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The effects of tamoxifen and soy on dark neuron production in hippocampal formation after pentylenetetrazole-induced repeated seizures in rats

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Abstract

Background: Regarding the similar modulatory effects of both soy and tamoxifen on the actions of estrogen which have previously reported, the aim of the present study was to investigate the effects of these two estrogen like compounds alone and in combination on dark neuron production in hippocampal formation of ovariectomized rats after pentylenetetrazole-induced repeated seizure. **Methods:** The rats were randomly divided into six groups: control, sham, OVX, OVX-soy (OVX-S), OVX-tamoxifen (OVX-T) and OVX-soy-tamoxifen (OVX-S-T). The animals of OVX-S, OVX-T and OVX-S-T groups received the soy extract (60 mg/kg; i.p.), tamoxifen (10 mg/kg) or both for 2 weeks before induction of seizures. The animals of these groups were also treated by soy extract, tamoxifen or both before each injection of PTZ (40 mg/kg) for 6 days. The animals of sham and OVX groups received saline plus tween instead of tamoxifen and soy extract. The animals of control group did not treat by PTZ, tamoxifen and soy. The rats were placed in Plexiglas cages separately and observed for 60 min. The brain tissues were then removed and subjected for histological studies. **Results:** A significant decrease in the seizure score was seen in OVX group comparing to sham. The animals of both OVX-T and OVX-S groups had a significant higher seizure score compared to OVX group. Co-treatment of the ovariectomized rats by both soy extract and tamoxifen decreased the seizure score compared to OVX-S and OVX-T groups. The results of histological study showed that the dark neuron number in CA1, CA2, CA3 and dentate gyrus (DG) of hippocampus area in OVX-T and OVX-S groups was higher than that of OVX group ($P < 0.05$–$P < 0.01$). In CA3, the produced dark neurons of OVX-S-T group were lower than that OVX-S group ($P < 0.01$). **Conclusion:** The results of present study showed that treatment of the ovariectomized rats by either soy extract or tamoxifen increased the seizure score as well as dark neurons. Co-treatment with soy extract and tamoxifen did not potentiate the effects of each of them alone. Co-administration of the tamoxifen and soy extract inhibited the effects of the soy extract and tamoxifen when they administered alone.

Keywords: Soy; Tamoxifen; Seizure; Dark neuron; Hippocampus

1. Introduction

Epilepsy is a chronic disorder, in which the indispensable feature is unprovoked and usually unpredictable recurrent seizures. Yet, it is stated that an epileptic seizure is the result of a temporary physiologic dysfunction of the brain caused by a self-limited, abnormal, hypersynchronous electrical discharge of cortical neurons. Also it is claimed that approximately 40 million people are affected with epilepsy in worldwide [1]. It has been shown that seizure induces the neuronal structure impairments in hippocampus formation includes the hippocampus and dentate gyrus [2].

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Hippocampal formation is sensitive to seizure-induced neuronal damage. Thus, memory impairment is one of the most important defects of epileptic patients [2].

Dark neurons, the neurons with dense cytoplasm and karyoplasms like necrotic cells, are resulted from various factors, including trauma to the brain [3]. It was also shown that dark neurons are produced during epilepsy and seizures [1,4]. Scientists believe that the produced dark neurons in epilepsy are due to excessive secretion of neurotransmitters [5]. Evidence shows that the dark neurons which are generated in advanced stages of epilepsy are irreversible and indentified as damaged neurons [6].

Estrogen has various effects on the nervous system and is essential in the development and the normal function of the brain [7,8]. Estrogen receptors (ERα and ERβ) are present in numerous regions of the brain including the hippocampus. Thus, it seems that this hormone affects the structure and function of the hippocampal neurons [9,10]. The female sexual hormones seem to act via various mechanisms such as modulation of gene expression, regulation of neurotransmitter release, or direct interactions with neurotransmitter receptors [11]. Thus, estrogen can affect neuronal excitability and seizure susceptibility [9]. But there are controversial results in the case of the effects of estrogen on seizure. In previous studies, both the proconvulsant [12–14] and anticonvulsant effects [15,16] of estrogen have been shown. It was previously shown that deletion of ovarian hormones attenuates seizure severity in a pentylenetetrazole (PTZ)-induced seizure model [14]. Furthermore, it has been shown that estrogen has neuroprotective effects against seizure-induced neuronal damage [15,17,18].

