Introduction

Neuraminidase inhibitors (NIs) are expected to reduce complications of influenza, especially in persons at higher risk for influenza complications,[1,2] and oseltamivir is included in the Model List of Essential Medicines.[3] Persons at higher risk include those with diabetes, neuropsychiatric illnesses, and respiratory, cardiac, renal, hepatic, or haematological diseases.[1,2] However, serious neuropsychiatric adverse reactions to oseltamivir, including sudden deaths and abnormal behaviours leading to accidental death, have been reported since the drug was introduced into medicine.[4–8] In Japan, oseltamivir has been contraindicated in principle for children and adolescents aged 10 to 19 years since 2007 due to concern about the risk of abnormal behaviours.[4–7] Adverse reactions to oseltamivir include sudden and delayed onset types and others.[6,7] Sudden death is another concern about oseltamivir use.[5–8]

The Cochrane’s neuraminidase inhibitors team showed in its latest review[9] that neuraminidase inhibitors (NIs), including oseltamivir and zanamivir, have symptom-relieving effects in adults, but it is unclear whether the same effects occur in children. Oseltamivir reduced a four-fold increase in antibody titre.[9,10] Evidence of reduction in hospitalization was not shown for either NI. Oseltamivir did not reduce complications, including pneumonia classified as serious or which led to study withdrawal. Both NIs reduced “laboratory confirmed symptomatic influenza infection,” but not influenza-like illness. Oseltamivir increased risk of nausea, vomiting, headache, psychiatric, renal, and diabetic/hyperglycaemic events, and pain in limbs, but zanamivir did not. Oseltamivir reduced diarrhea and apparent cardiac events, but induced prolonged QTc time (Bazett correction).[9]

This review discusses the biological base of adverse reactions, in particular, those of the delayed onset type.

Brief description of neuraminidase inhibitors

Neuraminidase inhibitors and medical use

Neuraminidase inhibitors (NIs) comprise inhaled zanamivir (Relenza, GlaxoSmithKline), oral oseltamivir (Tamiflu, Gilead Sciences, F. Hoffman-La Roche and Chugai in Japan), parenteral peramivir [11] (BioCryst Ltd and Shionogi), inhaled laninamivir (Daichi Sankyo Co. Ltd),[12] and others still under development.[13] Zanamivir and oseltamivir have been marketed in the US since 1999, and subsequently in other countries including Japan. Peramivir and laninamivir were put on the market in Japan in January and September 2010, respectively.
F. Hoffman-La Roche (Roche) donated oseltamivir to the World Health Organization (WHO), and the WHO decided to recommend stockpiling neuraminidase inhibitors (especially oseltamivir) for use in the event of a pandemic. Prior to the emergence of influenza A/H1N1 in 2009, governments worldwide stockpiled 220 million treatments of oseltamivir, swelling cumulative sales since the start of 2003 to CHF 7.6 billion. The use of NIs has increased dramatically since the outbreak of A/H1N1 in April 2009.

Between the first approval in the US and the 2006/2007 season, approximately 48 million patients received a prescription for oseltamivir worldwide, while 4 million patients received a prescription for zanamivir. Japan, the US, and the other countries consumed 76%, 21%, and 3% of the world total of oseltamivir, respectively. For children (suspension), 85% of the world total was consumed in Japan. Zanamivir was rarely prescribed (less than 1% of total antiviral prescriptions for influenza) in Japan before the Japanese Ministry of Health, Labour and Welfare (MHLW) contraindicated oseltamivir use in patients aged 10 to 19 years in March 2007. After this restriction, prescriptions of oseltamivir decreased substantially. After laninamivir was put on the market in Japan in September 2010, its share increased rapidly. In the 2013/14 season, the prescription shares of oseltamivir, zanamivir, laninamivir, and peramivir were 36%, 19%, 42%, and 3% in Japan, respectively.

Mode of action and pharmacokinetics in non-infectious state

Although NIs may reduce the ability of the virus to penetrate the mucus in the very early stage of infection, their main mechanism of action is thought to lie in their ability to interfere with the release and spread of progeny influenza virus from infected host cells by inhibiting neuraminidase of influenza viruses. It is generally believed that oseltamivir most likely reduces symptom duration by reducing viral load, and via the spread and release of cytokines. However, the full prescription information of oseltamivir (revised in April 2010) states “The concentrations of oseltamivir carboxylate required for inhibition of influenza virus in cell culture were highly variable depending on the assay method used and the virus tested. (…) The relationship between the antiviral activity in cell culture, inhibitory activity in the neuraminidase assay, and the inhibition of influenza virus replication in humans has not been established.” It is not clearly stated that the mechanisms of symptom relief are derived from the reduction of viral load.

Oseltamivir phosphate (OP) is an ethyl ester prodrug that requires ester hydrolysis for its conversion to the active form of the neuraminidase inhibitor, oseltamivir carboxylate (OC). The brand name drug of OP capsule (Tamiflu capsule) contains 75 mg of oseltamivir expressed as free base (OT), which is compatible with 98.6 mg of OP. OP dissociates in the gastrointestinal tract to form OT, which is absorbed and metabolized into OC by hepatic carboxylesterase (hCE).

In healthy volunteers, the area under the curve (AUC) of OP is 3 to 5% that of OC. The penetration of OP across the blood–brain barrier (BBB) is restricted (less than 10%) by P-glycoprotein (P-gp) in mature and non-infected animals.

When healthy adult volunteers were administered with 75 mg of OT (equivalent to 98.6 mg of OP), approximate average Cmax (ng/mL), Tmax (h), AUC (ng.h/mL), and elimination half-life (t1/2:1,h) of OP were as follows, respectively: 60 ng/mL, 0.7–2 h, 150–200 ng.h/mL, and 1.2–1.9 h. For OC, they were 200–300 ng/mL, 4–5 h, 3000–4000 ng.h/mL, and 5–10 h. The pharmacokinetic (PK) parameters in healthy children aged 3 years or older are not very different from those in adults. In the 5 patients with decreased creatinine clearance (<30 mL/min), after 6 days administration of 100 mg of OT (as free form), PK parameters (±SD) of OC were as follows: Cmax: 4052 (±1519) ng/mL, Tmax: 5.20 (±1.11), AUC0–12: 43086 ng.h/mL (±18068), and t1/2: 16.1 (±2.69).

