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Fatal neuropsychiatric adverse reactions to oseltamivir: Case series and overview of causal relationships

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Abstract. *Background*: Infection-associated encephalopathies such as Reye's syndrome have been one of the major public health problems in many countries. The not dissimilar neuropsychiatric adverse reactions, including deaths, observed with Tamiflu (oseltamivir phosphate: OP) have been another current problem especially in Japan.

Methods: Among the cases with neuropsychiatric adverse reactions to Tamiflu on which I was consulted, those cases in which medical charts, autopsy records and/or prescription certificates were available were analyzed and described. In order to obtain a complete view of the spectrum of neuropsychiatric adverse reactions attributed to Tamiflu and of existing knowledge of the causal relationship, adverse reaction case reports and accounts of personal experiences were collected using PubMed, Japonica Centra Revuo Medicina, the websites of MHLW, PMDA and FDA and other Internet sources. Information on animal toxicity and clinical trial findings was derived from the texts of the officially approved data sheet for Tamiflu.

Results and discussion: This paper reports eight cases in total: five of these died and three survived. Two died as a result of accidents resulting from abnormal behaviour. Three others died suddenly during sleep (two infants and one adult). One of the infants and the adult were found at autopsy to have severe lung oedema. A 14-year-old boy experienced agitation, cyanosis, loss of consciousness and seizures but recovered completely, while a 10-month-old girl showed retarded development and mental retardation after initially appearing to recover from the acute event involving loss of consciousness and seizure. A 15-year-old boy had a delayed onset of complications but developed prolonged neuropsychiatric adverse reactions after taking an almost complete course of Tamiflu; in this case the symptoms lasted for two weeks.

Following our review of known clinical cases of this type, which included 80 fatalities (among them 50 instances of sudden death and 8 cases of accidental death consequential upon abnormal behaviour, and in the light of our study of animal experiments and the latest laboratory findings, we propose to classify adverse reactions to Tamiflu as follows:

(1) *Sudden onset adverse reactions* typically occurring after taking one or two doses of Tamiflu; these result from the central nervous system suppressant action of oseltamivir, a pro-drug of oseltamivir carboxylate (OCB: an active metabolite). The group includes cases of sudden death during sleep or associated with respiratory suppression, sudden onset of abnormal behaviour and occurrence of other neuropsychiatric disorders having an acute onset but a short duration.

(2) *Delayed onset adverse reactions* occurring after taking several doses or a full course of Tamiflu, probably caused by OCB. Examples include delayed onset neuropsychiatric reactions with prolonged duration, pneumonia, sepsis, bleeding and *hyperglycemia.*

(3) *Allergic and miscellaneous* reactions involving various organs.

The mechanisms underlying the adverse reactions to oseltamivir and the causal relationships may be summarized as follows:

(1) Oseltamivir has a depressant effect on the central nervous system (CNS); the signs, symptoms and pathological findings are similar to those induced by hypnotics and sedatives (decreased body temperature, decreased spontaneous movements, slow/irregular breathing, cyanosis and pulmonary oedema). Severe sequels may reflect delayed neuronal damage resulting from temporary cardiopulmonary arrest.

(2) Abnormal behaviour, delirium, hallucinations and even suicide could be the consequences of disinhibition or loss of control induced by the CNS depressant effect.

(3) Delayed onset reactions to Tamiflu may be related to its inhibitory action on sialidase (neuraminidase), a key enzyme for antiviral activity and involved in a wide variety of mammalian physiological processes including immune functions, cell

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apoptosis and glucose metabolism reflecting its ability to influence the conformation of glycoproteins and gangliosides that are important components of cell structure and function.

Conclusion: Three sudden deaths during sleep and two near deaths with or without sequels, as well as two deaths from accidents resulting from abnormal behaviour in older children and adolescents shortly after taking Tamiflu are probably related to the central depressant action of oseltamivir. Late onset neuropsychiatric symptoms after taking a full dose of Tamiflu, which we observed in one case, may be related to the inhibition of human neuraminidase by OCB, an active metabolite of Tamiflu.

Keywords: Tamiflu, oseltamivir, adverse drug reaction, sudden death, influenza, animal toxicity, fever, delirium, organ damage, encephalopathy

Abbreviations

OCB: Oseltamivir carboxylate,
NAP: New drug approval package,
MHLW: Ministry of Health Labour and Welfare,
PMDA: Pharmaceuticals and Medical Devices Agency.

1. Introduction

Acute encephalopathy following viral infection, such as Reye's syndrome and/or influenza-associated encephalopathy, has been one of the major public health problems experienced not only in Japan but also in the US [8,69] and elsewhere. After the publication of warnings against and restrictions on the use of salicylates in young children, Reye's syndrome disappeared in the US [9]. Similarly, restrictions on the use of non-steroidal anti-inflammatory drugs (NSAIDs) as antipyretics in Japan in 2000 led to a dramatic decrease in case mortality of Reye's syndrome and/or of encephalitis/encephalopathy after viral infection [37].

However, further cases of sudden death associated with influenza in previously healthy children were reported both in the United States [8] and in Japan [84] during the winter of 2002/03.

After I warned of the possible involvement of Tamiflu (oseltamivir phosphate), an ethylester prodrug of oseltamivir carboxylate (OCB) as the cause of sudden death [33,34], eight families consulted me for an expert opinion on the cause of their children's deaths or the serious adverse events which they had experienced when using this drug, making their medical records available for my perusal. I presented three of these fatal cases at a scientific meeting in November 2005 [35].

The present paper will describe five deaths and three life-threatening cases of neuropsychiatric adverse reactions involving Tamiflu. The mechanism of such adverse reactions to Tamiflu and the causal relationship will be discussed.

2. Materials and methods

After the presentation of the above material at the scientific meeting in 2005 [35], and during the period up to the end of August 2007, the families of five additional cases made their medical charts, autopsy records and/or prescription certificates available to me for investigation.

I analyzed eight cases in all in which the necessary documents were available for study. Histories were again taken from the families.

In order to examine the full spectrum of neuropsychiatric adverse events associated with Tamiflu and to discuss the causal relationship, case reports and data on adverse events or reactions as well as accounts of personal experiences were collected from a wide range of sources. Information was derived variously from direct phone calls and e-mails to our center, by searches using PubMed, "Japonica Centra Revuo Medicina" (a Database of Japanese medical journals), the websites of the Japanese Ministry of Health, Labour and Welfare (MHLW), the Pharmaceuticals and Medical Devices Agency (PMDA) as well as the US Food and Drug Administration (FDA) and various other internet sources.

Data on animal toxicity and clinical trial findings were based on the studies cited in the "New drug approval package" (NAP) issued for Tamiflu capsules (used both for treatment and for prevention), and for Tamiflu dry syrup (in Japanese) [13–15].

The mechanism of adverse reactions to Tamiflu and the causal relationship will be discussed, focusing on the following points:

(1) the profile of adverse reactions to Tamiflu in human subjects,
(2) the drug's toxicity profile in animals,
(3) the similarity of the symptoms and findings in animals and in humans,
(4) the neuropsychiatric symptoms and forms of dyscontrol experienced with other CNS suppressants,
(5) differences between Reye's syndrome and influenza-associated encephalopathy,
(6) cases of non-Tamiflu related sudden death and seizure-inducing drugs,
(7) fever delirium and Tamiflu delirium,
(8) brain/lung oedema and hypoxia,
(9) sequels and appearance of delayed neuronal cell damage following cardiac arrest,
(10) delayed adverse reactions and inhibition of human sialidase (neuraminidase) by OCB,
(11) limitations of postmortem measurements of oseltamivir and OCB levels,
(12) methods of assessment for adverse reactions,
(13) what this paper adds to earlier reports,
(14) possible further study required to confirm causality and for other reasons.

For the statistical analysis EpiInfo (version 3-3-2) was used for case controlled study or cross sectional studies. The latest version of StatDirect was used for meta-analysis of the death rate during toxicity testing of non-steroidal anti-inflammatory drugs (NSAIDs) using infected animals.

2.1. Case reports

Eight cases are summarized in Table 1. Seven were sudden onset cases in which the events occurred soon after the first or second dose of Tamiflu, while one was a delayed onset neuropsychiatric case in which the adverse events occurred after the end of a full course of Tamiflu and continued for some two weeks.

Of the seven sudden onset cases, two were accidental deaths, probably after non-suicidal abnormal behaviour, three cases were sudden deaths during sleep, and the other two were life-threatening cases: one without any sequels after occurrence of abnormal behaviour, cyanosis, and seizure, and another with sequels after cyanosis and seizure.

2.2. Two cases with abnormal/strange behaviour and accidental death

Case 1. A 14-year-old boy had a body temperature 38.0°C (100.4 F) on the evening on February 4th 2005. It rose to 39.0°C (102.2 F), accompanied by other flu symptoms and was diagnosed as influenza

Table 1
Characteristics of the patients

Case no.	Age (Y/M)	Sex	Influenza type	Oseltamivir dose	Signs and symptoms (leading to death)	Outcome	Autopsy: findings	Body temperature** (°C)
1	14	M	A	75 mg × 1	Accidental death after abnormal behaviour	Death	Not done	37.5
2	17	M	A	75 mg × 1	Accidental death after abnormal behaviour	Death	Not done	39.2
3	2/9	M	A	2 mg/kg × 1	Sudden cardiopulmonary arrest during nap (10 min)	Death	Not done	34.0
4	3/3	M	A	2 mg/kg × 1	Death during nap	Death	Brain oedema and marked lung oedema without inflammation	?
5	39	M	B	150 mg × 1	Death during sleep	Death	Dilated heart and lung oedema without sign of inflammation and fibrosis	?
6	14	M	A	75 mg × 1	Abnormal behaviour, dyspnoea, cyanosis, seizure and weak breathing	Recovered without sequels		37.5
7	0/10	F	? *	2 mg/kg × 1	Flaccid extremities, loss of consciousness, cyanosis and seizure	Marked mental and physical retardation followed by gradual development		? (not high)
8	15	M	B	75 mg 1 day, 75 mg × b.i.d. 4 days, and 75 mg 1 day,	Lethargy at d 5, abnormal behaviour/delirium on day 6, hallucination on the third day and neuropsychiatric symptoms for some two weeks after the end of the Tamiflu course.	Recovered without sequels		Normal

* Influenza or adverse effect of influenza vaccine (Occurring 6 days after inoculation). ** Body temperature around the time of the event (degrees centigrade).

A by rapid testing at a clinic. After he had returned home and slept for two hours, his body temperature fell to 37.5°C (99.5 F). After he took a first dose of Tamiflu (one 75 mg capsule containing 75 mg of oseltamivir; equivalent to 98.5 mg of OP), he watched a video on TV for about 1.5 hours with his elder sister, and went to bed in his room. However, about 30 minutes later, his mother could not find him there. Noticing that the entrance door was open, she looked out into the stairway and heard a shout "a boy has fallen". His residence was on the ninth floor of a condominium. She went down to the ground level and found that the boy was her son.

