Effects of Oseltamivir Phosphate (Tamiflu) and Its Metabolite (GS4071) on Monoamine Neurotransmission in the Rat Brain

Kanako SATOH,* Ryouchi NONAKA, Akio OGATA, Dai NAKAE, and Shin-ichi UEHARA

Department of Environmental Health and Toxicology, Tokyo Metropolitan Institute of Public Health; 3–24–1 Hyakunin-cho, Shinjuku-ku, Tokyo 169-0073, Japan.

Received June 19, 2007; accepted July 10, 2007; published online July 10, 2007

As abnormal behaviors such as jumping and falling from balcony were reported in patients aged 10 to 19 years who administrated oseltamivir phosphate (Tamiflu) for treatment influenza infection, the Ministry of Health, Labor and Welfare in Japan notified that, as a rule, Tamiflu should not be prescribed to patients aged 10 to 19 years. To examine the relationship between Tamiflu and abnormal behaviors, we investigated the effects of Tamiflu and its carboxylic acid metabolite, GS4071, on the central nervous system, that is, on 3 neurotransmitters (dopamine, serotonin, and norepinephrine) in presynapses (inhibition of re-uptake, promotion of release) and postsynapses (guanosine 5′-triphosphate (GTP) γS binding), using rat brain synaptosomes. Neither Tamiflu nor GS4071 influenced the re-uptake/release of the 3 monoamines or GTP binding in postsynapses.

Key words Tamiflu; oseltamivir phosphate; GS4071; dopamine; serotonin; norepinephrine

As an anti-influenza virus agent, oseltamivir phosphate (ethyl-(3R,4R,5S)-3-(1-ethylpropyloxy)-4-acetamido-5-amino-1-cyclohexane-1-carboxylate phosphate, Fig. 1A) (proprietary name: Tamiflu®) was developed by Roche Laboratory Inc. (Switzerland). In Japan, the Ministry of Health, Labor and Welfare approved this agent in 2000. In February 2001 and July 2002, Chugai Pharmaceutical Co., Ltd., as a Japanese agency, started the sales of 75-mg Tamiflu® capsules and 3% Tamiflu® dry syrup, respectively. The action mechanism of Tamiflu is reported as follows: it suppresses viral release from the surfaces of infected cells by inhibiting neuraminidase, an enzyme essential for the proliferation of type A/B influenza virus, preventing viral infection/proliferation in other cells. This mechanism is similar to that of zanamivir hydrate (Relenza®, anti-influenza virus agent). Relenza is an inhalation agent, whereas Tamiflu is an oral preparation; therefore, the administration method is simpler, although the interval until Tamiflu reaches the infected site is prolonged. Furthermore, amantadine hydrochloride (Symmetrel®) is also administered as an oral anti-influenza virus agent. However, it is not effective for influenza infection other than type A influenza infection. Thus, Tamiflu can be simply administered to treat influenza infection, and may be useful for preventing and treating bird influenza infection. According to Roche Laboratory Inc., the sales situation of Tamiflu is as follows. Japanese patients (n=34000000) account for approximately 75% of the total world Tamiflu consumption (45000000 persons, as of March 12, 2007). American patients comprise the second highest percentage (20%). The amount of Tamiflu administered to children in Japan was 13 times higher than that in the United States. The usage of Tamiflu in Japan is numerous.

In 2007, abnormal behaviors such as jumping and falling were reported in 10- to 19-year-old patients administrated Tamiflu. Therefore, the Ministry of Health, Labor and Welfare in Japan notified that, as a rule, Tamiflu should not be prescribed to patients aged 10 to 19 years. On April 25, 2007, the Ministry of Health, Labor and Welfare published the “Reports on the Side Effects of Oseltamivir Phosphate (Tamiflu)”, which had been submitted between the start of sales and March 20, 2007. According to the report, abnormal behaviors were observed in 186 of 1268 patients with side effects (8 of them died). In the presence of influenza encephalopathy, abnormal behaviors similar to those after Tamiflu administration have also been reported. In Japan (1999, 2000), encephalopathy frequently develops in children aged less than 6 years (2.5/100000 persons), and the mortality rate (10 to 30% of patients with encephalopathy) and incidence of squeal (approximately 20% of them) are high. In children aged over 1 year, the side effects of Tamiflu are rare and slight. A study indicated that there was no association between Tamiflu and mortality/encephalopathy in infants aged less than 1 year. Concerning the relationship between Tamiflu and abnormal behaviors, another study reported that there was no significant difference in the incidence of abnormal behaviors between patients with and without Tamiflu (11.9% vs. 10.6%, respectively).