In the 12 patients who were haemo-dialyzed, PK parameters (±SD) after 75 mg of OT were as follows: Cmax: 2131 (±533) ng/mL, Tmax: 27.3 (±6.9), AUC0–last: 106314 (±26.029) ng.h/mL, and t1/2: 159 (these data were calculated before the next dialysis, 48 h after the previous dialysis; SD was not given). Thus, almost all OC is secreted in urine, and dose adjustment is necessary if the patients’ creatinine clearance is less than 30 mL/min.

Evidence on adverse reactions to oseltamivir in humans and in animals

To date, it is postulated that adverse effects of oseltamivir include sudden onset type, delayed onset type, and others. This chapter mainly summarizes the delayed onset type adverse reactions to oseltamivir reported in the clinical trials and epidemiological studies, which may help to explain possible adverse effects of other neuraminidase inhibitors. A very brief review of the sudden onset type neuropsychiatric adverse effects of oseltamivir is also provided.

Sudden onset type neuropsychiatric reactions

The sudden onset type reactions include nausea, vomiting, and hypothermia, as well as neuropsychiatric reactions such as abnormal behaviours, hallucination, and sudden respiratory arrest followed by cardiac arrest and death. These appear very shortly (from less than 1 h to 24 h at most) after the first dose of oseltamivir, and disappear rapidly unless they induce respiratory arrest and sudden death, or sequelae. They may disappear even if oseltamivir is continuously taken, although symptoms may reappear if the drug is taken several times. The underlying mechanisms of sudden-onset type reactions are discussed elsewhere.

Delayed onset type reactions with prolonged time for recovery

Delayed onset type reactions include disorders of various organs and systems such as renal, metabolic, cardiac, hepatic, haematological, immune, nervous, psychiatric, and general systems (fatigue or malaise). Most of the reactions of
this type appear at least a few days after commencement of oseltamivir intake, although QTc prolongation is closely related to the plasma concentration of oseltamivir carboxylate, even at first dose (details described below). Duration of symptoms from these reactions, especially neuropsychiatric ones, tends to be prolonged: for months or even years.[6]

**Inhibition of antibody production and reinfection**

Oseltamivir significantly reduced the odds of patients having a 4-fold antibody rise, by almost 20% (risk ratio by 8%), according to a meta-analysis of 8 reports (10 studies).[9,10] Heterogeneity was not significant ($I^2=4\%$).

Attenuation of secretory IgA (sIgA) was more marked.[32–35] Sawabuchi et al. reported that lower induction of sIgA against the influenza A virus was observed in children treated with oseltamivir in comparison with children treated without oseltamivir. The odds of a child’s sIgA level increasing more than 10-fold were non-significantly lower in children treated with oseltamivir in comparison with children treated without oseltamivir. The odds of a child’s sIgA level increasing more than 10-fold were non-significantly lower in children treated with oseltamivir (2/12) than in children without oseltamivir (3/3); odds ratio is 0.17 (95%CI: 0.01, 2.39, $p=0.13$) (calculated from the data shown in the Figure 1 of Ref. [32]).

Anti-influenza A virus sIgA attenuation was observed in children treated both with oseltamivir or with zanamivir.[33] These findings are consistent with evidence from animal tests using sub-clinical doses of oseltamivir in influenza A/ H1N1 infected mice.[34,35] Non-significant slight reduction of haemagglutinin (HA) specific IgG antibody in the serum and spleen was reported, while HA specific secretory IgA antibody (sIgA Ab) in nasal wash and bronchoalveolar fluids (BALF) was significantly reduced: by approximately 80% on day 12.[34]

In human clinical trials, zanamivir at the usual dosage did not reduce antibody (anti-HA Ab) production,[9] but attenuated sIgA antibody significantly.[33] In a double blind, placebo controlled trial with healthy volunteers designed to investigate the effect of zanamivir treatment (20 mg/day for 14 days) on the humoral immune response to influenza A/ H1N1 infected mice.[34,35] Levels of pro-inflammatory cytokines including IL-6, TNF-$\alpha$, IFN-$\gamma$, and other chemokines were almost completely suppressed in the viral challenge randomized controlled trial (RCT) using a very high dose (600 mg) of intravenous zanamivir before inoculation of influenza virus in human adults.[37]

Shinahara et al. [33] reported: “Even under the spread of a new virus subtype in 2009/2010, only 8.6% of the children of the no-treatment group were re-infected. However, the proportions of children treated the previous year with oseltamivir and zanamivir who developed re-infection in 2009–2010 were significantly higher at 37.3% and 45.0%, respectively ($p<0.01$), than those of the no-treatment group.”

Several cases of re-infection with the same influenza virus within one season were reported.[38,39] Kopel et al. [38] reported a 13-year-old boy with cerebral palsy who three times had episodes of fevering with positive 2009A/H1N1 influenza detected by the RT-PCR method. He was treated with the standard dose (75mg b.i.d for 5 days) of oseltamivir at the time of the first episode. A second course of oseltamivir was administered for 10 days with the dosage adjusted for age and doubled from that of the previous regimen. His HI titres were high, but the level of secretory IgA was not determined.

**Renal impairment**

Dose-related histological change in the renal tubules, increased urine volume, and increased relative weights of the liver and kidneys were observed at various doses in various animals treated with oseltamivir for various periods.[29]

For example, in the 6-month rat toxicity test, renal weights (both relative and absolute) increased, and histological examination revealed degenerating and regenerating changes in the renal tubular epithelia, basement membranes, and Bowman capsules; vacuolization in the renal tubular epithelia; and mineralization in the highest dose group (761 mg/kg). These histopathological findings were not reversed at 8 weeks after cessation of oseltamivir intake. In the medium-dose group (152 mg/kg), renal relative and absolute weights increased, and vacuolization in the renal tubular epithelia was observed.[29] According to the “Guidance for Industry” [40] for a person weighing 60 kg, conversion factors for Human Equivalent Dose (HED) for mouse, rat, ferret, marmoset monkey, rabbit, and dog are 12.3, 6.2, 5.3, 6.2, 3.1, and 1.8, respectively. Hence 152 mg/kg in rat is 24.5 mg/kg expressed by HED, NOAEL for renal impairment was 38 mg/kg. HED for 38 mg/kg is 6.1 mg/kg converted by body surface area and 5.5 mg/kg converted by AUC level of OC. These are 2.4 and 2.2 times higher than the usual dose for a person weighing 60 kg. Renal impairment in the highest-dose oseltamivir group was accompanied by increased water intake, increased leukocyte count, and increased bilirubin, BUN, creatinine, urine volume, and NAG/creatinine ratio.