His fingerprints were found on the bannister rail of the staircase leading down from the condominium. They showed that he had first climbed over the bannister and grasped it, from which it was concluded that he had first hung from the bannister and had then fallen the nine stories to the ground. His body was severely damaged, except for his head and he died from massive bleeding. There was no sign of his having consumed alcohol.

Case 2. A 17-year-old high school boy with high fever (39.0°C or 102.2 F) consulted his family doctor on February 4th 2004. He was initially treated with amantadine (50 mg b.i.d.), though rapid flu testing was negative. The next morning he had 39.7°C (103.5 F) and again consulted the doctor, at which time he tested positive for influenza A. He took a Tamiflu 75 mg capsule at home around noon. One and a half hours later, he complained of nausea. By about 2 p.m. his body temperature was 39.2°C (102.6 F), after which his father left home, leaving him alone. While all the family members were away from home, he suddenly went outside and jumped over the fence around his house. He ran on the several centimeters thick snow, then jumped over a concrete fence, crossed a railway line and jumped over a guardrail along a highway: passers-by noted that he was smiling. On the road he was run over by an oncoming truck and killed. These events occurred some three hours and forty-five minutes after taking Tamiflu and about nine hours after taking the last dose of amantadine (50 mg). There was no sign of his having consumed alcohol.

2.3. Three cases of sudden death during sleep

Case 3. A boy aged two years and nine months and weighing 13 kg, who had previously been in good health developed a temperature of 38.3°C (100.8 F) and was taken to the family doctor on February 5th 2005. He was influenza A positive as determined by rapid testing.

After having been alert and relatively well in the morning, he was given one dose of Tamiflu dry syrup (25.5 mg) together with one dose of other drugs, including cyproheptadine, carbocysteine and tipepidine hibenzoate. His temperature at this time was 39.2°C (102.6 F). He did not complain of vomiting or headache and he fell asleep ten minutes after taking the medicines. One and a half hours after taking the medicines, he woke up crying and complained of headache. He did not stop crying even when his mother took him in her arms to console him. It took forty to fifty minutes before he stopped crying and fell asleep again, some two hours and 20 minutes after taking the medicines. Two hours and 45 minutes after the treatment his mother noticed that he turned over in his sleep. Just ten minutes later she touched him and found him flaccid and not breathing. She called an ambulance and he arrived at the hospital some thirty minutes later. His body temperature at this time was 34°C (93.2 F). He was successfully resuscitated and his heartbeat resumed but he died next day (28 hours after admission to the hospital). His AST/ALT/LDH/CK levels were slightly elevated at admission and extremely high just before his death which was found to be due to hypoxic organ failure resulting from cardiopulmonary arrest. There was no sign of his having taken alcohol.

Case 4. A boy aged 3 years and 3 months and weighing 13.5 kg had generally been in good health though he suffered from atopic dermatitis without asthma. When he developed a body temperature of 38.5°C (101.3 F) that has persisted for several hours he was taken to the family doctor on December 27th 2002. At the clinic his temperature was found to be 39.6°C (103.3 F) and rapid testing led to a diagnosis of influenza A. He was treated with aminophyllin (50 mg) in 200 ml electrolyte solution and inhalation of procaterol with sodium cromoglycate for a mild wheezing bronchitis. Tamiflu 55 mg (4.1 mg/kg/day) and other drugs (including antihistamines and mucolytics) were prescribed for him. After coming back home at around 14:00, he took only one dose of Tamiflu (27.5 mg) of the various drugs prescribed and fell asleep shortly afterwards. He woke up after one hour and then slept again while watching a video on TV. At this time his mother remained in the room, checking him on occasion; after some time, believing he was asleep since he was lying on his left side, she switched off the video. At around 16:00 his mother found that he was lying face down; he now had rhinorrhea and was apparently not breathing.

He was taken by ambulance to a hospital emergency unit where he arrived at 16:34. He was intubated and treated with cardiac massage immediately on arrival, and was treated with three intravenous doses of 0.1 mg adrenaline and eleven intravenous doses of 1 mg adrenaline. Resuscitated nevertheless failed and he was pronounced dead at 17:15.

According to the autopsy report required by law, the major macroscopic findings comprised moderate lung congestion with marked pulmonary oedema, mild congestion of the spleen and of the renal pelvis. The brain was markedly congested and swollen (wt. 1331 g) especially in the pons and medulla, but no brain herniation was observed.

Histological examination showed that the lung tissue was slightly congested with some macrophagic infiltration, while the bronchial mucosa was slightly swollen and infiltrated with lymphocytes and neutrophils. There were no signs of pneumonia and the histological findings were compatible with bronchitis due to typical influenza virus infection and lung congestion after sudden cardiac arrest. A pulmonary lesion was not the cause of his death. No particular findings were observed in the heart and other organs except in the brain. Brain oedema was slight; no evidence of meningitis or encephalitis was found and no brain herniation appeared to be present. Diffuse microgliosis was observed in the brain and pons. Most of the astroglial fibers were segmented on GFAP staining, a typical but non-specific signs of disorganization of the blood–brain-barrier of unknown cause.

His glucose level was 196 mg/dl (10.9 mmol/l) at 16:41 and 466 mg/dl (25.9 mmol/l) at 16:52 though he had no diabetes mellitus. Rapid increase in the glucose level may be induced by very high doses of adrenaline (11.3 mg in total) such as were used for resuscitation. There was no sign of alcohol consumption.

Case 5. A 39-year-old previously healthy man developed flu-like symptoms on the evening of February 25th 2005. His body temperature was 37.4°C when he consulted his family physician at a hospital at 7:30 p.m. He was diagnosed as having influenza B and was treated with 0.5 g of intramuscular dipyrone and infused with 500 ml of maltose·lactated Ringer's solution containing 1 g of cefpirome sulfate over a couple of hours. He was then prescribed Tamiflu (two 75 mg capsules b.i.d.), cefcapene pivoxil hydrochloride (100 mg t.i.d.), naproxene (100 mg t.i.d.), ambroxol hydrochloride and throat lozenges. Returning home, he took one dose of each medication. 10 minutes later, at around 10:00 p.m., he went to bed. Next morning his mother found he was lying face upwards with open mouth and open eyes but leaning a little to his left, and apparently not breathing. His mother called an ambulance, but when it arrived the crew informed his mother that he was dead and took the body to the hospital where death was confirmed.

Autopsy was confirmed by a specialist in forensic medicine at the university medical school. Major findings of the autopsy and histology were as follows: dilated and heavy heart (448 g) without inflammation or fibrosis, pulmonary oedema without pneumonia, massive amounts of sputum in the bronchi and of a pinkish or brownish mucous in the trachea and larynx and a liquefied adrenal medulla. Viral testing revealed influenza B. Examination of the urine with triage testing proved negative for amphetamines, hypnotics, marijuana and antidepressants. Troponin testing in the urine also proved negative. The time of death was estimated at around 1:00 a.m., i.e. about three hours after taking the medicines. The cause of death was diagnosed as acute left heart failure due to dilated cardiomyopathy.

It is notable that this man had been entirely healthy before developing influenza. He had no trouble during or after the infusion of cefpirome in 500 ml of fluid over a couple of hours and he had shown no signs or symptoms of heart failure before taking one dose of each medication just before falling asleep. It can therefore be assumed that his heart failure and lung oedema set in after he had taken the medicines. There was no sign of his having consumed alcohol.

2.4. Two life-threatening cases with or without sequels

Case 6. A 14-year-old boy with a body temperature of 39.0°C (102.2 F) and other flu symptoms was diagnosed as suffering from influenza A by rapid testing at a clinic near the ski resort which he and his family had visited on 31st December 2005. He vomited about one hour after taking the first dose of Tamiflu (one 75 mg capsule) at 11:00 a.m. His father then took him back to the family home, which involved some 8 hours of driving, arriving at about 20:00. As soon as the boy arrived home, he took a second Tamiflu capsule. One hour after the second dose, he developed a headache and looked agitated saying “can’t breathe”, “Wau Wau” and something else that made no sense.

His father held him tight to calm him but his face became cyanotic and then he suddenly turned pale, his eyes turned upwards, his extremities became flaccid and he lost consciousness. By the time an ambulance arrived he had begun to breathe again but very weakly, and his father was very anxious that the breathing might fail again. In the ambulance the body temperature was 37.5°C (99.5 F) but after admission to hospital it rose again to 38.8°C (101.8 F), falling once more to 36.7°C (98.1 F) after taking paracetamol. Seven hours after he had taken the second dose of Tamiflu he again became agitated, shouting and with evident dyspnea and his eyes were again turned upwards. He recovered completely 15 hours after taking the second dose of Tamiflu. His electro-encephalogram (EEG) showed no evidence of encephalitis/encephalopathy. He now had no fever, there was no recurrence of neuropsychiatric and respiratory disorders and he was discharged on the third day of admission. There was no sign of alcohol consumption at any time.

Case 7. A 10-month-old girl who was born on March 12th 2002 weighed 3324 g at birth; there were no birthing complications. She grew steadily, could sit unsupported by the 6th to 7th month and began crawling backward for the first time in the 9th month and also began crawling forward a little later. She could stand supported and was making early attempts to walk and talk (“ma-ma-ma” and “ba-ba-ba”). She could hold a spoon and eat with it when her mother put some food on it. She also played with her toy telephone. At a routine check-up on December 26th 2002 her height was 71.2 cm and body weight was 9.0 kg. All these facts suggest that her physical, cognitive and emotional development was initially normal.

On 16th January 2003, she was inoculated with influenza HI vaccine 0.1 ml. In the evening on that day, her body temperature rose to 38.6°C (101.5 F), she had a running nose and productive cough;

her temperature thereafter fell until January 19th after which it rose again to 37.9°C (100.2 F). Her mother took her to a clinic. Rapid testing for influenza A and B was negative and the doctor prescribed d-chlorpheniramine maleate, tulobuterol, carbocysteine, tipepidine hibenzoate, pranoprofen and paracetamol suppositories. Her mother gave her all these drugs except pranoprofen and paracetamol only on the first day (January 20th) since her body temperature fell promptly. On January 22nd however her body temperature rose again and her mother returned with her to the clinic; her temperature was now 38.6°C (101.5 F) and a doctor diagnosed influenza though without specific testing; he prescribed Tamiflu 18 mg b.i.d. She was given an initial dose at 15:30. The mother, who was carrying the girl on her back, noticed something abnormal at 16:20. The mother tried to let her sit at this time but she could not sit unsupported. She fell down with flaccid extremities and lost consciousness with cyanotic lips and froth. The mother took her to the clinic again at 16:45, and when the doctor saw her she exhibited clonic seizures and was unconscious. She was treated with a 4 mg suppository of diazepam, and the seizures ceased within some 10 minutes. Her consciousness apparently recovered after one hour and three quarters (18:30) and the doctor recorded no abnormal findings when she left the clinic. The mother recalled that her body temperature was less than 38.6°C (101.5 F). She did not take another Tamiflu.

