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Neuroprotective effects of quercetin, rutin and okra (*Abelmoschus esculentus* Linn.) in dexamethasone-treated mice

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ABSTRACT

The administration of dexamethasone, a synthetic glucocorticoid receptor agonist, causes neuronal death in the CA3 layer of the hippocampus, which has been associated with learning and memory impairments. This study aimed to examine the ability of okra (*Abelmoschus esculentus* Linn.) extract and its derivatives (quercetin and rutin) to protect neuronal function and improve learning and memory deficits in mice subjected to dexamethasone treatment. Learning and memory functions in mice were examined using the Morris water maze test. The results showed that the mice treated with dexamethasone had prolonged water maze performance latencies and shorter time spent in the target quadrant while mice pretreated with quercetin, rutin or okra extract prior to dexamethasone treatment showed shorter latencies and longer time spent in target quadrant. Morphological changes in pyramidal neurons were observed in the dexamethasone treated group. The number of CA3 hippocampal neurons was significantly lower while pretreated with quercetin, rutin or okra attenuated this change. Prolonged treatment with dexamethasone altered NMDA receptor expression in the hippocampus. Pretreatment with quercetin, rutin or okra extract prevented the reduction in NMDA receptor expression. Dentate gyrus (DG) cell proliferation was examined using the 5-bromo-2-deoxyuridine (BrdU) immunohistochemistry technique. The number of BrdU-immunopositive cells was significantly reduced in dexamethasone-treated mice compared to control mice. Pretreatment with okra extract, either quercetin or rutin was found to restore BrdU-immunoreactivity in the dentate gyrus. These findings suggest that quercetin, rutin and okra extract treatments reversed cognitive deficits, including impaired dentate gyrus (DG) cell proliferation, and protected against morphological changes in the CA3 region in dexamethasone-treated mice. The precise mechanism of the neuroprotective effect of these plant extracts should be further investigated.

1. Introduction

Both animal and human studies have shown that glucocorticoids have profound effects on cognition (Lupien and McEwen, 1997; Roozendaal et al., 2006; McEwen, 2007). Sustained stressors or pathophysiological conditions, such as affective disorders, have detrimental effects on cognition. The impairing effects have been attributed mainly to glucocorticoids released from the adrenal cortex (Roozendaal et al., 2006). The influence of activated glucocorticoids on oxidative stress-induced neuronal cell death in vitro was investigated by employing hippocampus model systems (Behl et al., 1997). Elevated levels of endogenous glucocorticoids damage the brain, especially the hippocampus, which plays an important role in memory, mood and behavior. Chronic stress or prolonged exposure to high levels of corticosterone induces neuropathological alterations, such as dendritic atrophy in the hippocampus and striatal neurons (Conrad et al., 2007). Exogenous application of a high dose of corticosterone has been shown to elicit neuronal damage in the hippocampus CA3 subfield (Woolley et al., 1990; Haynes et al., 2001; McEwen, 2007). Glucocorticoids potentiate stress or ischemia-induced accumulation of excitatory amino acids in the

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extracellular space of the hippocampus (Chen et al., 1998). Excitatory amino acids play an important role in neuroplasticity and various neurological diseases related to cognitive dysfunction (Mattson, 2008).

A similar alteration was also noted following the administration of dexamethasone, a synthetic glucocorticoid receptor agonist that induces mood disorders, including psychosis, in some patients (Haynes et al., 2001). Increasing evidence suggests that there are harmful effects of dexamethasone after acute or prolonged administration. Sustained exposure to dexamethasone enhanced necrotic death of C6 glioma cells in rats (Morita et al., 1999) and damage to hippocampal neurons especially in the CA3 region (Sekita-Krzak et al., 2003; Danilczuk et al., 2006). Moreover, administration of dexamethasone at high doses of (120 mg/kg/day for 7 days) impaired long-term memory and motor coordination, reduced body weight and induced mortality in mice (Danilczuk et al., 2006). Additionally, glucocorticoids can exacerbate the neuronal loss associated with acute neurological insults such as hypoxia-ischemia, excitotoxicity, and metabolic disruption (Roy and Sapolsky, 2003). This exacerbation was accompanied by increased accumulation of glutamate in the synapse, excessive cytosolic calcium, and increased oxygen radical activity, markers usually attributed to pathways of necrotic cell death.

Recently, phytochemical in food and their effects on health, especially the suppression of reactive oxygen species by natural antioxidants from teas, spices, and herbs, have been extensively studied (Turner et al., 2002; Slemmer et al., 2008). Among these plants, *Abelmoschus manihot* (L.) Medic is a well-known traditional herbal medicine. It is used as an anti-inflammatory and myocardial ischemia protective drug in traditional Chinese medicine (Li et al., 2001; Fan et al., 2003). The major active ingredients of *A. manihot* L. Medic have been isolated, and identified as quercetin-3-robinobioside, hyperin, quercetin, myricetin, quercetin-3′-glucoside, gossypetin-3′-*o*-β-glucoside and isoquercetin (Wang et al., 2004). Okra (*Abelmoschus esculentus* Linn.), another traditional herbal medicine, consists of various polyphenolic components such as quercetin derivatives and (−)-epigallocatechin (Shui and Peng, 2004). These flavonols possess antioxidative properties according to HPLC and ABTS analysis (Shui and Peng, 2004), whereas quercetin and rutin act as efficient radical inhibitors and have a neuroprotective effect against ischemia and reperfusion-induced cerebral injury (Cho et al., 2006; Pu et al., 2007). However, the mechanism of neuroprotective effect of okra is still unknown. Our objective was to evaluate the neuroprotective effect of quercetin, rutin and okra in dexamethasone-treated mice.

