 are the lysosomes, cytosol, and plasma membranes, respectively, while NEU4 is localized in the lysosomal lumina or mitochondria and intracellular membranes. We previously discovered (14) that the primary structure of rat cytosolic sialidase, the first example of mammalian sialidase, contain several Asp boxes (Ser-X-Asp-X-Gly-X-Thr-Trp-) and the Arg-Ile-Pro sequence, the conserved sequences found in sialidases from microorganisms (22), despite having no particular similarity to those sialidases. The sequence alignment of other mammalian sialidases successively

Published ahead of print on 11 August 2008.

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cloned revealed that they all contain the conserved sequences. A recent study of the crystal structure of human recombinant NEU2 (3) has provided further evidence of a canonical six-blade beta-propeller structure, as observed for viral sialidases, with the active site in a shallow crevice, but there are some differences from viral and bacterial sialidases in amino acid residues recognizing the N-acetyl and glycerol moieties of 2-deoxy-2,3-dehydro-N-acetylneuraminic acid (NeuAc2en). The lysosomal sialidase NEU1 acts effectively on oligosaccharides, glycopeptides, and a synthetic substrate, 4-methylumbelliferyl N-acetylneuraminic acid (4MU-NeuAc), and NEU3 is a plasma membrane-associated sialidase that almost specifically hydrolyzes gangliosides, while the other two enzymes possess broad substrate specificity, acting on oligosaccharides, glycopeptides, glycoproteins, and gangliosides, as well as on 4MU-NeuAc. Maximal activities are obtained at a pH of about 4.6 for NEU1, NEU3, and NEU4 and at pH 5.5 to 6.5 for NEU2. Sialidases of mammalian origin not only have been implicated in lysosomal catabolism, playing a role as general glycosidases, but are also believed to play roles in the modulation of functional molecules involved in many biological processes, whereas the roles of the sialidases from microorganisms appear to be limited to nutrition and pathogenicity (4). Although many functional aspects of the mammalian sialidases are not fully understood, partly due to the enzyme instability and low activity, recent developments in sialidase research have clarified their important biological roles, including their roles in events involved in cell differentiation, cell growth and apoptosis, and malignant transformation (16).

As described above, human sialidases, while differing from the viral enzymes in their primary structures and enzyme properties, show tertiary structures and active-site amino acids similar to those of the viral sialidases. Therefore, in this study, we examined whether the antiviral drugs might have effects on any of the four types of human recombinant sialidases.

MATERIALS AND METHODS

Cell culture and sialidase transfection. Human kidney 293T cells (Riken BRC Cell Bank, Tsukuba, Japan) were maintained in minimal essential medium supplemented with nonessential amino acids and 10% heat-inactivated fetal bovine serum. Cell Bank, Tsukuba, Japan) were maintained in minimal essential medium supplemented with nonessential amino acids and 10% heat-inactivated fetal bovine serum.

Preparation and purification of the recombinant sialidases. The cells (2 × 10^7) transfected with FLAG-tagged sialidase cDNA as described above were collected, washed with phosphate-buffered saline, and sonicated on ice in 9 volumes of ice-cold lysis buffer. The lysates were centrifuged at 1,000 g for 10 min at 4°C, and the resultant supernatants (homogenates) were then used for measurement of the sialidase activity or for further purification. The lysis buffer A for NEU1 and NEU2 contained 20 mM potassium phosphate (pH 6.8), 0.15 M NaCl, 1 mM phenylmethylsulfonyl fluoride, and protease inhibitor cocktail (Roche), and the lysis buffer B for NEU3 and NEU4 was buffer A containing 1 mM EDTA and 1% Triton X-100. Purification of the recombinant sialidase proteins was performed using FLAG tag affinity chromatography as follows: NEU2 was purified from the cytosolic fraction after centrifugation of the homogenates at 100,000 × g for 1 h, followed by affinity chromatography. The cytosolic fraction of the cells was applied to an anti-FLAG M2 agarose column (1 ml) (Sigma), washed with 20 ml of lysis buffer A and successively with 10 ml of the buffer containing 1 M NaCl, and eluted with buffer A containing the FLAG peptides (100 μg/ml) and 10% glycerol. For NEU3 purification, the solubilized fraction after centrifugation at 20,000 × g for 15 min was applied to the column and then washed with lysis buffer containing 0.1% Triton X-100 (buffer C), followed by buffer containing 1 M NaCl, and then eluted with buffer C plus FLAG peptides and 10% glycerol.

