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The neuroprotective action of naringenin on oseltamivir (Tamiflu) treated male rats

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Abstract The aim of this study is to evaluate the protective action of naringenin (a flavonoid) on the brain functions of oseltamivir treated rats. 24 rats were divided into 4 groups as follows: control, naringenin treated rats (Nar, 50 mg/kg bwt/day), oseltamivir treated rats (Tam, 0.75 mg/kg bwt twice daily) and naringenin + oseltamivir treated rats (Nar + Tam). All the drugs were received via oral gavage for five days. The animals on the 5th day were trained in Y maze. Then, on the 6th day, rats were decapitated and the brain was excised for determination of total antioxidant capacity (TAC), total oxidant capacity (TOC), total nitric oxide (TNO), Ca ATPase, total cytochrome P450 (CYP450) contents and brain fatty acid binding proteins FABP7. The results showed a significant increase in the TOC, TNO and CYP450 in Tam treated rats while a significant decrease was noticed in TAC, Ca ATPase and FABP7 in the same group in comparison with the control. Nar + Tam treated rats exhibited a significant decrease in TOC, TNO and CYP450 and a significant increase in TAC, Ca ATPase and FABP7 in comparison with Tam treated rats. An improvement in Y maze behavior and all the investigated parameters was noticed in Nar + Tam treated rats as compared with the oseltamivir treated rats. The results suggest that Nar has a neurophysiological and behavioral protective effect on oseltamivir side effects on the brain functions.

Introduction

Infections caused by influenza viruses are a considerable threat to human health around the world. The outbreak of a novel influenza A (H1N1) virus has been identified in 2009. The means to prevent and control influenza include vaccines and anti-viral substances (Zhu et al., 2015). Currently, two classes of anti-viral drugs for chemoprophylaxis and treatment of human influenza viruses are approved by the U.S. Food and Drug Administration (FAD), including M_2 ion channel blockers and neuraminidase inhibitors (Murtaugh et al., 1974).

Oseltamivir (Tamiflu®) is the first orally available anti-influenza viral drug, works as a neuraminidase inhibitor. Oseltamivir is metabolized to oseltamivir carboxylate (OC) and other compounds in the body (He et al., 1999; Sweeny et al., 2000). Sialic acid cleaved by neuraminidase, may hinder cellular adhesion. Therefore, it is hypothesized that neuraminidase inhibited by OC, may participate in vital functions in the

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central nervous system (CNS), containing development of neuronal cells and impulse conveyance (Rodriguez et al., 2001; Crain and Shen, 2004; Da Silva et al., 2005). Whereas, the neurons of hippocampus treated with neuraminidase raises seizure beginning, its blockade reduces the seizure entry, suggesting that the neuraminidase contributes in the management of neuronal action (Rodriguez et al., 2001). Furthermore, neuraminidase activity in the hippocampus is increased during seizures (Isaev et al., 2007). These data showed that OC could lead to behavioral changes through effects on the CNS (Boyzo et al., 2003). Recently, a rise in information on neuropsychiatric adverse events (NPAEs) in influenza patients who were and were not treated with oseltamivir was recorded (Toovey et al., 2008).

Functional foods containing bioactive components can provide positive impacts on health (Dong et al., 2015), and influence physiological or cellular activities in animals and humans (Oh and Jun, 2014). Naringenin (4,5,7,trihydroxyflavanone7-rhamnoglucoside) is a natural flavanone, richly found in citrus and grapefruits, and exhibits antioxidant potential, superoxide scavenging, antiapoptotic, antiatherogenic and metal chelating activities (Jeon et al., 2001; Cavia-Saiz et al., 2010). Naringin is hydrolyzed by intestinal microflora to yield naringenin (4,5,7-trihydroxyflavanone). The latter is readily absorbed and also has good penetration across the blood brain barrier (Zbarsky et al., 2005).