2. Materials and methods

2.1. Materials

An ethanol extract of okra (*Abelmoschus esculentus* L. moench) was kindly provided by Dr. Piyanete Chantiratikul from the Department of Chemistry, Faculty of Science, Mahasarakham University. Rutin and quercetin were purchased from Fluka Chemie AG (Geneva, Switzerland). UTHO-Dexamethasone® (a standard preparation of 4 mg/ml dexamethasone sodium phosphate) was purchased from Utopian Co., Ltd. (Samuthprakarn, Thailand). Rabbit polyclonal antisera against NMDA receptors (NR2A/B; code No. AB1548) and the 5-bromo-2-deoxyuridine (BrdU) immunohistochemistry kit used were purchased from Chemicon International Inc. (Temecula, CA, USA). Biotinylated goat anti-rabbit IgG (Code No. PK 6101), normal goat serum, diaminobenzidine and an ABC Elite kit were purchased from VectorLabs (Burlingame, CA, USA). BrdU and other reagents were purchased from Sigma–Aldrich Chemical Company (St. Louis, MO, USA).

2.2. Preparation of okra extract

Okra was collected from the local market in June and dried at 60 °C for 24 h in a hot air oven. Dried okra was blended in a blender and was then subjected to Soxhlet extraction using ethanol as the solvent. Fifty grams of okra and 200 ml of ethanol were used in the extraction. Triplicate extractions were performed. The solvent was vacuum-distilled at 60 °C in a rotary evaporator and the remaining extract was finally dried at room temperature for several days to ensure the removal of any residual solvent. The final extract was a dark green powder. The major ingredients of okra (100 g) composed of catechin (56 mg), epicatechin (32 mg), procyanidin B1 (289 mg) and B2 (675 mg), quercetin (25 mg) and rutin (0.32 mg). The ethanol extraction yields of the samples were 1.97%. This has been reported in previous study (Khomsug et al., 2010).

2.3. Experimental animals

Male ICR mice (weighing 25–30 g) were obtained from the National Laboratory Animal Center of Mahidol University. The experimental protocols conformed to the Home Office (UK) Animal Scientific Procedure Act of 1986 and were approved by the animal care and use committee of Mahasarakham University. The experiments in the present study were designed to minimize the number of animals used and their suffering. Each mouse was housed in a single cage, allowed access to food and water *ad libitum*, and maintained under standard conditions at 25 ± 2 °C and 60 ± 10% relative humidity with a 12 h light/dark cycle.

2.4. Treatment of animals

Our preliminary study on various doses of dexamethasone (40, 60, 80 and 120 mg/kg) had been tried and we found that 40 mg/kg did not significantly alter the Morris water maze task, behavioral test whereas prolonged treatment of 80 or 120 mg/kg induced lethality. Prolonged treatment (21 days) of 60 mg/kg dexamethasone significantly altered the Morris water maze task, behavioral test but did not induce lethality. Therefore 60 mg/kg dexamethasone was chosen throughout this study. Dexamethasone at 60 mg/kg was chosen to prevent overdose treatment (Danilczuk et al., 2006).

Our preliminary study on various doses of quercetin (Que), rutin (Rut) and okra extract (Okr) (15, 30, 40 and 60 mg/kg) by p.o. 3 h (according to their bioavailability absorption reported by Shimoi et al. (2003) and Manach and Donovan (2004) prior to the treatment of 60 mg/kg dexamethasone, had been tried and we found that only 60 mg/kg of these three compounds significantly altered the Morris water maze task, behavioral test compared with the dexamethasone treated group. Therefore, 60 mg/kg of all three compounds was chosen throughout this study.

Different groups of animals were employed in the present study as follows: in the control group, mice were injected with 0.9% normal saline at 1 ml/kg/d for 21 d; in the dexamethasone group, mice were given dexamethasone (DEX) at 60 mg/kg by i.p. injection for 21 d; in the Que, Rut and Okr extract pretreated groups, mice were treated with Que, Rut and Okr 60 mg/kg/ml by p.o. 3 h prior to treatment with dexamethasone; and in the Que, Rut and Okr extract treated groups, mice were treated with Que, Rut and Okr 60 mg/kg/ml by p.o. for 21 d. The Que, Rut and Okr were dissolved in propylene glycol. Propylene glycol at 1 ml/kg p.o. was administered to the control group and the dexamethasone group before i.p. injection with 0.9% normal saline or dexamethasone, respectively.