Sialidase activity assays. The homogenates or purified fractions obtained above were used for measurement of sialidase activity using the synthetic substrate 4MU-NeuAc or ganglioside GM3 (NeuAca2-3Galβ1-4Glcβ1-1Cer) (Alexis Biochemicals). The activities for NEU1, NEU2, and NEU4 were measured routinely in 0.1 ml of a reaction mixture containing 10 μmol sodium acetate buffer (pH 4.6 for NEU1 and NEU4, pH 5.5 for NEU2) and 4MU-NeuAc, 0.1 mg bovine serum albumin, and enzyme. After incubation for 15 to 30 min at 37°C, the 4-methylumbelliferone released was determined fluorometrically (14). The reaction mixture for NEU3 activity contained 5 μmol sodium acetate buffer (pH 4.5), 10 nmol gangliosides GM3, 50 μM Triton X-100, 50 μg bovine serum albumin, and enzyme in 50 μl and was incubated for 20 to 30 min at 37°C. The sialic acids released from GM3 were measured by fluorometric high-performance liquid chromatography with malononitrile (27). Occasionally, NEU4 activity was also assayed with GM3 as the substrate in the same manner. Protein concentrations were determined by dye-binding assay (Bio-Rad Laboratories). One unit was defined as the amount of enzyme cleaving 1 nmol of sialic acid/TLC. After 48 h of transfection, the cells were cultured in serum-depleted medium for 5 h, followed by treatment of each drug for a further 5 h, and the cells were collected and subjected to thin-layer chromatography (TLC). Glycolipids were extracted from the cells (1 × 10^7) in sequence, with 2 ml of isopropanol-hexane-water (55:25:20 [vol/vol/vol]) and hydrolyzed with 0.1 M NaOH-methanol. After desalting was done using a Sep-Pak C18 cartridge, total lipid extracts were fractionated by TLC on high-performance TLC plates (Baker, Phillipsburg, NJ) in C-M-0.5% CaCl2 (60:40:9 [vol/vol/vol]) and visualized with orcinol-H2SO4. Densitometric analyses were performed using the Scion Image (Scion Corp., Frederick, MD) and Quantity One (Bio-Rad Laboratories) software programs.

Lectin blotting. To observe the changes in the amounts of the endogenous glycoproteins, lectin blot analysis was conducted with peanut agglutinin (PNA) and Ricinus communis agglutinin (RCA) (Honen, Tokyo, Japan). After 48 h of transfection, the cells were cultured in serum-depleted medium for 5 h, followed by treatment of each drug for a further 5 h, and the cells were collected and subjected to lectin blotting. Cell homogenates were resolved by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and transferred to polyvinylidene difluoride membranes. The blots were washed in a solution containing 10 mM Tris-HCl, pH 7.5, 100 mM NaCl, and 0.1% Tween 20 and incubated with the solution containing biotinylated lectins. After washing, the lectin-bound glycoproteins were visualized with horseradish peroxidase-streptavidin (Vector, Burlingame, CA) and A.

Inhibition assay of influenza virus sialidase activity. Inhibitory activity against the influenza sialidase activity was assessed using a fluorometric method with slight modification (21). Influenza viruses from human and animal isolates were propagated in allantoic cavities of 11-day-old chicken eggs for 48 h at 35°C and
purified by sucrose density gradient centrifugation as described previously (24). To obtain an appropriate virus concentration for use in the assay, the sialidase activity of each virus was first determined in a total volume of 10 μl containing 10 mM acetate buffer (pH 6.0), 80 μM 4MU-NeuAc substrate, and various concentrations of influenza virus solution. After incubation for 30 min at 37°C, the reaction was stopped and 100 μl of the solution was transferred to a 96-well black plastic plate, and the fluorescence was monitored. For the inhibition assay, 4 μl of each dilution of each inhibitor was preincubated with 4 μl of influenza virus solution for 1 h at 4°C before the addition of 2 μl of 400 μM 4MU-NeuAc substrate in 10 mM acetate buffer (pH 6.0) to begin the reaction.