Her temperature fell and other common cold-like symptoms disappeared except on 25th when her body temperature was 38.9°C (102.0 F) without taking any medicines.

On January 26th, her mother noticed that she did not crawl as she used to do before the events on 22nd. The movement of her upper extremities was not much affected but she crawled dragging her both legs. She could no longer stand supported; she did not put down her legs and did not try to stand. Her normal mental and physical condition which was acutely impaired at the time of the event has since that time ceased to develop satisfactorily. When she was two years and two months old, she became able to stand by grasping something. At the age of two and a half she could walk one or two steps. By the age of two years and eleven months she could stand up unsupported and could walk about 10 m, and she could walk by herself at around three years of age.

Following the acute events of January 22nd the child became very passive and ceased to speak. She spoke a little for a short period before her second years birthday, but thereafter she essentially lost the ability to speak until she was three and half years old. She can now say “papa” “bye bye” and “puapua” (this means mama. She cannot pronounce mama).

After the acute event she stopped trying to grasp a spoon and to eat by herself. It was not until she was 3 years and 5 months old that she could eat by herself again.

She is now 5 years old but she cannot put on clothes by herself, cannot excrete independently, cannot eat nor go up and down the steps alone. She is almost entirely dependent on others for her daily activity at home and in the society.

Magnetic resonance imaging (MRI), performed in April 2006, showed very slight atrophy in the right hippocampus, and poor development in the bilateral sylvian fissure was suspected. No seizure-spike was observed but basal waves were not completely normal by the EEG taken in April 2006.

2.5. A late onset case with neuropsychiatric symptoms lasting for two weeks

Case 8. A 15-year-old junior high school boy with a body temperature of 39.2°C was diagnosed as influenza B by a rapid testing by his family physician. Tamiflu 75 mg b.i.d. was commenced on the evening on February 8th together with paracetamol 400 mg b.i.d. and various other drugs to relieve the symptoms of flu. His body temperature fell to about 38.0°C next day and about 37.0°C on 10th, but he

could not go to school. On 12th his body temperature was within the normal range but he was lethargic throughout the day.

After he took the last dose of Tamiflu on 13th (6th day since commencement of Tamiflu), he went to school where he sat erect with his legs folded under him (Japanese sitting style) on the desk and began to sing loudly during a lesson. He could not communicate with his classmates or look them in the eyes. He seemed to be delirious. His parents took him back home where again he was lethargic, though no other abnormality of behaviour was apparent, and he was unwilling to return to school. After four days his parents took him to the physician who referred him to a general municipal hospital. There he was thought to be suffering from abnormal behaviour induced by Tamiflu and was admitted.

Routine examinations including urinalysis, complete blood count and blood chemistry, head CT, brain MRI, EEG etc. were normal. Serum ammonium level was also normal. During the physical examination before admission, he commented "There are insects on my mask" which led the staff to suspect that he was experiencing visual hallucinations.

On admission, signs and symptoms characteristic of delirium were observed: he tried to pull out his venous lines out or attempted to go home shouting "This is not a hospital this is a nursing home for elderly people". His doctor decided that he should be away from the hospital for several days (February 17th to 23rd), since he could not be maintained there.

On February 19th, his parents took him back to the hospital to be tested (SPECT), but he did not want to go inside the building. Finally he underwent a test but could not complete it because of his agitation during the procedure. On 20th he was referred to another hospital for a second opinion, but he could not await until his turn; rushing out of the hospital into the street he narrowly avoided being run over by a car.

After February 22nd he tried to attend school with his parents during his hospital leave and did so without any apparent trouble. He was formally discharged from hospital care on 23rd February. On 26th (Monday) and on 27th (Tuesday), he went to school and noticed that he had engaged in strange behaviours only after reading text messages from his classmates on his mobile phone. He was very much ashamed with this, but could not himself recall what he had done. After this he became fully normal and controlled. The entire episode had lasted for 18 days after commencement of Tamiflu, 16 days since the initial fever fell to normal, 14 days after the onset of lethargy, and 13 days after beginning of abnormal behaviour.

He was reluctant to attend the school's graduation ceremony on March 13th although he had been able to take an entrance examination for high school on March 7th. Once he realized that he had passed the examination, he gradually regained his usual cheerfulness.

There was no sign of alcohol consumption during the course of these events.

3. Discussion

Oseltamivir phosphate is easily dissociated in the GI tract to form oseltamivir which is absorbed from the gut and is extensively hydrolysed to OCB (Ro64-0802) and ethanol by liver microsomal carboxyesterase (hCE-1); apart from this, up to one fourth of oseltamivir is distributed via circulation and enters the brain tissue through the blood-brain barrier [13–15]. OCB is a potent selective inhibitor of influenza A and B virus neuraminidase [13–15], while oseltamivir phosphate and oseltamivir lack antiviral activity. The absolute bioavailability of OCB is 79.0% (SD 11.6%) [13–15].

3.1. Profile of adverse reactions to Tamiflu in human subjects

(1) Low body temperature.

One of the most prominent adverse reactions (AR) to oseltamivir observed in humans is low body temperature. According to the Chugai Pharm Co. (Chugai), 136 cases of reduced body temperature cases were reported to the company by June 2005. It is pointed out in the literature that low body temperature may be related to oseltamivir and the complication is not limited to children; that adult cases with low body temperature were also observed [85]. Chugai agrees that the reduction in body temperature is one of the adverse reactions to oseltamivir which may inhibit the body temperature regulating center in the brain [16].

Reduction in body temperature by inhibition of this regulating center undoubtedly means that oseltamivir readily passes the blood–brain barrier (BBB) and enters the brain not only of babies younger than one year old but also of older children and even adults who have not consumed alcohol when they are infected with influenza or other acute infectious diseases.

(2) Sudden onset psychiatric disorders including abnormal behaviour, delirium and hallucination.

Cases 1 and 2 are the typical cases showing a sudden onset of abnormal behaviour and occurrence of accidents. MHLW reported the case of a teenage girl with abnormal behaviour as a result of hallucination [62]: as soon as her body temperature fell, she ran to the window but her mother stopped her from jumping from the window, thus preventing an accident. MHLW warned of the possibility of abnormal behaviour by publishing this case in June 2004 [62]. Chugai had received 69 reports of hallucinations by June 2005 [16], though only 10 cases of hallucination and 8 of abnormal behaviour had been reported as adverse reactions to Tamiflu on the Japanese PMDA website up to March 2005 [77]. After my presentation of this issue at a scientific meeting in November 2005, 35 further incidents of abnormal behaviour were reported in three months between January and March 2006 [77].

The Japanese MHLW has announced that since 2001, when marketing of oseltamivir started in Japan and up to May 31st 2007 it had received 1377 reports of adverse reactions [63,64]. Of these, 567 were serious neuropsychiatric cases, including at least 211 showing abnormal behaviour [63,64]. Of 71 deaths reported by MHLW, accidental deaths from abnormal behaviour were noted in eight (five in teenagers, three in individuals aged 20 or over) [63,64].

FDA [27] holds reports on 103 neuropsychiatric cases (95 cases of these including 3 death cases are from Japan). 75 patients (73%) experienced neuropsychiatric symptoms after one or two doses of Tamiflu. The times of onset of symptoms from the administration of oseltamivir ($n = 58$) are as follows: 0.5 h = 12 (21%), 1–1.5 h = 12 (21%), 2–2.5 h = 8 (14%). 38 patients (66%) experienced symptoms within less than four hours, and 54 patients (93%) experienced them within some 6 hours after the last dose of Tamiflu. On the other hand, a few patients experienced adverse reactions after a full course of treatment dose and after 12 hours or more. It should be noted that the time elapsing from the commencement of Tamiflu until the onset of symptoms is very short in most of the cases.

The MHLW task force reported on October 26th 2006 the results of a survey analyzing 2846 children with flu in the winter of 2005/06, performing in order to the investigate causal relationship between oseltamivir and abnormal behaviour [63]. It calculated the frequency of abnormal behaviour in patients treated with Tamiflu and patients not yet treated for each period by dividing a day in three parts (morning, afternoon and night) for seven days and examining the cumulative frequency for the whole study period. It concluded that there was no significant difference between these two groups (11.9% vs. 10.6%: hazard ratio 1.16, 95% CI 0.90–1.49). There are however many limitations in this study. For instance, it is neither

a randomized controlled study nor a case-control study. It is merely a comparison of the situation before and after taking Tamiflu. It is not known which occurred earlier, the ingestion of Tamiflu or the event within each period of first Tamiflu intake for whole study period.

However, based on this study data, proportions of patients with abnormal behaviour in the afternoon (from noon to 6 p.m.) on the first day of fever can be calculated for patients known to have been treated with Tamiflu cases (treated) and those definitely not treated with the drug (pre-treatment and non-treatment cases: pre/non-treatment).

Proportions of children with abnormal behaviour, according to the information provided by physicians, were 0.45% in pre/non-treated cases and 1.82% in treated cases. According to the information provided by families, the proportions of children experiencing "Terror or fear" was 0.38% vs. 2.00%. The figures for "Hallucination" were 0.055% vs. 0.66%, for "Sudden screaming/delirious speech" was 0.60% vs. 2.35% and for "Anger" 0.55% vs. 2.03%. Thus relative risks (and the relevant 95% confidence intervals) were 4.02 (1.52–10.53), 5.22 (1.85–14.68), 11.99 (1.57–91.30), 3.89 (1.56–9.62) and 3.69 (1.40–9.67) respectively. However, these high relative risks were only clearly observed in the afternoon on the first day of fever and were not observed from the second day to the seventh day. This tendency coincides with FDA's analysis based on cases reported from Japan [26] and the high frequency of vomiting occurring only on the first day of the treatment [14].

The MHLW task force reported on December 25th 2007 the first preliminary results of a survey analyzing 10,316 children with flu in the winter of 2006/07 [98]. In this report there are many serious misclassifications of cases: for example, a part of cases with events were deleted from Tamiflu-prescribed group and added to non-prescribed group. This miscalculation yielded very low odds ratio (0.382: 95% CI 0.338–0.432, $p < 0.0001$). However, correct odds ratio is estimated at least 1.37 (95% CI 1.18–1.58) and up to 2.56 (1.83–3.61, $p < 0.0000001$) [39].

(3) Sudden death, hypoxia, respiratory depression and lung oedema.

Of 71 deaths reported by MHLW [64], the number of instances of "sudden death" according to its classification is 13. However, according to my analysis, in which cases of death following sudden cardiopulmonary arrests are included, the number of instances of "sudden death" was in fact 41. These 41 cases include one instance which was classified by MHLW as an "anaphylactic shock" "possibly related to Tamiflu" by MHLW but should be classified as sudden death by my classification. In this case a woman in her eighties was diagnosed as having suffered anaphylactic shock although no typical signs and symptoms of anaphylaxis such as urticaria, wheezing or evidence of laryngeal oedema were recorded on the case card. In addition, her family told the doctor that her level of consciousness suddenly declined just after taking Tamiflu, paracetamol and cefcapene pivoxil, leading to her death. However, neither the doctor nor the MHLW pointed to the presence of any typical signs and symptoms of anaphylaxis, and diagnosed her as anaphylactic shock simply because she suddenly died after taking the medicines.