**Metabolic disorders: hyperglycaemia and diabetes**

Increased glucose level in the highest dose group (1522 mg/kg) was observed in a rat oral 2-week test. The data were not described, but this increased glucose level was accompanied by increased leukocyte count, dose-related significant increase of BUN ($p<0.01$), increased weights of the kidney and the liver, mineralization of tubules of renal medulla (male: 8/10), and mild-to-moderate accumulation of lung alveolar macrophages.[29]

**Cardiac disorders: bradycardia and QT prolongation**

Oseltamivir decreased heart rate in the 9-month repeated toxicity test using marmoset monkeys. The average heart rate during treatment period with oseltamivir was 328 beats/min, which was an 11% and 16% decrease compared with the control group (368 beat/min) and the average of baseline and recovery phase (392 beat/min), respectively.[29]

In an experiment using beagle dogs to test the effects on cardiac functions such as QT time,[41] mean baseline QTc intervals (msec ±SE) were 417±16 in the control (vehicle) group ($n=4$) and 374±2 in the oseltamivir carboxylate (OC)
Delayed onset and prolonged type psychiatric symptoms

The present author reported the following case of psychiatric reaction.[6] A 15-year-old junior high school boy with a body temperature of 39.2°C due to influenza B diagnosed by rapid testing took Tamiflu 75 mg b.i.d. for 5 days. His body temperature was normalized on day 5, but he felt lethargic. After he took the last (10th) dose of Tamiflu in the morning on day 6, he went to school, sat on his desk, and began to sing loudly during a lesson. He could not communicate with his classmates. He seemed to be delirious. Four days after this episode, his parents took him to a general hospital. Before admission, he commented, “There are insects on my mask,” a sign of visual hallucinations. After admission, he tried to pull out his venous lines and attempted to go home, shouting “This is not a hospital but a nursing home for elderly people.” He could not wait until his turn for examination and rushed out of the hospital into the street, where he narrowly avoided being run over by a car. On day 16 he was discharged, and on days 19 and 20 he came to know that he had behaved abnormally from records on his mobile phone. It took 13 days after the beginning of abnormal behaviour for his psychiatric symptoms to be completely resolved.[6]

Among the four study reports of prophylaxis RCT analyzed in the systematic review,[9] the case of longest recovery time (longer) was reported in the oseltamivir and placebo groups, respectively. The pooled odds ratio was 3.37 (95% CI: 1.11–10.23, p = 0.032, I² = 0%).

No animal study was performed to confirm the association of psychotic reactions of delayed onset with prolonged recovery.

Other adverse effects (pneumonia, wheezing, gastric bleeding, and others)

Three of the six rats treated with intravenous OC (at 12 times higher level than clinical area under the curve) for 2 weeks developed acute alveolitis.[29] Of the 3, 1 exhibited wheezing on day 14 and was sacrificed the next day. Diffuse haemorrhagic alveolitis (pneumonia) and pulmonary microvascular thromboembolism were observed in this animal. The safe level of intravenous OC dose is lower than twice the AUC of the usual clinical human dose.

In the marmoset monkey 7-day oral toxicity studies,[29] all four animals treated with a 127-times-higher-than-HED dose of OT were sacrificed within 4 days (1 on day 2 and 3 on day 4) because they were near death after severe vomiting, sleep, hypoactivity and collapse. Macroscopic reddening of the stomach mucosa and histologically mucosal bleeding with erosions, ulcers, and atrophy were observed in the stomachs of all the animals.[29]

The safety index (animal AUC0–24 with no toxicity by human average AUC0–24 when taking 75 mg capsule b.i.d.) is 3 for the 4-week toxicity studies in rats, 3 for the 6-month oral toxicity studies in rats, 8 for the 2-week oral toxicity study in rats, and 10 for the marmoset monkey 7-day oral toxicity study.

Potential adverse reactions to oseltamivir and to other neuraminidase inhibitors

Potential adverse reactions to oseltamivir and to the other neuraminidase inhibitors are summarized in Table 1.

Mechanisms for symptom relief and the host’s endogenous neuraminidase

Mechanisms for symptom relief are mainly discussed in this section, and those for delayed onset type reactions are mainly discussed in the next section. Both mechanisms seem to be related to inhibition of the host’s neuraminidase.

Inhibition of the host’s neuraminidase and symptom relief

Symptom relief in RSV-infected mice by oseltamivir

Decisive evidence is shown by Moore et al.[44] who reported that administration of a clinically compatible dose of oseltamivir in mice challenged by a respiratory syncytial virus (RSV) that lacks a neuraminidase gene showed symptom-relieving effects (decreased weight loss) and inhibition of viral clearance. These effects were accompanied by decreased level of CD8+ T cell surface sialoglycosphingolipid (ganglioside) GM1, which is regulated by the
endogenous sialidase/neuraminidase in response to viral challenge, along with suppression of cytokines expression. To date, no such study has been conducted for zanamivir, laninamivir or peramivir.

**Decreased GM1 ganglioside and suppression of pro-inflammatory cytokines**

In the human phase II randomized controlled trial with experimental infection,[25] pro-inflammatory cytokines including IL-6, TNF-α, and IFN-γ were completely suppressed by oseltamivir administered 28 h after the experimental inoculation of the influenza virus, while reduction of viral titre in nasal lavages was partial.