RESULTS

Inhibition assays of human sialidase activities for oseltamivir and zanamivir. Our previous results from quantitative real-time PCR indicated that the expressions of NEU1, NEU3, and NEU4 are readily detectable but expression of NEU2 is hardly detectable in human tissues (30). To determine whether NEU2 is actually expressed in humans, we attempted to clone the cDNA, because NEU2 has been characterized only using a genomic clone constructed by ligation of two genomic fragment sequences (17). A NEU2 cDNA clone was obtained from a cDNA synthesized from poly(A)+ RNA from the human brain as the template by PCR and was employed for preparation of the NEU2 enzyme protein. Consistent with our previous data, the expression levels of four sialidases were found to be markedly different. The endogenous levels of four sialidases were compared with one another as they were measured quantitatively using standard curves of respective cDNAs; those from the brain and lung are shown in Fig. 1. NEU1, known as a target gene for sialidosis, was expressed at the highest level, followed by NEU3 and NEU4, which were generally expressed at 1/10 or 1/20 of the level of NEU1. Expression of NEU2 was observed at extremely low levels, being at most only 1/4,000 to 1/10,000 that of NEU1 in several tissues.

To obtain preparations of all four enzymes, we transiently transfected the respective expression plasmids into 293T cells. The cell homogenates and the enzymes, highly purified by FLAG peptide elution through FLAG affinity chromatography (Fig. 2A), were assayed for sialidase activity in the presence of oseltamivir phosphate, oseltamivir carboxylate (Toronto Research Chemicals Inc., ON, Canada), or zanamivir (26). Considering the substrate specificity, the substrates exogenously added routinely were 4MU-NeuAc for NEU1, NEU2, and NEU4 and the ganglioside GM3 for NEU3, because the former three can preferentially hydrolyze 4MU-NeuAc and NEU3 acts almost specifically on gangliosides (15). Due to the broad specificity of NEU4, the activity was also assayed here with GM3 and 4MU-NeuAc to exclude the effects of differences in the substrate. After 15 to 30 min of incubation, the released 4MU and sialic acids were determined using a fluorometer and using fluorometric high-performance liquid chromatography, respectively. The 50% inhibitory concentration (IC50) of each compound was calculated by plotting the decrease in activity against the log of the agent concentration. Oseltamivir phosphate did not affect any of the human sialidases (data not shown), since the prodrug oseltamivir is expected to be ineffective in vitro, and oseltamivir carboxylate also showed no appreciable inhibition of the sialidases even at the concentration of 1 mM (Table 1). Only the activity of NEU2 appeared to be inhibited, but with a very high IC50. In contrast, oseltamivir carboxylate was fully active against the influenza virus sialidases, with IC50s in the nanomolar range under the conditions described in Materials and Methods. In addition, a nonselective sialidase inhibitor, NeuAc2en, showed clear inhibition in the micromolar range. While zanamivir did cause substantial inhibition of the human sialidases, the effects were much less marked than those in testing against the viral enzymes. NEU2 and NEU3 seemed to be more susceptible than the other two sialidases to zanamivir. Interestingly, the IC50 was the lowest for NEU3, which is expressed abundantly in the brain and is colocalized in the plasma membrane with its substrate gangliosides. The Kmin and K values of the compounds for human sialidases were then compared using the homogenates (Table 2). Zanamivir was less inhibitory against NEU1 and NEU4, with K values approximately 300- and 20-fold higher, respectively.
FIG. 2. The effects of oseltamivir carboxylate and zanamivir on sialidase activities. (A) After SDS-PAGE of NEU2 (left) and NEU3 (right) enzymes purified by FLAG affinity chromatography, protein staining, Coomassie brilliant blue (CBB) for NEU2 and silver staining for NEU3, and Western blotting (WB) were performed to evaluate the degree of purification. The enzymes were assayed with increasing concentrations of 4MU-NeuAc or GM3 as a substrate with or without zanamivir (B) or oseltamivir carboxylate (C). The $K_m$ and $K_i$ values were calculated from these results.
2.4/s for NEU3. The $K_i$ for NEU4. In the experiments with human sialidases, each value for zanamivir or NeuAc2en represents the mean ± SD of three or four experiments. For virus sialidase, each value was obtained by performing two experiments. ND, not determined. The $K_i$ values measured with purified enzymes are described in the text.