In addition to the 71 deaths reported by MHLW, there were nine other sudden deaths which the ministry did not recognize as adverse reactions [38]. Of these nine cases four had already been recorded by the MHLW as adverse *events* but were not included as adverse *reactions*. Nor were my present Case 3, a literature case report by Fujii [29], an Internet report or two cases reported only by phone included in the cases that MHLW has disclosed to date.

Overall it would seem that, of the total 80 deaths on record, 50 were sudden deaths or deaths from sudden cardiopulmonary arrest (18 in those below 10 years old, 32 in those aged 20 or over). Of these,

21 were sudden deaths during sleep, 13 were sudden death with respiratory disturbance, and 17 were sudden cardiopulmonary arrests.

Of the eight cases presented here, three (Cases 3–5) died during sleep and two (Cases 6 and 7) were very nearly fatal, with severe cyanosis and seizure probably due to hypoxia from respiratory suppression.

A 3-year-old boy described in the literature [29] died within an hour while his parents were taking him to a hospital by their car after they noticed his abnormal respiration.

After the news release of my presentation in November 2005, I received phone calls and e-mails from a total of more than 50 people who had experienced (either personally or in their families) adverse reactions to Tamiflu. Among these, there were two fatalities. One was a 53-year-old male with dyspnea, cyanosis and subsequent cardiopulmonary arrest while still in the ambulance. The other was a 60-year-old male with dyspnea and cyanosis, who died in hospital after cardiopulmonary resuscitation.

Of the eight cases of my report, two (Cases 4 and 5) were autopsied and both showed marked lung oedema, changes that are frequently observed among dead rats after treatment with Tamiflu (occurring in one experiment in 9 of 18 animals treated), pointing to sudden deaths from hypoxia due to central suppression of respiration.

The following two cases whose case reports were disclosed on the MHLW website [63] are also important in considering the continuity of the spectrum covering respiratory suppression and sudden deaths due to Tamiflu.

A two years old boy (MHLW-B04026215) with hydrocephalus and an Arnold–Chiary malformation and a VP-shunt took oseltamivir phosphate 18 mg as Tamiflu dry syrup five times in four days. He became unusually lethargic on day 4 and stopped taking the drug. Late on day 4 his temperature fell to some 35°C and he suddenly suffered cardiac arrest with facial pallor. After about twenty minutes he was resuscitated in an ambulance. At a hospital his body temperature was 34°C and lung oedema was observed on chest X-ray without pneumonia; the latter recovered easily after adequate oxygenation. Brain oedema was however also observed and after repeated incidents of cardiac arrest and resuscitation, he died 85 days after the commencement of Tamiflu, probably due to hypoxic multi-organ failure.

Another previously healthy boy (MHLW-B05005388) took Tamiflu syrup for two days. His age appears to have been about ten months old, in view of the dose level of Tamiflu (16.5 mg) and the fact that he could toddle by grasping fixed objects. On day 3, he became flaccid just after waking. During medical examination he developed pulmonary arrest; he was therefore intubated and given artificial respiration manually. After three further incidences of cardiopulmonary arrest and resuscitation with the aid of a ventilator, lung oedema was observed by chest X-ray; this was relieved by the next day following adequate oxygenation. He could be weaned from the artificial ventilator on day 5 after the principal event, but he had sequels which rendered him bedridden although he could drink and eat something.

The mechanism of lung oedema is discussed below.

The spectrum and continuity of symptoms due to respiratory suppression after taking oseltamivir (i.e. sudden deaths with or without lung oedema, sudden arrests with sequels and complete recovery) are summarized in Table 2.

(4) Cases with multiple neuropsychiatric symptoms.

There are several cases exhibiting a variety of combinations of multiple neuropsychiatric symptoms occurring after taking Tamiflu; manifestations include low body temperature, hallucinations, abnormal behaviour, suppressed activity, suppressed respiration, cyanosis, dyspnea and subsequent seizure.

Among the cases reported by telephone or e-mail is that of a woman in her thirties who noted that as her body temperature fell to 34.1°C, she tried to call her family for help but could not, and subse-

Table 2

Spectrum and continuity of symptoms due to respiratory suppression – sudden death, sequels or complete recovery after taking oseltamivir

1. Sudden death without pulmonary oedema (the hypoxic period prior to death may have been too short to allow for development of lung oedema).
2. Sudden death mainly during sleep with pulmonary oedema (Cases 4, 5).
3. Sudden cardiopulmonary arrest for a substantial period; resuscitated but transient lung oedema ensued and patient died after several weeks or months due to hypoxic multiorgan failure (MHLW-B04026215).
4. Sudden cardiopulmonary arrest for a substantial period; resuscitation followed by transient lung oedema with sequels rendering patient bedridden (MHLW-B05005388).
5. Probably sudden cardiopulmonary arrest for a period with seizure; apparent recovery followed by sequels (retrograde development with subsequent retardation and gradual development).
6. At least one episode of loss of consciousness and possible hypoxic seizure; complete recovery without sequels (Case 6 and many similar cases in MHLW's reports).
7. Asthenia, dyspnea and/or cyanosis without seizure followed by complete recovery (many cases in MHLW's reports).

quently lost consciousness. After regaining consciousness, she could not move and experienced visual and auditory hallucinations.

Sugaya reported a case with low body temperature and cyanosis [86]. Among the series of cases reported in the present paper, Case 5 experienced delirium, severe dyspnea, cyanosis, suppressed respiration and subsequent seizure. His body temperature was 37.4°C. He experienced a second episode with delirium, agitation, dyspnea and subsequent seizure 6 hours after the first episode. In this case the body fell to 36.7°C about 100 min after taking paracetamol.

A Japanese case (case #5769078) reported by the FDA [26] involved a 15-year-old male patient treated with oseltamivir 75 mg b.i.d. for influenza. He experienced delirium, involuntary movement, seizure and subsequent loss of consciousness and collapse. His temperature was 38.1°C on arrival at hospital. After admission, his temperature went down to 37.6°C with stable vital signs. He experienced a second episode of delirium with abnormal behaviour at midnight but he did not remember this incident. The first EEG showed no abnormality but a second EEG showed a spine-like spike.

This case was reported as one of seizure. As noted above however, various other symptoms were also present, notably two episodes of delirium (one with decreased temperature and antegrade amnesia, loss of consciousness and collapse), with improvement over the next two days.

(5) Reactions with delayed onset and/or a prolonged course.

Case 8 is one of the typical cases with delayed onset and prolonged course. There are substantial numbers of cases in which neuropsychiatric reactions appear only after several days of treatment with Tamiflu. However, in some cases neuropsychiatric symptoms appeared after taking a few doses and they continued for more than a week or even for as long as several months. For example, a 9-year-old boy was treated with two doses of Tamiflu for his flu A [44]. Even after his body temperature had decreased to normal, he had not fully recovered and he experienced reduced consciousness with amnesia for a week. These symptoms recurred about a month later without any triggering factor and on this occasion lasted about a week.

There are in total 22 known deaths which are neither sudden deaths nor accidental deaths resulting from abnormal behaviour [59,60]. Of those, four are deaths from sepsis following exacerbation of pneumonia after possible respiratory suppression. Nine cases are possibly related to exacerbation of mainly

pneumonia. Gastroinestinal (GI) bleeding occurring six days after one dose of Tamiflu 75 mg was the main cause of death in a case treated with dialysis due to renal failure.

GI bleeding was observed in eight patients in all including three cases classified as instances of sudden death (two boys under 10-year-old and a man in his thirties) [63,64]. Bleeding was one of the complications in four adult cases classified under the headings severe infection or sepsis [63,64].

Beside these disorders recorded as causes of death, hyperglycemia is one of the typical delayed reactions to Tamiflu. This conclusion is based on the analysis of several randomized controlled trials and the adverse reaction is described in the New Drug Approval Package (NAP) of oseltmivir (in Japanese): Tamiflu capsule for treatment [13].

(6) Reactions of allergic origin and other possible complications.

A further five deaths were associated with disorders of an allergic nature: two involved fulminant hepatitis with hepatic failure (one with a positive and one with negative drug-induced lymphocyte stimulation test: DLST), and the three others involved toxic epidermal necrolysis, pancytopenia and agranulocytosis respectively [63,64].

Acute hemorrhagic colitis induced by oseltamivir with positive DLST for oseltamivir was reported [100].

The clinical course of death could in three cases not be classified, as the available information was insufficient.

(7) Summary of profile of adverse reactions to Tamiflu.

In view of the above, the serious adverse reactions to oseltamivir reported so far may be roughly classified into three groups:

(1) Sudden onset adverse reactions related to the central nervous system suppressant action of oseltamivir:
   (a) *Sudden death, mainly during sleep, after complaints of dyspnea or abnormal respiration, or death with sudden pulmonary or cardiopulmonary arrest.* Somnolence, sleep, vomiting, headache and/or hypothermia may be frequently observed as initial prodromal symptoms. Dyspnea, cyanosis, agitation, or loss of consciousness with grand mal seizures may be often observed just before the sudden death. However, sudden death might also occur during apparent sleep.
   (b) *Abnormal behaviours and other acute onset neuropsychiatric disorders.* Low body temperature, hallucinations, agitation and/or difficulty of movement may be observed before abnormal behaviour is noted. Visual, auditory or pain hallucination may occur and even suicidal ideation or suicidal attempts have been reported.

(2) Delayed onset serious adverse reactions such as delayed onset neuropsychiatric reactions with prolonged course, pneumonia, sepsis, bleeding and hyperglycemia. Reactions of this type usually occur after taking several doses or a full course of Tamiflu. This type of complication could however occur even after taking a single dose in cases of severe renal failure because of a prolonged high plasma concentration of OCB.
(3) Reactions of allergic origin such as fulminant hepatitis, toxic epidermal necrolysis, agranulocytosis, pancytopenia and others.

3.2. The toxicity profile of Tamiflu in animals

(1) Deaths.

Sudden deaths were observed in at least three animal toxicity studies submitted to the MHLW [14,15]:

(a) In a dose finding toxicity study on 7-day-old rats, 18 of 24 treated with 1000 mg/kg of oseltamivir phosphate (OP) (i.e. 761 mg/kg of oseltamivir (OT)), died within seven hours after the treatment. Vacuolization of liver cells was observed in all dead rats and lung oedema was also observed in nine of the 18 dead rats on histological examination. No death was observed in 500 mg/kg (381 mg/kg of OT) or lower dose groups including the vehicle group.

(b) In a series of 7-day-old rat toxicity studies, it was noted that two to three hours after the first dose of OP had been administered, 2 of 14 rats died in the 700 mg/kg (533 mg/kg of OT) group and 3 of 14 rats died in 1000 mg/kg group. Symptoms such as decreased body temperature, decreased spontaneous movements and slow and/or irregular breathing were observed in 6 of 14 rats in the 700 mg/kg group and 12 of 14 rats in 1000 mg/kg group. Tremor was observed in one rat and collapse was observed in another rat among 14 rats of 1000 mg/kg group.