Attenuating induction of pro-inflammatory cytokines including IL-6, TNF-α, and IFN-γ is related to decreased secretion by immune cells including dendritic cells,[45] by polymorphonuclear leukocytes,[46] and by CD8 T-cells.[44,47] Reduced cytokine induction is derived from decreased expression of GM1 ganglioside in these cells related to inhibition of the host’s endogenous neuraminidase (or sialidase).[44–47] especially Neu 3 (the third subtype of neuraminidase mainly expressed in plasma membrane).[45]

**Decreased GM1 ganglioside and pain**

Crain et al. [48] reported that oseltamivir at 100 to 1000 times lower HED than the clinical dose may affect the host’s neuraminidase and reduce endogenous GM1 ganglioside, leading to some reactions in the host. They suggest that “Clinical administration of oseltamivir at doses that result in inhibition of influenza may also have an additional effect by decreasing GM1 levels in nociceptive neurons”.[48]

**Animal infection model: symptoms, inflammatory/ cytokine response, and viral load**

**Ferret model: reduction of febrile, inflammatory response with little viral load change**

The ferret model is one of the best animal models for human influenza infection. Roche used this model and reported as follows in the protocol (Module II) of most clinical study reports for treatment randomized controlled trials [43*a]:

Adult ferrets (four per group) were inoculated with a virulent influenza strain. Ro-0796 was administered orally at a dose of either 5mg/kg or 25-mg/kg b.i.d. for 3 days starting 2 h post exposure. A control group of four ferrets received vehicle alone. In this experiment, Ro 64-0796 was shown to reduce the febrile response and reduce the number of inflammatory cells in nasal washing in a dose dependent manner. However, neither dose was demonstrated to reduce the viral titres obtained from the lungs or nasal washings of infected animals.

Ro-0796 refers to oseltamivir phosphate.

According to the published paper of oseltamivir,[49] the area under the curve (AUC) of the viral load of both 5 mg/kg/day and of 25-mg/kg/day oral doses and peak viral load in the 5 mg/kg/day group were not reduced significantly, although average peak viral titres of the 25 mg/kg/day group was reduced significantly.

In contrast, AUC of the febrile response was reduced significantly in both oseltamivir dose groups, dose-dependently. In addition, the total number of inflammatory cells in nasal wash obtained from infected animals in both oseltamivir dose groups was continuously reduced for more than 96 h.

The conversion factor for ferret dose to human equivalent dose (HED) for a person weighing 60 kg in the “Guidance for Industry” [40] is 5.3. Therefore, 5 mg/kg and 25-mg/kg for adult ferrets correspond to approximately 1 mg/kg and 5 mg/kg.

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**Table 1. Spectrum of adverse reactions to oseltamivir and other neuraminidase inhibitors.**

| System                      | Symptom group      | Symptoms and reactions                                                                 |
|-----------------------------|--------------------|----------------------------------------------------------------------------------------|
| Sudden onset type reactions (only to oseltamivir) | Gastrointestinal | Nausea, vomiting, Hypothermia, sleep, headache, vomiting, bulging of fontanelle |
| Digestive                   | Mild to moderate symptoms | Sensory disturbances, impairment of cognition, abnormal behaviour (suppressed or excitatory), unconsciousness, paranoia, delusion, hallucination, psychosis, depression, aggression, agitation, delirium, suicidal (ideation, attempt, complete) |
| Central nervous system      | Psychiatric symptoms | Cytokine inductions and chemokines (IL-6, IFN-γ, TNF-α, etc) |
| Renal                       | Respiratory suppression | Cyanosis, difficulty of breathing, hyperpnea, hypopnea, irregular breath, respiratory failure, respiratory arrest, cardiorespiratory arrest and death |
| Delayed onset and/or prolonged type reactions (all neuraminidase inhibitors) | Antibody | Reduction of antibody production, especially secretory IgA at respiratory tract |
| Immune/inflammatory/infectious | Cytokine | Attenuated induction of cytokine and chemokines (IL-6, IFN-γ, TNF-α, etc) |
| | Cell | Reduction of inflammatory cells in nasal wash and lung, reduction of CD8+ T cell in lung |
| Renal                       | Reinfecction etc. | Reinfection of influenza, pneumonia and exacerbation of other infections |
| Metabolic                   | Histology/function | Degenerating and regenerating changes in the renal tubular epithelia and Bowman capsules, increased urine volume and kidney weight, proteinuria, etc. |
| Cardiac                     | Diabetic | Hyperglycaemia, exacerbation of diabetes mellitus, new onset diabetes |
| Neurological                | Contraction | Decreased heart rate, bradycardia, QT prolongation |
| Psychiatric                 | Nociceptor | Pain in limbs or other parts of the body |
| Delayed onset/prolonged type | Delayed onset/prolonged type | Delayed onset psychiatric symptoms (abnormal behaviours, psychosis hallucination, delusion, agitation, schizophrenic reactions, depression, etc.) |
| Digestive                   | Gl tract | Bleeding |
| Others                      |                   | Bleeding (hepatic and/or haematological impairment) |
| ---                          |                   | --- |
kg for humans. These are almost equivalent doses and 4 to 5 times higher than the human single dose of oseltamivir (75 mg/60 kg).

Hence, the clinical dose of oseltamivir may reduce febrile response and the number of inflammatory cells in a nasal washing without significant reduction of viral titres obtained from the lungs or nasal washings of infected animals.

* *a: Module II of the most CSRs for adult treatment RCTs including 2 pivotal RCTs (WV15670, WV15671) and others (WV15673/15697, WV15707, WV15708, WV15730, WV15758, WV15759/15871, WV15799, WV15812/872, WV15819/15876/15978, WV15825), except 3 CSRs (M76001, WV16277 and ML16369). These full CSRs are available in ref [43] http://data-dryad.org/resource/doi:10.5061/dryad.77471.

**Mouse model; mild influenza and lack of evidence of reduction of viral load**

Oral administration of 10 mg/kg of OP per day caused a 100-fold reduction in lung homogenate viral titres in mice infected with a 90% lethal dose of some strains of influenza A or B viruses, and enhanced survival.[29,49] Similar experiments were reported for peramivir.[50–52]

However, in a study by Wong et al. [53] using mice infected with mild influenza (inoculated with a non-lethal dose of influenza virus), which is a better model for testing the effects of oseltamivir in human seasonal influenza, a clinically compatible dose of oseltamivir (10 mg/kg = approximately 0.8 mg/kg as HED) administered (in 3 different experiments) at 4 hours before inoculation, 24 h after inoculation, or 48 h after inoculation showed no significant effect on viral titres at day 5 post-inoculation.