To evaluate psychoactive drug activity quickly, we reported an assay system for investigating the influence on the central nervous system using synaptosomes prepared from the rat brain, a system for examining the influence of various chemicals including psychoactive drugs on 3 neurotransmitters (dopamine (DA) system, serotonin (SHT) system, and norepinephrine (NE) system) in presynapses (inhibition of re-uptake, promotion of release). Many of the monoamine receptors, including DA, SHT, and NE receptors, are considered to belong to the superfamily of guanosine 5′-triphosphate (GTP) binding protein-coupled receptors in postsynapses (GTP binding). Abnormal behaviors seen in the case of Tamiflu administration to treat influenza closely resemble those seen in the case of the acute psychoactive drugs intoxication, a pleasurable mix of stimulant-like and hallucinogen-like effects. Oral administration of Tamiflu, an ethyl ester prodrug, is converted to the active form, (3R,4R,5S)-3-(1-ethylpropyloxy)-4-acetamido-5-amino-1-cyclohexane-1-carboxylic acid (GS4071, Fig. 1B) in vivo. As an animal experiment it is reported that Tamiflu passes the brain barrier, we investigated the influence of Tamiflu and its metabolite GS4071, which were supplied by Prof. Shibasaki (the Uni-
versity of Tokyo, Tokyo, Japan), on DA, 5HT, and NE as well as GTP binding using a psychoactive drug-assay system to examine the relationship between Tamiflu and abnormal behaviors.

MATERIALS AND METHODS

Reagents Tamiflu and GS4071 were a kind gift from Prof. Shibasaki. They were synthesized according to Fukuta et al.12) Methamphetamine (MAP) and cocaine were purchased from Takeda Pharmaceutical Company Limited and Dainippon Sumitomo Pharma Co., Ltd., respectively. Chemicals were dissolved in dimethyl sulfoxide (DMSO, final concentration: 0.1%). 

3H-DA (2.20 TBq/μmol), 

3H-5HT (1.11 TBq/μmol), 

3H-NE (1.93 TBq/μmol), and [35S]GTPγS (46.25 TBq/μmol) were purchased from PerkinElmer Inc. (MO, U.S.A.). Other reagents used in the study were of the highest grade commercially available.

Animals Male Sprague Dawley rats (crj:CD (SD)) at 5 weeks old were obtained from Charles River Japan (Kanagawa, Japan). After the rats were preliminary bred for one week, they were killed under ether anesthesia and their brains were quickly removed. All animal studies were performed in accordance with the UFAW Handbook on the Care and Management of Laboratory Animals.

Preparation of Cerebral Synaptosomes for Re-uptake and Release Assay The striatum and cerebral cortex were dissected from the rat brain. Crude synaptosomes were obtained by the methods described in our previous reports.10,14) The synaptosome from the striatum was used for the assay of re-uptake and release of DA, and that from the cortex was used for the assays of 5HT and NE. For the release assay, 1 μM reserpine was added to 0.32 M sucrose and buffer.

The re-uptake and release assays were started immediately after the preparation of synaptosomes. Protein concentrations were determined by the modified Lowry method using a Bio-Rad assay kit.

Preparation of Cerebral Synaptosomes for GTPγS Binding Assay The whole brain dissected on ice was homogenized. Crude synaptosomes were obtained by the modified methods described in previous13) and our personal reports. The synaptosome was divided into aliquots and stored at −80 °C until use.

3H-DA, 3H-5HT, and 3H-NE Re-uptake and Release Assays The re-uptake and release assays were conducted using the methods described in our previous reports.10,14) The final concentration of 0.1% DMSO had no effect on the activity. Specific uptake or release was calculated by subtracting the non-specific uptake (DA; 260, 5HT; 500, NE; 720 dpm) or release (DA; 5210, 5HT; 4960, NE; 2200 dpm) content from the total uptake (DA; 11600, 5HT; 2960, NE; 8480 dpm) or release (DA; 10160, 5HT; 7560, NE; 6770 dpm) content. From these results, the drug concentration giving the % of 5-HT maxima was determined by dividing DA-, NE-, or chemical-induced maximal binding using the 5-HT-stimulated maximal binding value (2110 cpm) as a reference compound.

Statistical Analysis IC50 and EC50 values were determined using the sigmoidal dose-response curve fitting obtained by a software, KaleidaGraph ver. 4 (Synergy Software, PA, U.S.A.). The data represented the mean values of three independent experiments (n = 3).