(c) In a series of toxico-kinetic tests, young rats were treated with a single OP dose of 1000 mg/kg. Seven of 56 seven-day-old rats died between 10 minutes and 4 hours after a single dose of OP. Symptoms such as decreased body temperature, paleness and decreased spontaneous movements were observed in 8 of 56 rats. One of twenty eight 14-day-old rats died 10 minutes after the treatment. No particular abnormality was however in the surviving or dead animals. No drug-related deaths were observed among twenty eight 24-day-old rats or twenty eight 42-day-old rats.

(2) Symptoms suggesting central nervous system suppression.

Symptoms such as decreased body temperature, decreased spontaneous movements and slow/irregular breathing before death and frequent findings of lung oedema at autopsy suggest that the major cause of death is probably respiratory suppression due to central nervous system suppression.

(3) 64 times higher concentration in the immature brain.

The ratio of maximum concentration (Cmax) of OT in the brain of 7-day-old rats to that of mature (42-day-old) rats was about 64 and the ratio of Cmax of OT in 7-day-old rats' brain to plasma was about 0.81. A ratio of Cmax of active metabolite (OCB) in the brain of 7-day-old rats to that of mature (42-day-old) rats was only 3.1. The ratio of Cmax of the active metabolite in 7-day-old rats' brain to that in plasma was 0.72 [15].

(4) The non-fatal rat dose was 10–20 times higher than clinical dose.

Non-fatal dose in rats (500 mg/kg of OP) is about 100 times the recommended human dose in children (5.3 mg/kg/day as OP or 4 mg/kg/day as OT) calculated on a mg/kg basis for OP, but it is only 10–20 times the recommended dose in terms of $AUC_{0-24\ h}$ of OCB. No data are available for comparison between concentrations of oseltamivir in the brain and plasma of 7-day-old rats and those measured in human infants less than one year of age.

(5) Other toxicities: pneumonia, GI bleeding and renal toxicities [13].

Three of six rats treated with 100 mg/kg (equivalent to free form) of OCB intravenously for two weeks developed acute alveolitis. Of the three, one exhibited wheezing on day 14 and was sacrificed the next day. Diffuse hemorrhagic alveolitis (pneumonia) and pulmonary microvascular thromboembolism

were observed in this animal. The $AUC_{0-\infty}$ of 100 mg/kg of OCB administered intravenously was 53.9 μg·h/ml, which is only 12 times higher than the average human AUC_{0-24} (4.6 μg·h/ml) on day 7 in individuals treated with 75 mg oseltamivir b.i.d. The $AUC_{0-\infty}$ of safe level i.v. doses (20 mg/kg) in rats was 8.55 μg·h/ml, i.e. less than twice the usual human AUC.

Lung oedema with congestion was observed in one female dead rat among ten treated with 2000 mg/kg of OP for three days in an oral toxicity test for two weeks.

Leukocytosis, an increased glucose level, histological change in renal tubules, increased relative weight of liver and kidney were also observed in various doses and various animals treated for various periods.

In the marmoset monkey 7-day oral toxicity tests, one of four animals treated with 2000 mg/kg of OP was sacrificed on day two, because of dying after severe vomiting, sleep, slow movement and collapse. Other three animals were all sacrificed on day four and the test for this dose group was discontinued. In all of the animals killed, reddening of the stomach mucosa macroscopically and mucosal bleeding with erosions, ulcers and atrophy was observed in the stomach histologically. In the animal killed on day two, these findings in duodenum and jejunum were also observed with macroscopically swollen small intestine. In stead of 2000 mg/kg, new dose group was started at the level of 1000 mg/kg of OP. In this dose group, reddening macroscopically and atrophy histologically of the stomach mucosa were observed. Vomiting was observed in the 500 mg/kg OP group, but this level was considered as non-observed adverse effects level (NOAEL) by the manufacturer, though the lowest level (100 mg/kg) should be the real NOAEL.

The safety index (animal AUC_{0-24} with no toxicity by human average AUC_{0-24} when taking 75 mg capsule b.i.d.) is only 3 for the four-week toxicity studies in rats and the six month oral toxicity studies in rats, 8 for the two week oral toxicity study in rats and 10 for the marmoset monkey 7-day oral toxicity study.

(6) Interaction with alcohol (acting as a partial agonist and antagonist).

Recently, in 28 weeks old rat experiment, oseltamivir (50 mg/kg ip) administered two hours prior to injection of ethanol (3.3 g/kg ip) shortened the duration of the loss of righting reflex (LORR) induced by ethanol, while the rectal temperature measured one hour after ethanol injection was significantly lower in rats treated with oseltamivir than in those not receiving oseltamivir treatment [46]. In the CA1 region of hippocampal slices, oseltamivir (100 μM) induced paired-pulse facilitation in population spikes without changes in excitatory postsynaptic potentials. Similarly, 3 μM OCB facilitated neuronal firing, though the facilitation did not involve GABAergic disinhibition [42]. These results apparently mean that oseltamivir may act as an agonist while OCB acts as an antagonist to ethanol.

However, none of eight human cases presented here was reported to have taken alcohol. Only two cases among hundreds of serious neuropsychiatric adverse reactions to Tamiflu (including instances of sudden death) were reported to have been taking alcohol.

The quantity of ethyl alcohol released when 75 mg of oseltamivir is fully metabolized to OCB is only 11 mg. This may be too small to affect one's neuropsychiatric state including respiration compared with the levels resulting from ethanol injection in animals (3.3 g/kg ip as above) or human consumption of alcohol (about 20–25 g in a bottle of beer or two glasses of wine).

3.3. Similarity of symptoms and findings in animals and in human

Table 3 shows that oseltamivir has almost exactly the same effects in humans and in animals except for the psychiatric symptoms which are difficult to demonstrate in animal toxicity studies. The spectrum

Table 3
Similarity of symptoms and histological findings in animals and human subjects after treatment with oseltamivir-p

Symptoms and findings		Humans	Animals: rats and marmosets*
General symptoms	Temperature	Low temperature	Lowering of temperature
	Movement/ behaviour	Could not move, could not speak even when attempting to do so (suppressed behaviour), abnormal behaviour (excitatory behaviour)	Decreased spontaneous movement (suppressed behaviour)*
	Sleep	Somnolent	Prone to sleep*
	Respiration	Suppressed respiration, abnormal respiration, shallow and weak respiration, irregular and mixed patterns (deeper and lighter respiration) respiratory arrest	Slow and weak respiration, irregular respiration
	Face appearance	Pale, cyanosis, blackish hue	Cyanotic before death
	collapse	Collapse, cardiopulmonary arrest	Collapse*
	Death	Death	Death
Psycho-sensory symptoms	Abnormal behaviour Hallucination/ delirium	Abnormal behaviour, Hallucination/delirium	These symptoms may be difficult to detect in animal experiments and have never been investigated in animal toxicity studies of oseltamivir-p.
	Loss of consciousness	Decreased level or loss of consciousness, anterograde amnesia	
	Visual abnormality	Besides visual hallucinations, misleading visual impressions of real objects (size, patterns).	
	Auditory abnormality	Normal sounds appear very loud or non-existent sounds are experienced. Patient may cover both ears to protect from supposedly loud noises.	
Pathological/ histological findings	Lung, heart and brain	Marked pulmonary oedema is often observed in the sudden and autopsied death cases (8 of 11 including our cases). Temporary pulmonary oedema may be observed in the resuscitated cases. Brain oedema and/or bleeding may also be observed in sudden death cases.	Lung oedema was observed in nine of 18 dead rats. No macroscopic or histological abnormalities were reported for the brain. But it does not mean the possibility. GI bleeding is frequently observed in the high dose group of marmoset monkey.

of effects in humans and in animals including lung oedema is exactly the same as that of central nervous suppressants such as benzodiazepines and barbiturates.

3.4. Neuropsychiatric symptoms and disinhibition due to CNS suppressants

Respiratory suppression and abnormal behaviour are different effect profiles of central nervous system suppressants.

It is well established that benzodiazepines and barbiturates that induce respiratory suppression may cause bizarre uninhibited behaviour including anxiety, irritability, hallucinations, and hypomanic behaviour or even hostility and rage as a result of disinhibition or dyscontrol reactions [7]. Paranoia, depression and even suicidal behaviour may also occasionally accompany the use of benzodiazepines and barbiturates [7].

Table 3
(Continued)

Symptoms and findings		Humans	Animals: rats and marmosets*
Significant differences between fatal and surviving cases	Very slight differences seem to determine death or survival	Even life-threatening cases recovered without sequels except in two instances, but there are many fatalities. There seem to be very slight differences between fatalities and surviving cases. Most of the surviving cases recovered within a few days, though delirium and psychiatric symptoms occasionally continued for more than a few months.	None of the surviving animals cases had pathological changes. No deaths were observed in the 500 mg/kg group while few died in the 700 mg/kg group and most died in the 1000 mg/kg group. No abnormal findings were observed except vacuolization in liver cells of all dead rats and lung oedema in 9 of 18 dead rats
	Timing of onset of reaction	Symptoms appear at the first dose or on the first day of administration in most cases. Symptoms usually subside even on continuation, as transport of OP to the brain decreases in parallel with the improvement in the influenza. Symptoms occurred on day 2 to day 3 in some cases.	In rats before weaning, most deaths occur following the first dose. As animals grow older, BBB function develops and oseltamivir is prevented to enter into the brain by increased efflux transporter function of BBB. In some mature marmoset cases, symptoms appeared on day 2 to day 4.

* Two male and two female marmosets which weighed around 400 g were treated with 2000 mg/kg of oseltamivir-p. Of these four, one exhibited suppressed behaviour, fell asleep, collapsed and died on day 2. The remaining three were sacrificed on day 4 (therefore, all were reported as "dead"). All animals hemorrhaged in the GI tract (erosions, ulcers, hemorrhage and atrophy). No toxico-kinetic data including Cmax and AUC were available for this experiment.

These forms of disinhibition or "dyscontrol" may all be viewed as different expressions of a broad spectrum of effects exerted by central nervous system suppressants such as benzodiazepines and barbiturates.

3.5. Differences from infection-associated encephalopathy including Reye's syndrome and/or influenza-associated encephalopathy

Following restrictions on the use of NSAIDs as antipyretics for children in Japan in 2000 [37], the proportion of NSAIDs users among cases of Reye's syndrome and/or influenza-associated encephalopathy decreased from about 30% to below 10% and the proportion of case fatalities resulting from influenza-associated encephalopathy decreased from about 30% to about 10%. Two years (i.e. two winter seasons) elapsed during which the proportion of case fatality of influenza-associated encephalopathy decreased, before the marketing of Tamiflu dry syrup for children in Japan commenced in September 2002.