Wong et al. [53] observed that oseltamivir markedly and significantly reduced lung inflammatory cell response and induction of pro-inflammatory cytokines and chemokines such as TNF-α, IL-1β, IL-6, granulocyte–macrophage colony-stimulating factor (GM-CSF), keratinocyte-derived chemokine (KC), macrophage inflammatory protein-1α (MIP-1α), and monocyte chemotactic protein-1 (MCP-1) whether administered prophylactically or therapeutically. However, these were accompanied by small non-significant effects on viral titres. Based on these findings, the researchers discussed the possibility of intrinsic anti-inflammatory effects of oseltamivir.[53]

No animal study has been conducted of the infection model with mild and non-lethal doses of the influenza virus for zanamivir, laninamivir, or peramivir. Only animal studies of the infection model using lethal doses of the influenza virus are available.

**In vitro findings showing inhibitory effects on immune cells**

Peripheral T-lymphocytes from healthy adult whole blood were incubated with antigen presenting cells (APCs) pre-sensitized with influenza viruses and were tested for their proliferation ability with and without oseltamivir carboxylate. Proliferation of the T-lymphocytes was suppressed by 15% and 20% when incubated with 1 μM and 10 μM of oseltamivir carboxylate, respectively, compared with the control.[29]

Concentration of oseltamivir carboxylate (OC) of 1 μM is compatible with the human clinical concentration of OC.[29,30] The Pharmaceuticals and Medical Devices Agency (PMDA) and the Summary Basis of Approval (SBA) did not refer to any published paper for these findings.[29,30] No published papers with these data could be found.

**Mechanism for delayed onset type reactions**

**Delayed onset and prolonged type of psychiatric and other neurological symptoms**

Psychiatric and nervous symptoms that occur in the very early phase of the treatment such as acute behavioural change and respiratory depression leading to death may be due to the effects of unmetabolized oseltamivir phosphate (OP) on the central nervous system (CNS). If OP has affinity to NMDA receptors [31,54,55] and is used for an extended period of time, it may induce schizophrenic reactions in humans, as shown in the prophylaxis RCTs of oseltamivir,[9,43] by a mechanism similar to that of the sudden onset type reactions.

The symptoms that occur in the late phase of treatment with prolonged duration, such as psychosis, confusion, and aggression, and are frequently observed in the prophylaxis trials (shown in the section “Other adverse effects (pneumonia, wheezing, gastric bleeding, and others)”) may also be due to the effects of oseltamivir carboxylate (OC) on CNS. Pain in the limbs [9] may also be induced by both the mechanisms.

Izumi et al. reported that systemic injection of oseltamivir (50 mg/kg i.p.) significantly altered the duration of loss of lightning reflex following ethanol injection in rats. Ethanol injection in the presence of oseltamivir also resulted in enhanced hypothermia.[56] Izumi et al. also reported that combination of oseltamivir with other neurostimulants alter synaptic plasticity and this may contribute to behavioural changes associated with the drug.[57]

As described in section “Cardiac disorders: bradycardia and QT prolongation”, QT prolongation is closely related to the plasma concentration of oseltamivir carboxylate.

Taking these into account, it may be possible that oseltamivir carboxylate directly alters the cell excitability of both neurons and heart muscles, although it is not known whether the alteration is derived from inhibition of the host's endogenous neuraminidase or from other mechanisms, including effects on other receptors or enzymes. Among receptors or enzymes that were tested by Lindeman et al.,[58] those that showed apparent dose-related increase are listed in Table 2.

Muraki et al. [55] demonstrated that oseltamivir, but not oseltamivir carboxylate, directly blocks human neuronal nicotinic acetylcholine receptors. Hisa et al. [59] found that oseltamivir, but not oseltamivir carboxylate, competitively and selectively inhibited human MAO-A. They estimated the Ki value to be 25 to 28 μM, and IC50 was shown to be between 50 to 100 μM in their paper, while Lindeman et al. reported that both oseltamivir and oseltamivir carboxylate lacked clinically relevant pharmacological activities on a panel of 155 other molecular targets, including MAO-A. Differing results between the study by Lindeman et al. and those by Muraki
et al. or Hiasa et al. may be derived from the different assay methods used.

Accordingly, it is possible that there exist target receptors or enzymes that oseltamivir carboxylate specifically acts on.

**Attenuated antibody production, immune suppression and re-infection**

Attenuated antibody production [32–34] may be derived from the same mechanisms as those for reduction of cytokine induction related to inhibition of the host's neuraminidase due to decreased expression of GM1 ganglioside in various immune cells.[44–47] These findings are supported by the fact that both oseltamivir and zanamivir reduce antibody production at certain doses both in humans and other animals. Marois et al. [47] demonstrated that influenza specific CD8$^+$ effector T cell recruitment was reduced up to 81% in the lungs of mice treated with oseltamivir (5 or 50 mg/kg twice daily; EC$_{50}$ 49 nM in vitro) compared to saline controls. They also showed that oseltamivir administration significantly decreased the pools of tissue-resident and circulating effector memory (93.7%) and central memory CD8$^+$ T cells (45%) compared to saline controls. During heterologous secondary infection, a decreased memory CD8$^+$ T cell pool combined with reduced generation of secondary influenza-specific effectors in the lymph nodes resulted in 10-fold decreased CD8$^+$ T cell recall responses, which increased mouse morbidity and delayed viral clearance. Furthermore, they reported that antiviral administration led to a significant 5.7-fold decreased production of functional anti-influenza antibodies. They summarized that oseltamivir treatment affects the kinetics, magnitude, and nature of innate, adaptive, and memory immune responses during the mouse-adapted influenza (PR8;H1N1) infection in the mouse model. They suggested that administration of oseltamivir in infected individuals might reduce the generation of protective immunity against reinfection and, thus, lead to the development of disease.[47]

The evidence of re-infection in the subsequent season [33] or within the same season [38,39] supports the adverse effect of both neuraminidase inhibitors on the immune system.