### TABLE 1. IC$_{50}$s of oseltamivir carboxylate, zanamivir, and NeuAc2en for activities of human and influenza virus sialidases

<table>
<thead>
<tr>
<th>Sialidase</th>
<th>Oseltamivir carboxylate</th>
<th>Zanamivir with:</th>
<th>NeuAc2en with:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>4MU-NeuAc</td>
<td>GM3</td>
<td>4MU-NeuAc</td>
</tr>
<tr>
<td><strong>Human sialidases</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NEU1</td>
<td>&gt;10,000 μM</td>
<td>2,713 ± 239 μM</td>
<td>ND</td>
</tr>
<tr>
<td>NEU2</td>
<td>&gt;6,000 μM</td>
<td>16.4 ± 20 μM</td>
<td>ND</td>
</tr>
<tr>
<td>NEU3</td>
<td>&gt;10,000 μM</td>
<td>6.8 ± 3.1 μM</td>
<td>ND</td>
</tr>
<tr>
<td>NEU4</td>
<td>&gt;10,000 μM</td>
<td>600 ± 127 μM</td>
<td>ND</td>
</tr>
<tr>
<td><strong>Sialidases from:</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A/PR/8/34 virus (H1N1)</td>
<td>7.09 nM</td>
<td>1.56 nM</td>
<td>ND</td>
</tr>
<tr>
<td>A/Aichi2/68 virus (H3N2)</td>
<td>1.55 nM</td>
<td>2.66 nM</td>
<td>ND</td>
</tr>
<tr>
<td>A/DK/HK/313/78 virus (H5N3)</td>
<td>4.95 nM</td>
<td>3.97 nM</td>
<td>ND</td>
</tr>
</tbody>
</table>

$^a$ NEU3 was evaluated using the ganglioside GM3 as a substrate because of its strict preference, and in addition to GM3, 4MU-NeuAc was used as another substrate for NEU4. In the experiments with human sialidases, each value for zanamivir or NeuAc2en represents the mean ± SD of three or four experiments. For virus sialidase, each value was obtained by performing two experiments. ND, not determined. The $K_m$ values measured with purified enzymes are described in the text.

**Effects of oseltamivir and zanamivir on desialylation of endogenous substrates in cells.** In addition to the inhibition test with an exogenously added substrate in the assay tube described above, we examined whether desialylation of endogenous substrates by NEU2 or NEU3 was blocked in the presence of these drugs in the cell culture. The homogenates of sialidase transfected and mock-transfected cells were subjected to lectin blotting or TLC to analyze the changes in the amounts of glycoproteins or gangliosides, respectively. As shown in Fig. 3, the significant changes were observed in the transfected cells compared with those in the control cells. A marked increase in several bands of RCA and PNA lectins, recognizing Gal-GlcNAc and Gal-GalNAc, respectively, was observed in the NEU2-transfected cells. Considering that the increase in the intensities of the glycoprotein bands occurred as a result of hydrolysis by the transfected sialidase, it is in agreement with substrate preference of NEU2. However, no significant changes were observed in the intensities of the corresponding protein bands (Fig. 3A), either after the addition of oseltamivir phosphate or after the addition of oseltamivir carboxylate at a concentration of 500 μM, as expected from the results of the activity assays. Although zanamivir at this concentration was effective in the activity assays, as shown in Tables 1 and 2, the drug did not seem to inhibit NEU2-mediated hydrolysis of endogenous glycoproteins. This may not be contradictory, because the activity of the drug is known to be extracellular and the drug does not undergo metabolism. In line with the substrate preference of NEU3 for gangliosides, the glycoprotein patterns were altered by NEU3 transfection, especially in a marked decrease in GM3 amounts compared with that in the mock-transfected cells (Fig. 3B). The reduction in the GM3 hydrolyzing activity was scarcely detected in treatment with oseltamivir phosphate or in that with oseltamivir carboxylate, while zanamivir significantly abrogated GM3 reduction by the NEU3 enzyme. The zanamivir effects may be reasonable, because NEU3 is localized in cell surface membranes. The cells did not show any morphological changes after the addition of these drugs to the culture. These results suggest that oseltamivir at this concentration inhibits neither the hydrolytic reac-

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$^a$ Each value represents the mean ± standard deviation of three or four experiments. The ganglioside GM3 was used as a substrate for NEU3 because of its strict preference. ND, not determined. The $K_m$ and $K_i$ values measured with purified enzymes are described in the text.
FIG. 3. Effects of oseltamivir and zanamivir on desialylation of endogenous substrates of 293T cells. (A) Alterations of glycoproteins by transfection of NEU2 into 293T cells were observed by PNA and RCA lectin blotting. Intensities of several protein bands (right panel) indicated were markedly increased in the NEU2-transfected cells compared to the mock-transfected cells, but no significant changes were observed in the NEU2-transfected cells after treatment with any of the drugs at the concentration of 500 μM. (B) The glycolipids were examined by TLC in the NEU3-transfected cells. The transfected cells showed a marked decrease in GM3 amounts compared to mock-transfected cells. Neither addition of oseltamivir phosphate nor that of oseltamivir carboxylate changed the patterns, while zanamivir inhibited GM3 hydrolysis by NEU3. The relative intensities of the bands corresponding to GM3 in TLC are shown in the right panel.
tions of the sialidases inside the cells nor the sialidase activity following exogenous addition of substrates.