Recently, Nar was found to be a neuroprotective effect in the intracerebro-ventricular streptozotocin-induced model of Alzheimer's disease and 6-hydroxydopamine model (of Parkinson's disease and Scopolamine-induced amnesia and to prevent oxidative damage in various pathophysiological conditions due to their ability to penetrate into the brain (Khan et al., 2012). In addition, Flavonoids have powerful antioxidant activities against reactive oxygen species (Rice-Evans et al., 1996) and nitrogen species (Oldreive et al., 1998; Kerry and Rice-Evans, 1999). Moreover, Kumar et al. (2010), has reported that Nar has a neuroprotective potential against D-galactose induced cognitive impairment and oxidative stress in mice.

Since the previous works proved the brain physiopathology of oseltamivir, there are few works that discussed the protection of these hazards. The present work aims to evaluate the neuroprotective effect of Nar on the behavior and brain pathophysiology health hazards resulting from oseltamivir treatment, through evaluating the Y maze, total antioxidant capacity (TAC), total oxidant capacity (TOC), total nitric oxide (TNO), Ca ATPase, total cytochrome P450 (CYP450) contents and brain fatty acid binding proteins (FABP7).

Materials and methods

Experimental animals

This study was carried out using twenty-four adult, *Wister strain*, male rats weighing 140 ± 10.5 g purchased from the animal house of National Organization for Drug Control and Research (NODCAR) they were 4 months old. Animals were housed in plastic cages, each cage contained six rats. Animals were kept under a controlled temperature of 25 ± 2 °C with relative humidity 50–55% and 12 h light–dark cycle throughout the experiment. The animals were allowed to adapt to the laboratory conditions one week before the beginning of the experiment and a commercial pelleted diet was used during the experiment. The experimental protocols and procedures were approved by Ain Shams University authorities and followed Egyptian rules for animal protection. All efforts were made to minimize animal suffering, to reduce the number of animals used, and to utilize alternatives to in vivo techniques, if available.

Drugs

Naringenin ($C_{15}H_{12}O_5$) was supplied as a white powder (assay ⩾95%) from Sigma St. Louis, MO, USA, (CAS-NO 67604-48-2). Oseltamivir was supplied as "Tamiflu" 75 mg capsules manufactured by ROCHE, Roche Products (New Zealand) Limited, PO Box 109113 New market, Auckland 1149, New Zealand.

Experimental design

The animals were divided into four main groups; six rats each: 1 – Control group (C) daily-received a vehicle (water containing 0.1% w/v sodium benzoate). 2 – Naringenin (Nar) group, received 50 mg/kg/day dissolved in water (Khan et al., 2012 and Chtourou et al., 2015). 3 – Oseltamivir (Tam) received 0.75 mg/kg twice daily dissolved in water containing 0.1% w/v sodium benzoate used as a preservative. 4 – Naringenin + Oseltamivir (Nar + Tam) received the same previous doses of both (Nar, 50 mg/kg/day + Tam, 0.75 mg/kg twice daily). Animal groups were treated daily for 5 days from the beginning of the experiment by oral gavage. These doses used in this study were calculated from the human equivalent pharmacological dose according to Reagan-Shaw et al. (2008). The Y maze test began on the 5th day of the experiment before decapitation.

Y maze task

The Y maze consisted of three equal arms tagged A, B and C. The angle between the arms is 120°, each division of the maze is 40 × 15 × 30 cm, long X wide X high respectively (Roghani et al., 2006). The floor and sides of each arm is formed of wood. The maze employed to evaluate spontaneous alternations. Each rat was put in one of the sections and permitted to move freely among the three divisions for 5 min. The number of arm entries and the number of trials are documented to estimate the alternation percentage. The number of maximum spontaneous alternations is calculated as the total number of arms entered minus two, the correct alternation is calculated as the successive entries into the different three arms on overlapping triplet sets (i.e., ABC, CBA, BAC), the percentage alternation is calculated as {(actual alternations/maximum alternations) × 100}, and the percentage of correct alternation is calculated as {(correct alternation/maximum alternations) × 100}. An entry is calculated when all the rat four limbs are within the arm. The maze is cleaned with 70% alcohol and allowed to dry between sessions. The Y maze was utilized to evaluate the general locomotor activity, short-term memory and stereotypic behavior (Kokkinidis et al., 1976; Maurice et al., 1994, and Roghani et al., 2006).