These findings are also consistent with the evidence on the mechanism of action of oseltamivir from animal models,[49,53] a randomized controlled experimental human influenza study,[25] and in vitro findings showing inhibitory effects on immune cells.[29]

**Other adverse reactions: renal, metabolic, cardiac, prolonged psychiatric and bleeding disorders, pneumonia, etc**

In mammalian cells, four types of sialidase (neuraminidase) have been identified. They are classified according to their major intracellular localization as intralyosomal sialidase (NEU1), cytosolic sialidase (NEU2), plasma membrane-associated sialidases (NEU3), and mitochondrial sialidase (NEU4).[60,61]

Hepatic NEU3 may be associated with sensitivity to insulin and glucose tolerance through modification of ganglioside...
composition and peroxisome proliferator-activated receptor gamma signaling.[62]

Clinical administration of oseltamivir at doses that result in inhibition of influenza may also have an additional effect by decreasing GM1 levels in various cells, including immune cells,[44–47] nociceptive neurons,[48] insulin or peroxisome proliferator-activated receptor gamma signaling,[62] and possibly other important cells in the kidney, liver, heart, or central nervous system.

The evidence from these reports suggests that reduction of human endogenous sialidase (neuraminidase) activity by oseltamivir carboxylate may cause delayed onset type adverse reactions to neuraminidase inhibitors. These include not only inhibition of antibody and pro-inflammatory cytokine induction, but also prolonged neuropsychiatric reactions, hyperglycaemia, renal and hepatic impairment, pneumonia, and exacerbation of infection, such as re-infection of influenza, gastrointestinal tract haemorrhage, and others.

**Difference between oseltamivir and other neuraminidase inhibitors in delayed onset type reactions**

Sufficient plasma concentration of oseltamivir carboxylate, a metabolite of orally administered oseltamivir phosphate, acts on the host's endogenous neuraminidase to reduce (or suppress) the immune response even at the dose of 20 mg b.i.d. for 5 days.[25] However, bioavailability of inhaled zanamivir is 11%, estimated using the data of area under the curve (AUC) from inhalation and intravenous administration over 30 min while peak concentration (Cmax) was 3.1% of that of intravenously administered zanamivir.[63] According to the data from the summary basis of approval of zanamivir in Japan,[64] bioavailability of inhaled zanamivir is calculated to be approximately 9 to 72% (geometric mean = 25%). The low bioavailability of zanamivir may be the major reason why reduction of antibody rise was not observed in the systematic review of zanamivir.[9]

However, if zanamivir is administered at a high dose or for an extended period, or if the patient is very susceptible, inhaled zanamivir might reach a concentration high enough to reduce the immune response. In fact, 20 mg/day of inhaled zanamivir for 14 days showed significant reduction of antibody titres to the H1N1 compared with a placebo.[36] Levels of various pro-inflammatory cytokines and chemokines were almost completely suppressed in the viral challenge RCT using a very high dose (600 mg) of intravenous zanamivir before inoculation of influenza virus in human adults.[37]

Although no report which examined the suppression of cytokine induction by laninamivir was found in either published papers or the summary basis of approval,[65] several reports indicated that pro-inflammatory cytokine induction was suppressed by peramivir administration in the animal models of lethal influenza virus infection.[49–51] Prolonged median survival was reported in these animal infection models with almost 100% mortality. However, prolonged survival was not stated in the ferret infection model of laninamivir.[65] Moreover, viral titres in the nasal wash were higher at 72 h after inoculation of influenza virus in ferrets administered with inhaled laninamivir 4 h after inoculation compared with vehicle control, while they were lower at 24 and 48 h after inoculation.[65]

The AUC at the maximum animal dose of zanamivir (191 µg h/mL after intravenous 90 mg/kg) [64] in rats was only 2.2 times higher than that of healthy human male adults [63] (86.6 µg h/mL after intravenous administration of 600 mg over 30 min). The maximum dose of inhaled laninamivir in the toxicity tests was 7.4 times higher than the usual clinical dose based on the AUC.[65] Hence, it may be hard to detect renal toxicity of zanamivir and laninamivir using existing animal toxicity studies.

According to the Japanese summary basis of approval,[66] the kidney is a toxic target organ of peramivir. NOAEL of peramivir for renal toxicity in rabbits was 100 mg/kg, which is 32 mg/kg in HED (conversion factor 3.1) or 2.7 times higher than the maximum clinical dose of peramivir (600 mg/50 kg). The safety margin for renal toxicity was estimated as 3.0 and 2.1 based on the AUC in single-dose and multiple-dose (7 days) oral rabbit toxicity studies.[66] It is very important to use sensitive animals to detect early signs of toxicity.[67] For example, canaries were used by miners to detect the increased level of carbon monoxide.[68]

Inhibitory effects on antibody production have been reported for oseltamivir and zanamivir. Inhibitory effects of cytokines or chemokines have been reported for oseltamivir, zanamivir, and peramivir. Toxicities in kidneys have been reported for oseltamivir and peramivir. On the other hand, toxicities affecting other cells and cell functions, including effects on ganglioside GM1, have not been investigated, except for oseltamivir.

Based on these findings, it can be said that neuraminidase inhibitors in general may act as inhibitors of the host's endogenous neuraminidase and thereby induce various delayed onset type reactions as shown in the case of oseltamivir.

**Summary and conclusion**

Neuraminidase inhibitors (NIs) are generally believed to relieve influenza symptoms by inhibiting the neuraminidase of the influenza virus. However, a clinically compatible dose of oseltamivir relieves symptoms in mice infected with RSV that lacks neuraminidase. The underlying mechanisms of these phenomena are explained by the inhibition of the host's endogenous neuraminidase, leading to reduction of GM1 ganglioside in immune cells. This may cause subsequent reduction of pro-inflammatory cytokines such as IL-6, interferon, and TNF-α. These mechanisms may be closely related to symptom relief without significant reduction of viral load. They also cause attenuated antibody production of secretory IgA antibodies and plasma HI antibodies of influenza, and may be the cause of re-infection of influenza within the same or the subsequent seasons.