I have collected nine papers reporting on 15 animal experiments designed to investigate the effects of NSAIDs on mortality in infected animals [19,23,45,46,49,52,71,72,83]. One experiment was excluded because proportions of death of both groups were 0; reports on 14 experiments were therefore examined. Various NSAIDs were tested including ibuprofen, flurubiprofen, mefenamic acid, indomethacin, salicylates and so on. Various microorganisms including viruses, bacteria and protozoas were used. Proportions of death from these experiments were meta-analyzed. Peto odds ratio for NSAIDs use on proportion of death in infected animals was 7.54 with 95% confidence interval (CI): 4.50–12.66 ($p < 0.0001$) and I^2 (inconsistency) = 9% (95% CI: 0–52.1%) [36]. Other evidence suggesting NSAIDs as a major cause of fatal influenza-associated encephalopathy is to be found in a case-control study reported in the Japanese Task Force's paper "A case control study on factors related to onset and severity of influenza-associated encephalopathy" [81]. Three children among four fatal cases from influenza-associated encephalopathy took NSAIDs, while among 84 controls (flu without encephalopathy) only five (6.0%) had taken

NSAIDs. A strong association between NSAID use and fatal influenza-associated encephalopathy was thus observed: the crude odds ratio was 47.4 (95% CI; 3.29–1458, $p = 0.0019$) [36], though the task force reported that the study could not demonstrate any definite relation of NSAIDs to the occurrence of influenza-associated encephalopathy. The odds ratio for paracetamol was not significant (OR 2.25; 95% CI; 0.19–58.6) [74].

The clinical course of sudden death and accidental death from abnormal behaviour after taking Tamiflu is very different from that seen in Reye's syndrome or influenza-associated encephalopathy. It is reported that the latter usually continue for less than two or three days until proving fatal [85], but they run for at least a half day or one day even in the most severe cases. However, in the Tamiflu cases, an infant may stay well for the first few hours after taking a single dose of the drug, but soon later he or she may deteriorate suddenly and stop breathing within ten minutes. This is one of the most important differences between the previously so-called "influenza-associated encephalopathy" or "infection-related encephalopathy" and this newer complication.

This new type of encephalopathy among infants was first found in the winter of 2002/2003 just after the marketing of Tamiflu dry syrup for children had started. However a similar adult case of sudden death had been already reported in March 2001 [MHLWB01-529], just after the Tamiflu capsule was marketed in February 2nd 2001 in Japan. A man in his sixties who had usually been healthy developed a 39°C fever; he was suspected of having flu and was treated with Tamiflu 75 mg b.i.d. Several hours after taking the second dose of Tamiflu he got worse and consulted another hospital. Although his condition was not deemed so serious as to merit urgent treatment, he suddenly went into arrest immediately after arriving at a further hospital and died about two hours later from multi-organ failure.

In Case 5, the patient was treated with NSAIDs (dipyrone and naproxen). These might have influenced the development of myocardiopathy by enhancing the induction of cytokines in viral infection [57]. The fatal course may however be too short for acute left ventricular failure to be established without the contribution of lung oedema caused by Tamiflu.

Times of onset of most sudden deaths and of neuropsychiatric symptoms [25] are very similar. These facts also suggest that the majority of sudden deaths and neuropsychiatric symptoms after taking Tamiflu are different from the pattern observed with infection-associated encephalopathy including Reye's syndrome and/or influenza-associated encephalopathy

3.6. Non-Tamiflu-related sudden death and seizure-inducing drugs

It has been claimed that sudden death could occur due to influenza itself. However, in spite of a thorough search, I have never seen any report of sudden death caused by influenza. Sudden deaths that are believed to be caused by influenza are actually induced by the drugs used to treat it. Six child cases of sudden death were observed during the 2002/03 winter season [84]. All of these cases were found dead during sleep; three died during daytime naps and the other three at night. Although four of them took only a single dose of Tamiflu and an 8-year-old boy took amantadine, one boy aged a year and seven months had according to the original reported taken no drug [84]. This "non-drug" case is sometimes referred as an example of sudden death caused by influenza. However, it was found later that the boy had in fact been given theophylline [99]. The cause of the sudden death of this infant was thus probably either cardiac arrhythmia and/or hypoxia due to a seizure caused by theophylline.

Seizure is a well documented dose-related toxic reaction to some drugs including both amantadine and theophylline [7,24]. In experiments with a pentylenetetrazol convulsion model, it has been found that amantadine in a dose of 25 mg/kg and particularly in a dose of 100 mg/kg potentiates convulsive seizures

[58]. In an electroshock test, amantadine decreased the convulsive threshold [52]. As to theophylline: interferon reduces the drug's clearance and increases its elimination half-life in human subjects [95]. The concentration of theophylline thus increases when one has influenza especially with high fever.

In the case of a one-year-and-seven-month-old boy, when an ambulance doctor arrived and saw him within a few hours after death, the doctor found rigor mortis already in his body [99]. It is also well documented that if one has experienced a seizure and/or high fever just before death, rigor mortis tends to appear earlier than usual. Evidence suggesting that this boy may well have experienced a seizure prior to death is the fact that his twin brother, who also had influenza during theophylline treatment for his asthma, experienced a seizure one hour after the mother noticed his brother's death [99].

I believe that sudden death during sleep occurs only in those patients treated with oseltamivir, other central nervous suppressants, seizure-inducing drugs including theophylline and hypoglycemic drugs and/or proarrhythmic drugs.

3.7. Fever delirium and Tamiflu delirium

Delirium or psychosis is not a rare complication of infection [72] and it has been claimed by some specialists in pediatrics that delirium after Tamiflu treatment may in fact be fever delirium [86]. There are however many reported cases in which delirium or hallucination after taking Tamiflu occurred at low body temperatures: for example, as low as 34–35°C.

In order to analyze the relationship between body temperature and abnormal behaviour, I analyzed two groups of suspected delirium cases, namely cases with no drug history and Tamiflu-treated cases collected from phone-calls/e-mails and from the Internet. 67 delirious cases were collected in total, 15 non-drug cases and 52 Tamiflu-treated cases including 35 phone-calls/e-mail cases. Information about body temperature was available for 12 non-drug cases and 35 oseltamivir cases. The differences between fever delirium and abnormal behaviour after Tamiflu treatment are summarized in the Table 4(A). 80% of instances of delirium or abnormal behaviour occurred in the absence of fever or after the temperature had started to fall after taking Tamiflu, while only one among 12 non-drug cases occurred in the absence of fever (Odds ratio = 44.0; 95% CI: 4.37–1081.12, $p = 0.000018$).

To perform another comparison, I searched PubMed and "Japonica Centra Revuo Medicina" (a database of Japanese medical journals) using the key words "fever" and "delirium" and found four papers [48,72,73,88] in which the temperature of patients with delirium was described. All papers were

Table 4

Comparison of temperature during delirium (with Tamiflu, untreated or published cases of apparent fever delirium in the literatures)

	Treated with Tamiflu	(A) No drug treatment	(B) Published cases of fever delirium
Delirium in absence of fever	28 (80%)	1[a] (8%)	81 (37.0%)
Delirium with high fever	7 (20%)	11 (92%)	138 (63.0%)
Odds ratio of delirium occurring without fever on Tamiflu treatment as compared with controls (95% confidence interval[b] and p value[c])		44.0 (4.37–1081) $p = 0.000018$	6.81 (2.68–18.02) $p = 0.0000018$

[a] One case with only nasal symptoms before fever developed. [b] Cornfield 95% confidence limits for OR using EpiInfo Version 3-3-2. [c] Fisher's exact test 2-tailed p-value using EpiInfo Version 3-3-2.

from Japan and reported a total of 226 fever delirium cases among which temperatures were known in 219 cases. Although authors did not state whether NSAIDs antipyretics and antihistamines were used or not, there is no doubt that many of these patients were treated with them. However, the proportion of patients with a body temperature lower than 39.0°C during fever delirium was only 37.0% (81/219) which is far less than the 80% (28/35) in patients with delirium treated with Tamiflu. Odds ratio was 6.81 (95% CI: 2.68–18.02, $p = 0.0000018$ Table 4(B)). Only 10.9% of fever delirium patients reported in papers (24/219) had temperatures below 38.0°C.

3.8. Brain/lung oedema and hypoxia

(1) *The drug in the brain and its elimination by efflux transporters in the blood–brain barrier*.

Drugs acting on the central nervous system (CNS) have to cross the blood–brain barrier (BBB) or the blood-cerebrospinal fluid barrier. Recent studies have shown that this is not only a static anatomical barrier but also a dynamic one in which efflux transporters play a role [4,7,87] on the apical cell membrane of brain capillary endothelial cells adjacent to the blood lumen.

The fact that oseltamivir levels with a Cmax 64 times higher are found in the brain of 7-day-old rats compared with 42-day-old rats [15] indicates that the drug might not be easily eliminated from the endothelial cells by the immature efflux transporters.

(2) *Increase of intra-cranial pressure*.

There are several reasons to conclude that Tamiflu increases intra-cranial pressure. For example, a 5-month-old male infant who was treated with Tamiflu for prevention of influenza vomited one and a half hours after receiving the drug and his mother noticed his fontanelle was bulging [26]. After the second dose of Tamiflu in the next evening, he did not vomit but his fontanelle bulged again. His mother described how "the large fontanelle bulged one or two hours after administration of oseltamivir in the evening and returned to the usual size by the next morning". She said that this phenomenon was repeatedly observed throughout the treatment period of eight days [26]. This is one of the firm pointers to an increase in intra-cranial pressure induced by oseltamivir itself, since the infant did not have influenza and at this age the BBB might not yet have matured.

In a randomized controlled trial of Tamiflu for the prevention of adult influenza, headache, nausea and vomiting were complained of significantly more often in the Tamiflu group than in the placebo group [15]. The Number Needed to Harm (NNH) for inducing headache, nausea and vomiting was 25, 24 and 55 respectively. This again suggests that Tamiflu increases intra-cranial pressure.

In a randomized controlled trial of Tamiflu for treatment of influenza in children, vomiting only on day 1 was observed significantly more often in the Tamiflu group than the placebo group (odds ratio 3.4: 95% CI 1.9–6.1) [14]. NNH for inducing vomiting on day 1 was 15, while the odds ratio for vomiting on day 2 or later was 0.8 (95% CI: 0.47–1.4). This once more suggests that Tamiflu increases intra-cranial pressure only on day 1 when used to treat influenza.

(3) *Brain oedema and aquaporins*.

Recent studies have shown that aquaporins (AQPs) play an important role in the induction and resolution of oedema in various organs and tissues [5,45,51,54,60,75] including brain [54,45,75] and lungs [5,54,75]. AQP4 is upregulated in response to cerebral oedema induced by various agents or factors [54,75]. AQP4-mediated transcellular water movement is crucial not only for the development of brain oedema after intoxication and ischaemic stroke, but also for fluid clearance in vasogenic brain oedema [54].

(4) *Lung oedema and aquaporins*.