Gonadal hormones depletion due to menopause leads to various problems in women such as hot flashes and cognitive deterioration [19]. For some menopausal women estrogen replacement therapy (ERT) is used to avert these defects [20,21]. Still, there are evidence that ERT in some women increases the risk of uterine cancer and neoplasms of the breast. This happens especially after long-term use of this therapy [22–24]. Another treatment may be the phytoestrogens that exists in some vegetables like soy [25]. Isoflavones are the natural compounds of phytoestrogens which have structural similarities with estrogen and bind to estrogen receptors and therefore, show some estrogen-like effects [26]. Soy contains isoflavones genistein, daidzein, glycitein and their respective glucosidic conjugates [27]. It has been shown that soy prevents the inhibitory effects of diabetes on the Na^+, K^+-ATPase activity [28]. Soy isoflavones was also showed to affect memory without significant side-effects on the vaginal and uterine [29]. Moreover, soy improved the memory performance of hypercholesterolemic mice by decreasing the blood lipid levels and modulating the metabolism of neurotransmitters such as acetylcholine and amino acids in the brain [30].

Studies have shown that in response to soy extract the production of new cells in the dentate gyrus increased [31]. Pretreatment with the phytoestrogens also inhibited the cell death in hypoxia or oxygen–glucose deprivation [32]. Increasing in the mRNA for antiapoptotic Bcl-2 family member by soy and decreasing in the levels the proapoptotic proteins have also reported in frontal cortex [29,33]. In contrast to these findings, the attenuation of nerve growth factor expression has also been reported [34]. Finally, it has been shown that soy therapy in ovariectomized rats exacerbate seizure phenomena [35].

Selective estrogen receptor modulators (SERMs) are drugs such as ospemifene, tamoxifen and raloxifene which depending on their chemical structure and the specific properties of the target tissues show both agonist/antagonist estrogen actions [36,37]. Tamoxifen is frequently used for the prevention and treatment of all stages of hormone-dependent breast cancer [38]. The maintenance of bone density and its cardio protective effects are examples of agonistic effects of tamoxifen [39]. While, it behaves as an antiestrogen in the breast tissue [40] and it also prevents of ovariectomy-induced downregulation of Bcl-2 and upregulation of Bax expression [41].

In the central nervous system, tamoxifen was shown to reduce infarct size and neurobehavioral deficits due to middle cerebral artery occlusion in rats [42]. It also attenuated the microglial inflammatory responses and decreased the irradiation-induced brain damage [43]. Tamoxifen was also shown to have the estrogenic role in hypothalamic differentiation during the neonatal period [44]. Moreover, it enhanced choline acetyltransferase mRNA expression in several basal forebrain regions in a manner which was similar to the effects of estrogen [45]. Tamoxifen also has prevented of the neuronal cell by inhibiting of glutamate release [46]. It has also been suggested that tamoxifen acts as an antihormone in seizure and raises the electroconvulsive threshold in female mice [47].

Regarding the similar modulatory effects of both the soy and tamoxifen on the actions of estrogen which have previously reported [48], the aim of the present study was to investigate the effects of these two estrogen like compounds alone and in combination on dark neuron production in hippocampal formation of ovariectomized rats after pentylenetetrazole (PTZ)-induced repeated seizure.

2. Materials and methods

2.1. Animals and groups

The experiments were carried out on 40 virgin female Wistar rats weighing 180–220 g. The animals were confined at random in metal cages with water and food ad libitum, and maintained at a temperature of 22 °C under a 12-h light–dark cycle.

The animals were randomly divided into six groups ($N = 6–8$ in each) as follows: control, sham, OVX,