**DISCUSSION**

In the present study, we explored the inhibitory effects of oseltamivir and zanamivir on four human sialidases using their recombinant proteins. The effects were examined by two methods, namely, measurement of the inhibitory activity in sialidase activity assays and estimation of the reduced desialylation of endogenous glycophorin and glycolipid substrates inside the cells. Recent observations have suggested a possible inhibitory potential of oseltamivir against endogenous sialidases. In mice, the drug decreased the GM1 of activated CD8+ T cells infected with a respiratory syncytial virus (18) and also blocked GM1-mediated opioid hyperalgesia induced by low doses of morphine (5). In rats, it exhibited neuroexcitatory effects, especially under conditions of simultaneous administration of ethanol (10). With these animal models, however, no direct inhibitory effects of the drugs on endogenous sialidases could be shown. We do not yet know the molecular mechanisms underlying these phenomena at present, but our data suggest that they may not be the direct consequences of sialidase inhibition. Decreased GM1 may not always be due to sialidase inhibition and may also involve other processes, such as disturbance of the ganglioside synthetic pathway. In a report on the effects of the drugs on the sialidases of PC12 cells, Tamifu was found to inhibit the sialidase activity induced by nerve growth factor-dependent Trk receptor activation (29). Although we tried to examine the inhibitory effects under the same experimental conditions and confirmed induction of sialidase activity toward 4MU-NeuAc in a nerve growth factor-dependent manner, we were not able to detect any inhibition of the activity in the cells at a Tamifu dose of 1 mM. This ineffectiveness of Tamifu is probably due to the absence or low expression of carboxypeptidase 1 (23) and P-glycoprotein (19) in the cells, which are involved in activation and transport of the prodrug oseltamivir, respectively. Studies using recombinant NEU2 sialidase (11) have shown that a variant with a nonsynonymous single nucleotide polymorphism frequently observed in some Asian populations is associated with lower sialidase activity and higher sensitivity to oseltamivir than the wild-type enzyme. This is of particular interest in the context of the frequent occurrence of abnormal behavior and deaths associated with Tamifu use in Japan. However, in addition to the extremely low expression of NEU2 in human tissues, including the brain, as shown in Fig. 1, our present data demonstrate that very high concentrations of oseltamivir carboxylate are needed to inhibit the sialidase activity, which is 14- to 20-fold higher than the concentration reported by Li et al. (11). The other sialidases were not susceptible to the drug, even in the millimolar range, despite the drug inhibiting viral sialidases in the nanomolar range. In contrast, zanamivir inhibited NEU3 at micromolar levels. In consistency with this, Nan et al. (20) reported that less than 1 mM zanamivir inhibited the endogenous sialidase activity of human T lymphocytes, although no information is available with regard to the type of sialidase involved in this phenomenon.

Taken together, the data indicate that oseltamivir carboxylate does not significantly inhibit constitutively expressed NEU1, NEU3, or NEU4 human sialidases while zanamivir exhibits inhibitory effects in the micromolar range against NEU3 as the most sensitive form. We cannot arrive at any firm conclusions at present regarding the potential association between Tamifu and the abnormal behavior and deaths reported among Japanese teenagers with influenza virus infection treated with the drug, but our results do indicate that the drug might not exert any direct effects on the ganglioside-specific plasma membrane-associated sialidase NEU3, expressed abundantly in the brain. The present results were obtained with only recombinant enzymes, and we also need to stress that the observed effects might not directly reflect the effects that might be observed under physiological conditions inside the cells. Since the plasma concentration of oseltamivir carboxylate is reported to be 1.2 μM following oral administration of the 75-mg capsule twice daily to patients (http://www.rocheusa.com/products/tamiflu/), endogenous sialidase is unlikely to be a direct target molecule. In the case of zanamivir, the plasma concentration is observed to be 0.05 to 0.43 μM within 1 to 2 h following administration of a 10-mg dose (http://us.gsk.com/products/assets/us_relenza.pdf). Our results also suggest negligible effects, although it should be borne in mind that the concentration in the respiratory tract after inhalation may be much higher than that achieved in the blood. In conclusion, it may be desirable to examine newly discovered drugs targeting viral sialidases for their effects on endogenous human sialidases, in order to minimize potential side effects in patients.

**REFERENCES**