Epithelial Na^+ channel (ENaC), Na^+/K^+-ATPase pumps, and several aquaporin water channels are the best described molecular transporters in the lung when pathological conditions lead to the development of pulmonary oedema. Lung oedema results from the impairment of alveolar cells and/or capillary endothelial cells, both of which actively transport fluids from the alveolar space to the blood vessels. In acute lung injury (ALI), especially in severe sepsis (systemic inflammatory response by infection), an inflammatory process damages the capillary endothelium rather than the alveolar epithelium, resulting in high permeability of the lung capillaries to fluid, which leads to clinical pulmonary oedema. In contrast to the endothelium, the alveolar epithelium is often spared in ALI, and the rate of alveolar fluid clearance (AFC) in ALI can therefore be maintained and perhaps even increased [102].

(5) *Lung oedema, brain oedema and hypoxia*.

Although the role of Tamiflu in the development of lung oedema in two of the autopsied cases is not completely clarified, it is at least possible that severe hypoxia induced by the respiratory suppressive action of oseltamivir might have contributed to the induction of lung oedema just prior to respiratory arrest in both cases. The difference between dead and surviving humans and animals is very impressive, although intermediate cases also occur [63,64]: fatal cases exhibited severe and frequent lung oedema both in animals and human subjects, while complete recovery was observed in surviving animals and most of the human cases. In the present clinical series for example, fatal Cases 4 and 5 had severe lung oedema, while Case 6 and case #5769078 reported by the FDA completely recovered without sequels [26]. There are many such cases in the published data [63,64] (Table 2).

There are some intermediate cases in one of which a boy died after serious hypoxic brain damage with transient lung oedema just after he had been resuscitated. Another boy had very severe sequels following the occurrence of temporary lung oedema just after he had been resuscitated.

Lung oedema following severe hypoxia is frequently observed in various diseases such as acute asphyxia [6], sleep apnoea syndrome [11,12,10,28,98], and high-altitude disease [2,41,42,94], but also immediately after extubation [47], and under the influence of drugs including sedatives [64,76] and others [19,83]. When severe hypoxia results from high-altitude disease, brain oedema is observed in addition to lung oedema [41], and has often resulted in death [41]. High-altitude pulmonary oedema (HAPE) is the most common cause of death from exposure to high altitudes [1,41].

Hypoxia downregulates the synthesis and activity of both ENaC and Na^+/K^+-ATPase time- and concentration-dependently in cultured alveolar epithelial cells [20,76]. Interestingly, this effect is completely reversed after reoxygenation [20,76].

AQP-independent water transport, involving either alternative transcellular water channels or paracellular pathways, plays a major role in Alveolar Fluid Clearance [91].

3.9. Sequels and delayed neuronal cell damage following cardiac arrest

Case 7 and MHLW's two cases (B04026215 and B05005388) had sequels or hypoxic multi-organ failure after cardiopulmonary arrest and resuscitation. Though it is not known whether oseltamivir causes this type of sequels, I believe that the clinical courses of these three cases are compatible with that of the neurological sequels following global ischemia [3,22,55]. However various other disorders such as acute disseminated encephalomyelitis (ADEM) [23,31], Rett syndrome [71,93] and various causes of apparent life threatening events (ALTE) in infants [61] should be differentiated as a cause.

A wide spectrum of neurological sequels may follow global ischemia, ranging from brain death, vegetative states, and impairment of higher intellectual function to syndromes characterized by amnesia and

cortical blindness, post-anoxic myoclonus, delayed leukoencephalopathy, and spinal stroke [3] including paraplegia [22] or lower-limb paralysis [55].

129 patients underwent surgical repair of thoracoabdominal aneurysms, with an overall 30-day mortality rate of 35% [22]. Spinal cord ischemia occurred in 25 cases (21%) of 116 who survived operation. Partial ischaemia occurred in six cases (25%), and complete paraplegia occurred in the remainder [22].

Animals experiencing 12 minutes of hemorrhagic shock followed by 5 minutes of cardiac arrest showed severe neuronal damage in the *Cornu Ammonis* field CA1 region of the hippocampus, severe hind-limb paralysis and neuronal damage in the lumbar spinal cord 6 hours to 7 days after resuscitation [55]. In view of the results of this experiment, it was suggested that hind-limb paralysis after global ischemia might result from spinal cord damage.

Loss of intellectual ability including speech and behaviour, minimal atrophic change in the right hippocampus and minimal development in the sylvian fissure, suspected in the light of MRI findings in the present Case 7 are compatible with damage in the hippocampal area. The account of this patient's crawling movement ("dragging her legs") some days after the event is compatible with paraplegia or hind-limb palsy after global ischemia. Thus it is reasonable to suppose that this girl may have suffered delayed neuronal cell damage in the central nervous system, especially in the hippocampal area and in the lumbar spinal cord, due to global ischemia after cardiopulmonary arrest caused by the oseltamivir-induced inhibition of respiratory function. The very slight worsening observed after the mother first noticed her behavioural and mental abnormalities may well rule out the possibility of acute disseminated encephalomyelitis (ADEM) since in that condition a monophasic peak of symptom worsening is more usually observed [23,31]. The very slight changes found on the MRI similarly seem incompatible with that condition.

More recently, this girl has shown some gradual development, which means that her development retardation is not progressive. Absence of stereotypic hand movements also argues against the presence of Rett syndrome [93]. In the present case, the abnormality occurred abruptly after the event. Retardation of an infant with Rett syndrome starts after apparently normal development for more than 6 months, but not so abruptly [71] as in her case. Her breathing disturbance appeared shortly after she took Tamiflu. She has never shown breathing disturbances while awake except during the acute event. These are other reasons why a diagnosis of Rett syndrome seems inappropriate in her case.

Seizure, gastro-oesophageal reflux disease (GORD), respiratory syncytial virus (RSV) infection, pertussis or other lower and upper respiratory tract infections (LRTI and URTI), aspiration pneumonia, asthma and other causes including ear, nose and throat problems, cardiac problems including cardiac arrhythmia or QT prolongation, inborn metabolic disorders were listed as causes of hypoxia in a review by McGovern [61]. Ingestion of drugs or toxins was reported in eleven patients (1.5% of all diagnoses in that paper).

Seizure may not have been a primary cause of the hypoxia seen in Case Nr. 7 but could have been secondary to hypoxia due to respiratory suppression. She had no gastro-oesophageal reflux and was not so severely infected as one would expect with RSV infection or pertussis. Patients whose causes of life-threatening events are suspected to be LRTI, URTI or other forms of infection may be treated with various medicines. In such cases, the possibility that these medicines themselves have induced the life-threatening event cannot be ruled out. Patient Nr. 7 did not aspirate and had no asthma. Her QT interval was 0.397 s and she has never had syncope suggesting Adams-stokes syndrome either before or after the event. She has never been suspected to have any inborn metabolic disorder.

3.10. Delayed reactions and inhibition of human sialidase (neuraminidase) by OCB

Li et al. [59] reported that they identified a nonsynonymous SNP (single nucleotide polymorphism) in dbSNP database, R41Q, near the enzymatic active site of human cytosolic sialidase HsNEU2, a homologue of the virus neuraminidase that is the target of OCB. This SNP could increase the unintended binding affinity of human sialidase to OCB, thus reducing sialidase activity. Theoretically administration of oseltamivir to people with this SNP (present in 9.29% of Asian populations but absent in European and African American subjects) might further reduce their sialidase activity and these authors suggest that this Asian-enriched sialidase variation caused by the SNP, probably in homozygous form, may be associated with certain severe adverse reactions to oseltamivir [59].

In the CA1 region of hippocampal sections, OCB facilitated neuronal firing, without involving GABAergic disinhibition, and OCB produced further facilitation following administration of ethanol [46]. From these findings, the authors indicated that oseltamivir has an effect on the central nervous system, especially when combined with other agents such as ethanol.

Neither of the two papers cited above [46,59] refers to sudden deaths during sleep as a major component of the adverse reaction profile of Tamiflu to which the central inhibitory effect of unchanged oseltamivir probably contributes. However, reduction of human cytosolic sialidase activity by OCB might be a possible mechanism involved in delayed type adverse reactions to Tamiflu including not only neuropsychiatric reactions but also hyperglycemia, pneumonia, exacerbation of infection, renal and hepatic disorders, GI tract hemorrhage and others [63,64].

In mammalian cells, four types of sialidase have been identified. They are classified according to their major intracellular localization as intralysosomal sialidase (NEU1), cytosolic sialidase (NEU2), and plasma membrane-associated sialidases (NEU3) and mitochondrial sialidase (NEU4) [96,97].

Hepatic NEU3 may be associated with sensitivity to insulin and to glucose tolerance through modification of ganglioside composition and peroxisome proliferator-activated receptor gamma signaling [101].

Introducing a review on sialidase and cancer [66], the authors make the following general point:

> Sialic acids are generally found in the terminal position of the carbohydrate groups of glycolipids and glycoproteins. They have been proposed to play important roles in various biological processes by influencing conformation of glycoproteins, recognizing and masking biological sites of the molecules and cells. The removal of the sialic acids catalyzed by sialidase is an initial step in the degradation of glycoproteins and gangliosides. Sialidases of mammalian origin, therefore, have been implicated not only in lysosomal catabolism but also in regulation of functional molecules involved in many biological phenomena [62,73] through modulating sialoglycoconjugates. In fact, the lines of evidence for their involvement in cellular events including cell differentiation, cell growth, and apoptosis have been accumulated. Alterations in sialylation during malignant transformation have been observed to be closely associated with malignant phenotype in terms of metastatic potential and invasiveness, although no definite conclusion between sialic acid contents and malignant properties could be drawn because of some controversial experimental results.

Sialidase is also involved in a wide variety of other physiological processes, including immune functions [82,89,92] such as helper T cell, neutrophil, cytokines and so on [92]. It might be involved in the late onset pneumonia, and in the aggravation of infection leading to sepsis with multiorgan failure including precipitation of a disseminated intravascular coagulation syndrome (DIC).

Long term impairment of Immune function may increase the susceptibility to cancer development. The pharmaceutical company concerned has reported on two long term carcinogenesis studies, cited in

the new drug approval package (NAP) of oseltamivir capsules for preventative use [15]: they comprise two-year studies in mice and in rats.

In the two-year mouse carcinogenesis study, the proportion of deaths and of liver cell tumours, both seen in male mice, significantly increased in a dose-dependent manner, although the pharmaceutical company denied that there was a causal relationship. The "safe" level was 125 mg/kg, which is only 6 times higher than the human therapeutic dose level (calculated on the basis of the AUC of OCB) and this may explain the chronic toxicity observed in the animals.

In the two-year carcinogenesis study of Tamiflu in rats (75 males and 75 females for each dose level: 0 mg/kg, 0 mg/kg, 50 mg/kg, 150 mg/kg, 500 mg/kg), the proportion of animals developing lymphoma (male: numbers are 0, 1, 1, 1, 3 respectively), epithelial tumor of the thymus (female: 0, 1, 1, 2, 3), angioma (male: 2, 1, 1, 3, 5) was significantly increased in a dose dependent manner, although the pharmaceutical company denied that there was causality. A completely safe dose derived from the rat carcinogenesis study may be 50 mg/kg which is only 1.6 times higher than the clinical dose level for treatment based on the AUC of OCB. The intermediate level is about 5.8 times higher.