Mechanisms for delayed onset type reactions with prolonged course, such as psychosis and other psychiatric reactions, and renal, metabolic (hyperglycaemia or diabetes), and
cardiac reactions (QT prolongation), may be related to inhibition of the host’s endogenous neuraminidase.

**Disclosure statement**

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**References**

[1] CDC. Updated Interim Recommendations for the Use of Antiviral Medications in the Treatment and Prevention of Influenza for the 2009–2010 [Internet]; [cited 2016 Mar 23]. Available from: http://www.cdc.gov/h1n1flu/recommendations.htm

[2] CDC. Influenza antiviral medications: summary for clinicians, (last updated Feb 25 2015) [Internet]; [cited 2016 Mar 23]. Available from: http://www.cdc.gov/flu/professionals/antivirals/summary-clinicians.htm#modalIdString_DCSTable_1

[3] World Health Organization. WHO Model List of Essential Medicines.19th edition (May 2015) [Internet]; [cited 2016-03-23]. Available from: http://www.who.int/medicines/publications/essentialmedicines/EML2015_8-May-15.pdf?ua =1.

[4] Maxwell SR. Tamiflu and neuropsychiatric disturbance in adolescents. BMJ. 2007;334:1232–1233.

[5] Hama R. Discussion of the causal relationship between oseltamivir phosphate (Tamiflu), and sudden death and death from abnormal behavior. Paper presented at the session of Japanese Society for Pediatric Infectious Diseases in Tsu, Mie Prefecture (held on 2005-11-12) [Internet]; [cited 2016 Mar 23]. Available from: http://www.npjpj.org/english/no59.html.

[6] Hama R. Fatal neuropsychiatric adverse reactions to oseltamivir: case series and overview of causal relationships. Intern J Risk Safety Med. 2008;20:5–36.

[7] Hama R. Oseltamivir’s adverse reactions: fifty sudden deaths may be related to central suppression. BMJ. 2007;335:559.

[8] Hama R, Jones M, Hayashi K, et al. Oseltamivir and early deterioration leading to death: a proportional mortality study for 2009A/H1N1 influenza. Int J Risk Saf Med. 2011;23:201–215.

[9] Jefferson T, Jones MA, Doshi P, et al. Neuraminidase inhibitors for preventing and treating influenza in healthy adults and children. Cochrane Database Syst Rev. 2014;4:CD008965.

[10] Jefferson T, Jones MA, Doshi P, et al. Neuraminidase inhibitors for preventing and treating influenza in healthy adults and children. Cochrane Database Syst Rev. 2012;1:CD008965.

[11] Kohno S, Yen MY, Cheong HJ, et al. (S-021812 Clinical Study Group), et al. Phase III randomized, double-blind study comparing single-dose intravenous peramivir with oral oseltamivir in patients with seasonal influenza virus infection. Antimicrob Agents Chemother. 2011;55:S267–S276.

[12] Sugaya N, Ohashi Y. Long-acting neuraminidase inhibitor lanamivir octanoate (CS-8958) versus oseltamivir as treatment for children with influenza virus infection. Antimicrob Agents Chemother. 2010;54:2575–2582.

[13] Hayden F. Developing new antiviral agents for influenza treatment: what does the future hold? Clin Infect Dis. 2009;48:53–51.

[14] Roche. Media release. Roche donates a further 2 million treatment courses of antiviral Tamiflu to the WHO for regional stockpiling (2006 Jan 17) [Internet]; [cited 2016-03-23]. Available from: www.roche.com/med-cor-2006-01-17.html

[15] Jack A. Roche steps up production of Tamiflu after virus scare. Financial Times. 13 May 2009; p. 17.

[16] Hoffmann-La Roche Inc. FDA Pediatric advisory committee briefing document for Tamiflu (2007) [Internet]; [cited 2016 Mar 23]. Available from: http://www.fda.gov/ohrms/dockets/ac/07/briefing/2007-4325b_02_15_Sponsor%20Background%20Package%20Roche.pdf.

[17] Glaxosmithkline FDA Pediatric Advisory Committee Meeting Relenza® (zanamivir) Inhalation Powder. Sponsor Background (Briefing Information) (2007-11-27) [Internet]; [cited 2016 Mar 23]. Available from: http://www.fda.gov/ohrms/dockets/ac/07/briefing/2007-4325b_02_16_Sponsor%20Background%20Package%20GSK.pdf.

[18] Ministry of Health, Labor and Welfare (MHLW): advisory panel for drug safety (held on 2014-10-29), usage review of neuraminidase inhibitors (in Japanese) [Internet]; [cited 2016 Mar 23]. Available from: http://www.mhlw.go.jp/file/05-Shingikai-11121000-lyakushokuhinkyo-Soumuka/0000063406.pdf.

[19] Bhata A, Kast RE. How influenza’s neuraminidase promotes virulence and creates localized lung mucosa immunodeficiency. Cell Mol Biol Lett. 2007;12:111–119.

[20] Matrosovich MN, Matrosovich TY, Gray T, et al. Neuraminidase is important for the initiation of influenza virus infection in human airway epithelium. J Virol. 2004;78:12665–12667.

[21] Moscona A. Neuraminidase inhibitors for influenza. N Engl J Med. 2005;353:1363–1373.

[22] Ohuchi M, Asaoka N, Sakai T, et al. Roles of neuraminidase in the initial stage of influenza virus infection. Microbes Infect. 2006;8:1287–1293.

[23] Liu C, Eichelberger MC, Compars RW, et al. Influenza type A virus neuraminidase does not play a role in viral entry, replication, assembly, or budding. J Virol. 1995;69:1099–1106.

[24] Full prescription information of oseltamivir (revised in April 2010) [Internet]; [cited 2016-03-23]. Available from: http://www.accessdata.fda.gov/drugsatfda_docs/label/2011/021087s057lbl.pdf

[25] Hayden FG, Treanor JJ, Fritz RS, et al. Use of the oral neuraminidase inhibitor oseltamivir in experimental human influenza: randomized controlled trials for prevention and treatment. JAMA. 1999;282:1240–1246.