RESULTS AND DISCUSSION

In this study, we examined that Tamiflu and GS4071 on DA, 5HT, NE-reuptake and release assays, and GTP binding assay using rat brain synaptosomes. We compared the influence of Tamiflu and GS4071 on 3H-DA, 3H-5HT, and 3H-NE re-uptake with that of a stimulant, MAP, and a narcotic, cocaine (Figs. 2A—C). Both MAP and cocaine potently inhibited DA, 5HT, and NE re-uptake, and their IC50 values were similar to those previously reported.10) However, neither Tamiflu nor GS4071 influenced re-uptake of the 3 monoamines. In release assay, MAP potently promoted DA/NE release, but cocaine did not influence the 3 monoamines. MAP’s EC50 values and the finding of cocaine were consistent with the results of our previous study.10) Neither Tamiflu nor GS4071 promoted the release of H-DA, H-5HT, and H-NE (Figs. 2D—F). Subsequently, we studied a [35S]GTP binding assay under conditions which facilitated the adequate responses of DA, 5HT, and NE. Neither MAP nor cocaine promoted G-protein binding, as previously reported. Also, neither Tamiflu nor GS4071 bound the G-protein binding of DA, 5HT, and NE receptors (Fig. 3).

In a symposium held by the Japanese Society of Pharmacological Epidemiology (JSPE) (May 20, 2007), some investigators reported that there was no association between Tamiflu administration and abnormal behaviors based on statisti-

![Fig. 2. Inhibition of Re-uptake and Stimulation of Release of Monoamines by Tamiflu and GS4071](image-url)
6 years in Japan (2.5/100000 persons) is higher than that less than 1 years, 0.30 persons aged 1 to 4 years, and 0.11

Fig. 3. Representative Concentration–Response Curves for [125]GTPγS Binding by Tamiflu and GS4071 in the Rat Whole Brain Membranes

Specific [125]GTPγS binding was measured in the presence of various concentrations of Tamiflu and GS4071 as described in Materials and Methods. The results are expressed as a percentage of the specific binding of 5HT at 10−6 M. The S.D. values are less than 5.0%. ▲ Tamiflu; ○ GS4071; ● methamphetamine; ■ cocaine; △ DA; □ 5-HT; ○ NE.

cal data, and that Tamiflu decreased the incidence of pneumonia to 1/3. Others suggested the relationship between Tamiflu administration and abnormal behaviors. A consensus has not been reached. Concerning anti-influenza virus agents other than Tamiflu, the Ministry of Health, Labor and Welfare also announced the incidences of abnormal behaviors after administration on May 14. According to this, 10 and 6 patients with abnormal behaviors after administration of Relenza/amantadine hydrochloride (denominators unpublished) have been reported since 2000 and 1998, respectively.2) In the package inserts of amantadine hydrochloride, side effects on the central nervous system (hallucination, delusion) are described. They may be probable, since it is described that the agent inhibited DA re-uptake while promoting its re-release/synthesis in an animal (rats) experiment in the new drug application. However, in the new drug application of Tamiflu (materials regarding its pharmacological effects), it is described that Tamiflu may not influence any symptoms/activities, the central/autonomic nervous systems, smooth muscle, nor immune system based on the results of general pharmacological and toxicity studies using animals, and that neither Tamiflu nor its carboxylic acid metabolite (GS4071) influenced 19 receptors involved in nausea/vomiting in an in vitro study regarding the central nervous system.

In the United States, 153 patients (0.21/100000 persons) aged less than 18 years died of influenza in the season between 2003 and 2004.17) They consisted of 0.75 persons aged less than 1 years, 0.30 persons aged 1 to 4 years, and 0.11 persons aged 5 to 17 years per 100000 persons. The incidence of influenza encephalopathy in children aged less than 6 years in Japan (2.5/100000 persons) is higher than that in the United States. This reflects differences in genetic backgrounds.4,6) As the number of patients in whom Tamiflu was prescribed was not reported,3) we estimated the mortality rate according to abnormal behaviors as 0.024/100000 persons from Roche’s report.2) The incidence of influenza encephalopathy is much higher than that of the abnormal behaviors occurred in those who were administered Tamiflu to treat influenza. Neither Tamiflu nor GS4071 inhibited the re-uptake of 3 monoamines in presynapses, promoted their release, or influenced G-protein binding in postsynapses in our in vitro assay system. It is thus indicated that mechanisms underlying the abnormal behaviors due to Tamiflu are different from those underlying the effects of psychoactive drugs. It has been suggested that Tamiflu inhibits encephalopathy-related death and the onset of pneumonia in children.18) Therefore, this agent may be useful for treating influenza infection in high-risk groups consisted of children or elderly persons.19) We propose that the relationship between Tamiflu and abnormal behaviors should be examined quickly using influenza-infected animals, and then Tamiflu must be administered, considering its risks and benefits.

REFERENCES

2) http://www.mhlw.go.jp/shingi/2007/05/s0502-1.html/B%27& extension=html&search_lang=ja
5) http://www.city.shinjuku.tokyo.jp/division/340700yobo/infuruenza. html#_top