There is only one reported case of acute hemorrhagic colitis induced by Tamiflu but in that instance an allergic mechanism was involved [100]. However, among the 80 fatal cases now known, bleeding episodes were described in eight, including one case without severe infection, sepsis or multiorgan failure (see Section 2.5).

Plasminogen binds neuraminidase and is activated to form plasmin. Neuraminidase is therefore a plasminogen receptor and its plasminogen-binding activity determines the pathogenicity of the WSN virus (a mouse-adapted human isolate A/WSN/33 (WSN), a neurovirulent influenza virus strain that causes systemic infection when inoculated intranasally into mice) [30].

One certainly cannot exclude the possibility that activation of normal human plasminogen by human neulaminidase (sialidase) might be inhibited by OCB, in which case normal fibrinolysis might be affected, resulting in abnormal coagulopathy including DIC.

3.11. Limitations of postmortem measurement of oseltamivir and OCB level

One problem encountered in current work is the limited sensitivity of the methods most commonly used to detect the relevant substances in the system. On July 3rd 2006, for example a 14-year-old boy died from an accident resulting from abnormal behaviour after taking Tamiflu; the case was reported by the media on July 4th 2006. In the course of the autopsy required by law the concentration of oseltamivir and OCB in the brain and in the plasma was measured by an HPLC-UV method which can measure 100 ng/g or more of oseltamivir and OCB as reported by Fuke et al. [30]. The concentration of OCB in the femoral blood was 400 ng/ml. The highest concentration in all specimens was in the liver at 18300 ng/g. In all the brain samples taken, however, the concentrations did not attain the minimum detection level of the method. It may be noted in this connection that the average Cmax after administering one capsule of Tamiflu (75 mg) to a healthy volunteer is 60 ng/ml which is well below the level detectable with the method used by Fuke (100 ng/g). Moreover, oseltamivir is rapidly hydrolyzed in the circulation. In plasma samples taken from rats and mice the half life of oseltamivir was found to be only 20–60 min. In human postmortem blood, the half life may be longer than in that of rats or mice, but oseltamivir may be almost completely changed into OCB in human postmortem blood and tissues within 24 h. Therefore, Fuke et al. would hardly detect unchanged oseltamivir in the postmortem tissues and plasma, even if they used more sensitive methods as HPLC/MS/MS (quantification limit is about 1 ng/g). Causality in a case such as the above cannot readily be excluded unless the latter methods are available.

3.12. Methods of assessment for adverse reactions

Where a causal relationship between a drug and an adverse event cannot be excluded, the latter should, according to the definition adopted by the International Conference on Harmonization, be referred to as an "adverse reaction" [43].

Only a short period has so far elapsed since oseltamivir (Tamiflu) was first marketed in Japan and elsewhere. Initially, sudden death and death resulting from abnormal behaviour were not recognized as occurring specifically in those treated with Tamiflu. Today, no-one can realistically deny that there is a causal relationship. These "events" should therefore now be classified as "adverse reactions" even if the causal relationship still requires further elucidation. It may be noted that the classifications of possible adverse reactions as "rather negative" or "unlikely", that the MHLW employs in Japan are not equivalent to "completely excluded"; they leave open the possibility that there may be a causal relationship, even though MHLW uses them to throw doubt on causality.

When many similar reports of supposed adverse events are collected, the causality of these events should not only be individually assessed; there should also be a collective analysis. Individual reports should be compared with others, and their similarities and differences should be discussed. In addition, the spectrum of clinical events should be compared with the spectrum of symptoms observed in animal toxicity studies. To date, however, MHLW and its scientific advisory panel have analyzed such events only individually and have practically denied that there are causal relationships. Nor have FDA, MHLW and their advisory panels discussed the similarity of symptoms in human and in animals both dead and surviving, despite the very close similarity between the symptoms observed in humans and in animals, as discussed in this paper. However, in view of the criticism advanced by the victims and the mass media after the occurrence of a series of fatal accidents, MHLW has decided that causality will now be reassessed.

One must stress that sudden deaths, occurring within 10 min in a hitherto normal child, have never been reported in cases of influenza-associated encephalopathy or Reye's syndrome except following the use of seizure-inducing drugs. In most persons with abnormal behaviour, the body temperatures during the delirious phase were much lower in those with fever delirium.

The causal relationship between Tamiflu and death (sudden death during sleep and accidental death after abnormal behaviour) thus seems to be very strong. Delayed types of adverse events such as delayed onset or prolonged neuropsychiatric symptoms which begin even after full dose of Tamiflu and last for a week or more even for months, pneumonia after the end of taking Tamiflu, bleeding disorders, hyperglycemia, renal disorders and so on may be related to Tamiflu and more especially to OCB.

If one takes these facts into account it would seem that, even if in cases of sudden death and/or abnormal behaviour or delayed reactions there are confounding factors such as complications, treatment with other medicines and high fever, the causal relationship with Tamiflu should not be seriously doubted so long as the patient is known to have taken Tamiflu before the onset of the symptoms.

3.13. What this paper adds to earlier reports

This paper is built primarily around a case series of eight patients, based on their medical records and history taking from bereaved families. However, in most of the cases of sudden or accidental death, the doctors concerned arrived at their diagnoses only by taking the history from the families.

The strength of the present paper lies in the inclusion of cases manifesting a variety of clinical courses and a variety of causes: three are sudden deaths, two are deaths after abnormal behaviour, two are near

fatal cases with or without sequels and one a case of a late onset neuropsychiatric disorder that lasted two weeks. The evidence relating to these cases is augmented by the disclosure by MHLW of the relevant adverse reaction reports that the pharmaceutical company in question had submitted to the authorities (i.e. MHLW). In addition, brief results of animal toxicity tests conducted by Roche were made available (in Japanese: TK data were corrected very recently by the manufacturer at the meeting of the MHLW's scientific panel [17]).

This paper adds the following three major points to earlier reports: this is the first original paper presenting a case series of fatal or near fatal adverse reactions to Tamiflu; it is also the first paper overviewing the full spectrum of adverse reactions to Tamiflu, and the first paper discussing the causality and underlying mechanisms of the full spectrum of reactions to Tamiflu. It is true that some evidence of these matters has been published earlier: Shiomi briefly described four such cases but presented them only as "adverse events", not recognized as adverse reactions to Tamiflu [84]; two fatal cases of abnormal behaviour and one of sudden death were reported earlier by myself at a scientific meeting [35]; 71 other cases have been listed on the Internet [63,64], and several fatal cases have been personally collected. However, this is the first original paper dealing comprehensively with these matters and examining data both from animals and from man.

3.14. Possible further studies to confirm causality and provide further elucidation

The number of patients suffering adverse reactions to Tamiflu may be substantial; the fact that 5 fatal cases were observed in Osaka alone in one season could mean that some 50–60 fatalities per year might occur nationally in Japan. These adverse reactions are important and serious, and the association between such grave but rare reactions and the drug should be investigated epidemiologically, e.g. using a case-control study. In the past, no such case-control study could be carried out; it was very difficult to select matched controls, because most people in Japan who consulted physicians for flu or flu-like symptoms were immediately treated with Tamiflu. However, Tamiflu is not necessary for the treatment of seasonal flu; this view is accepted in Europe, but not in Japan. However, in March 2007 the Japanese authorities advised against prescribing Tamiflu to adolescents aged 10–19 years. If therefore oseltamivir is indeed to be used for seasonal flu during a coming winter in Japan, a case-control study could be carried out since a substantial number of people might not use Tamiflu knowing its harmful effects, while others would still use it. This may be one of the best methods to assess the association further, though serious reporting bias would be confounded because of the legal threats.

Another piece of evidence that could confirm the causal relationship between sudden death and the use of Tamiflu could well be obtained by performing animal infection-toxicity studies. In those studies, the proportion of oseltamivir concentrations in the brain to plasma would be very high in the animals infected with an influenza virus or similar agent or treated with lipopolysaccharide. Also, if a toxicity study using infected animals is conducted, the ratio of concentration in the brain of an infected animal to that of a non-infected animal would be very high, comparable to the differences in concentration observed in infant rats before weaning as compared with mature rats.

4. Conclusion

1. It can be concluded that oseltamivir has central nervous system suppressive action resembling that of hypnotics, sedatives and anesthetics. Signs and symptoms and histo-pathological findings in animal studies with doses 10–20 times higher than those used clinically (calculated on an AUC

basis) are similar to those observed in human cases, including pulmonary oedema, decreased body temperature, decreased movements and slow/irregular breathing.
2. In addition, delirium, abnormal behaviour, hallucinations and even suicide could be included in those symptoms, as resulting from the disinhibitory effects of central nervous system depressants, including oseltamivir, leading to loss of control.
3. One can therefore conclude that sudden onset type of reactions such as sudden death and death from accidents due to abnormal behaviour in older children and adolescents, especially when observed shortly after taking the first dose of Tamiflu, are probably related to its use.
4. Dyspnoea with cyanosis followed by seizure and cardiopulmonary arrest after taking Tamiflu with or without sequels may be related to the drug. Severe sequels may be a consequence of delayed neuronal cell damage after cardiopulmonary arrest due to the acute toxicity of oseltamivir.
5. Delayed/prolonged type of adverse reactions, which usually begin after an almost complete course of Tamiflu, are probably related to the inhibition of human sialidase (neuraminidase) by OCB. They include neuropsychiatric symptoms of delayed onset (which often last a week or more and may be exacerbated in the absence of any evident secondary trigger), pneumonia, exacerbation of infection (frequently leading to sepsis with multi-organ failure and death), bleeding disorders, hyperglycaemia and renal disorders.
6. Even where in cases of sudden death and/or abnormal behaviour there are confounding factors such as treatment with other medicines or high fever, it would be unwise to exclude a causal relationship with Tamiflu where this is known to have been taken before the onset of the symptoms.

Cases 1–3 in this paper were presented at a session of the Japanese Society for Pediatric Infectious Diseases in Tsu, Mie Prefecture, November 12th 2005.

After the submission of this paper, the families of two additional cases made their medical charts, autopsy records available to me for investigation by the end of 2007: A 44-year-old man died during sleep with severe pulmonary oedema at autopsy and a 29-year-old woman died with low body temperature (34°C) with severe cyanosis and collapse followed by seizure with pulmonary oedema at autopsy [40]. MHLW also added two adult death cases: both from exacerbation of infection [65]. After the submission of this paper, three investigators [18,68,74] found equivocally that P-glycoprotein is the efflux transporter of oseltamivir at the BBB.

Conflict of interest

Rokuro Hama provided scientific opinions for 8 cases where applications were made for adverse reaction relief. He has received no funding from the pharmaceutical industry or the Japanese government.

Written consent was obtained from the patients involved or their relatives for publication of his study.

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