[26] Morimoto K, Nakakariya M, Shirasaki Y, et al. Oseltamivir (Tamiflu) efflux transport at the blood-brain barrier via P-glycoprotein. Drug Metab Dispos. 2008;36:6–9.

[27] Ose A, Kusuhara H, Yamatsugu K, et al. P-glycoprotein restricts the penetration of oseltamivir across the blood-brain barrier. Drug Metab Dispos. 2008;36:427–434.

[28] MHLW. Documents for the fifth 2007 advisory Panel on Drug Safety (held on 2007-12-25) (in Japanese) [Internet]; [cited 2016 Mar 23]. Available from: http://www.mhlw.go.jp/shingi/2007/12/s1225-7.html.

[29] Japanese SBA. Tamiflu capsule for treatment [Internet]; [cited 2016 Mar 23]. Available from: http://www.pmda.go.jp/drugs/2000/g001202/index.html.

[30] PMID. Examination results by PMDA (Tamiflu capsule for treatment) [Internet]; [cited 2016 Mar 23]. Available from: http://www.pmda.go.jp/drugs/2000/g001202/07repo01.pdf.

[31] Hama R, Bennet C. The mechanisms of sudden-onset type adverse reactions to oseltamivir. Acta Neurologica Scandinavica (revised for publication).

[32] Sawabuchi T, Suzuki S, Iwase K, et al. Boost of mucosal and systemic immune responses by clarithromycin enhances mucosal and systemicimmune responses in pediatric patients with influenza treated with antiviral neuraminidase inhibitors: a retrospective analysis. PLoS One. 2013;8:e70060.

[33] Takahashi E, Kataoka K, Fuji K, et al. Attenuation of inducible respiratory immune responses by oseltamivir treatment in mice infected with influenza A virus. Microbes Infect. 2010;12:778–783.
[35] Takahashi E, Kataoka K, Indalao IL, et al. Oral clarithromycin enhances airway immunoglobulin A (IgA) immunity through induction of IgA class switching recombination and B-cell-activating factor of the tumor necrosis factor family molecule on mucosal dendritic cells in mice infected with influenza A virus. J Virol. 2012;86:10924–10934.

[36] Cox RJ, Mykelti-Vedt E, Sjursen H, et al. The effect of zanamivir treatment on the early immune response to influenza vaccination. Vaccine. 2001;19:4743–4749.

[37] Fritz RS, Hayden FG, Calfee DP, et al. Nasal cytokine and chemokine responses in experimental influenza A virus infection: results of a placebo-controlled trial of intravenous zanamivir treatment. J Infect Dis. 1999;180:S86–S93.

[38] Kopel E, Mandelboim M, Amitai Z, et al. Possible recurrent pandemic (H1N1) 2009 infection. Israel. Emerging Infect Dis. 2010;16:1321–1322.

[39] Perez CM, Ferres M, Labarca JA. Pandemic (H1N1) 2009 reinfection, Chile. Emerging Infect Dis. 2010;16:156–157.

[40] U.S. Department of Health and Human Services, Food and Drug Administration, Center for Drug Evaluation and Research (CDER). Guidance for Industry-Estimating the Maximum Safe Starting Dose in Initial Clinical Trials for Therapeutics in Adult Healthy Volunteers, 2005[Internet]; [cited 2016 Mar 23]. Available from: http://www.fda.gov/downloads/Drugs/Guidances/UCM078932.pdf.

[41] EMA. Marketing Authorization Application, Table of Contents—Volume 81. 1007–Brewster M. Ro 64–0802/002 (GS–4071) cardiovascular and respiratory evaluation in the anaesthetized dog following intravenous administrations (DHB08601).RR W–142974. 1999 (disclosed document from EMA).

[42] Hama R, Jones M, Jefferson T. Concerns about the randomization in the prophylaxis trials of oseltamivir (rapid response), 2015[Internet]; [cited 2016 Mar 23]. Available from: http://www. bmj.com/content/348/bmj.g2545/rr.

[43] The Dryad Digital Repository[Internet]; [cited 2016 Mar 23]. Available from: http://datadryad.org/resource/doi:10.5061/dryad. 77471.

[44] Moore ML, Chi MH, Zhou W, et al. Cutting edge: oseltamivir blocks binding interaction with ICAM-1. J Leukoc Biol. 2011;90:313–321.

[45] Crain SM, Shen KF. Neuraminidase inhibitor, oseltamivir blocks GM1 ganglioside-regulated excitatory opioid receptor-mediated hyperalgesia, enhances opioid analgesia and attenuates tolerance in mice. Brain Res. 2004;955:260–266.

[46] Mendel DB, Tai CY, Escarpe PA, et al. Oral administration of a prodrug of the influenza virus neuraminidase inhibitor GS 4071 protects mice and ferrets against influenza infection. Antimicrob Agents Chemother. 1998;42:640–646.

[47] Onishi M, Kitano M, Taniguchi K, et al. Intravenous peramivir inhibits viral replication, and leads to bacterial clearance and prevention of mortality during murine bacterial co-infection caused by influenza A(H1N1)pdm09 virus and Streptococcus pneumoniae. Antiviral Res. 2015;117:52–59.

[48] Tanaka A, Nakamura S, Seki M, et al. The effect of intravenous peramivir, compared with oral oseltamivir, on the outcome of post-influenza pneumococcal pneumonia in mice. Antivir Ther. 2015;20:11–19.

[49] Kitano M, Kodama M, Itoh Y, et al. Efficacy of repeated intravenous injection of peramivir against influenza A (H1N1) 2009 virus infection in immunosuppressed mice. Antimicrob Agents Chemother. 2013;57:2286–2294.

[50] Wong ZK, Jones JE, Anderson GP, et al. Oseltamivir treatment of mice before or after mild influenza infection reduced cellular and cytokine inflammation in the lung. Influenza Other Respir Viruses. 2011;5:343–350.

[51] Hama R. Pharmacological toxicological clinical and epidemiological evidences of neuropsychiatric adverse reactions to oseltamivir and misleading analysis due to serious miscalculation in Japanese MHLW's 2006/07 epidemiological study report. (In Japanese). Jap J Clin Pharmacol Therap. 2009;40:135–145.