

Effect of Estradiol and Soy Extract on the Onset of PTZ-Induced Seizure in Ovariectomized Rats: Implications for Nurses and Midwives

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Abstract

Background: In many epileptic women, the ovarian cycle influences the risk and severity of seizures. Previous studies have reported inconsistent findings regarding the neural excitatory effects of estrogen. Soybean is a rich source of plant estrogens. Therefore, soy intake can affect the risk and severity of seizures in women. Nurses and midwives need to be aware of the effects of diets containing estrogen in pregnant and post-menopausal women, as well as during the sexual cycle periods.

Objectives: Due to the limitations of human studies, this study aimed to investigate the effect of estradiol and soy extract on the onset of pentylentetrazol (PTZ)-induced seizures in ovariectomized rats.

Materials and Methods: In this experimental study, sixty female Wistar rats were randomly divided into six groups of ten. All of the groups except for the negative control groups were ovariectomized. One group received estradiol (100 µg/kg), one group was treated with alcoholic extract of soy (20 mg/kg), and the other group received vehicle over 28 days. PTZ (90 mg/kg) was used to induce seizure in all groups. Behavioral changes were observed over 30 minutes. All data were analyzed using a two-way analysis of variance with Tukey's post-hoc test.

Results: The mean ± SD of the clonic seizure threshold was 61.84 ± 8.53 in the group that received estradiol, while it was 71.70 ± 10.85 in the control group, 64.69 ± 3.23 in rats with laparotomy without removing the ovaries, 51.30 ± 6.84 in rats with laparotomy and removing the ovaries, 52.7 ± 7.18 in ovariectomized rats that received sesame oil as vehicle, and 59.2 ± 4.91, in the soy groups. Therefore, estradiol significantly increased the clonic seizure threshold, while the alcoholic extract of soy had no effect.

Conclusions: We concluded that the chronic administration of estradiol has an anticonvulsant effect in the animal model. However, alcoholic extract of soy has no effect on the onset of clonic seizures.

Keywords: Soybean, Estradiol, Ovariectomized, Seizure

1. Background

Sex steroids such as estrogen have regulatory effects on neuronal excitability (1). The frequency and severity of seizures can be altered by changes in sex hormones (2), and they can be worse during certain phases of the menstrual cycle (3). Catamenial epilepsy is a subset of epilepsy that includes women whose seizures are exacerbated by their menstrual cycle (4). The prevalence of epilepsy is 50 in 100,000 in developed countries (5). Due to the effects of sex hormones, for many decades it was believed that there is a direct relationship between estrogen levels and seizure (6), and that estrogen induces excitatory actions in the central nervous system (4). However, various studies have suggested both proconvulsant (7) and anticonvulsant (8) roles for estrogen.

Almost 50% of women who experience convulsion are

of childbearing age (9). During ovulation, reducing the ratio of progesterone to estrogen might predispose women to seizure. This ratio is reduced during the menstrual cycle, and it is increased during the early and middle luteal cycle (6). Changes in seizure frequency among men and women may occur in cycles of eight to 46 days and, interestingly, these changes sometimes occurs in cycles of 28 days, which is similar to the female sexual cycle (10).

Women with epilepsy receive antiepileptic drugs such as carbamazepine, oxcarbazepine, and phenobarbital. These medications increase the risk of osteoporosis (11). The estrogen level will decrease following ovariectomy and menopause, which can result in hot flashes and osteoporosis. Hormone replacement therapy (HRT) is usually used to relieve such symptoms (11). Studies have shown that the type of estrogen used in HRT is important, and that equine

conjugated estrogen might increase the risk and severity of seizures (12).

A traditional food source in Asia for many years, soybean is a rich source of phytoestrogens (13). Phytoestrogens contain isoflavones, coumestans, and lignans (14). Isoflavones display similar properties to endogenous estrogens and they can bond with estrogen receptors (15). However, the estrogenic activity of phytoestrogens is poor. Therefore, they are known as selective estrogen receptor modulators (14). These selective estrogen receptor modulators act as agonists or antagonists in different tissues (16). Nurses and midwives play important roles not only in controlling seizures during pregnancy, but also in educating and caring for women with convulsion. They should be aware of the latest knowledge concerning the treatment and prevention of convulsion in women in order to not only educate them, but also to monitor them and reduce the side effects of anticonvulsants. Given the side effects of anticonvulsant drugs and HRT, as well as the effects of phytoestrogens and soybean on estrogen receptors, a question remains regarding whether soybean can be used as an extrinsic source of estrogen without any associated estrogenic side effects. If this were the case, then soy could be used to reduce the risk and severity of seizure in women.

2. Objectives

Due to the limitations of human studies, this study aimed to investigate the effects of the chronic administration of estradiol and alcoholic extract of soy on pentylentetrazol (PTZ)-induced seizure in ovariectomized female rats.

3. Materials and Methods

3.1. Study Design and Animal Groups

In this experimental study, sixty female Wistar rats weighing 170 - 220 gr were studied. The animals were housed in standard cages under standard temperature (25°C) and humidity (50% - 60%) with a 12-hour light and dark cycle. The animals were fed with standard chow and they had free access to water. The rats were randomly divided into six groups of ten as follows: 1, CON (intact female rats); 2, SHAM (laparotomy in the female rats without removing the ovaries); 3, OVX (laparotomy in the female rats accompanied by removal of the ovaries); 4, VEH (ovariectomized rats that received sesame oil as a vehicle); 5, EST (ovariectomized rats that received 100 µg/kg estradiol); and 6, SOY (ovariectomized rats that received 20 mg/kg alcoholic extract of soy).

3.2. Preparing the Materials

To prepare the hydro alcoholic extract, 100 g of powdered soybean varieties (Glycine max, Barij Essence Pharmaceutical Co.) was prepared and dissolved in 800 mL of 70% ethanol. It was then kept at room temperature for 72 hours. Then, the extract was separated through the funnel. The dried extract was placed in water, alcohol, and other additional material to evaporate for 16 hours. In order to produce the drug, a certain amount of powder was dissolved in water and a dose of 20 mg/kg was achieved.

3.3. Surgery and Treatments

The study was conducted at the physiology research center, Kashan University of Medical Sciences. The female rats were anaesthetized using an intraperitoneal injection of Ketamine 100 mg/kg/ + xylazine 10 mg/kg. Then, under aseptic conditions, the researchers laparotomized and or ovariectomized the relevant animals. Two days after recovery (17), estradiol (100 µg/kg) was injected subcutaneously and soy extract (20 mg/kg) was gavaged daily for 28 days in the appropriate groups.

3.4. Behavioral Study

Pentylentetrazole (Sigma Aldrich, St. Louis, Missouri, USA) was dissolved in sterile 0.9% saline to prepare a fresh solution. In all study groups, the animal model of seizure was achieved via the intraperitoneal injection of 90 mg/kg pentylentetrazole. The rats were placed in a Plexiglas cage following PTZ injection and they were observed for 30 minutes. The seizure stages were classified as myoclonic twitch, clonic seizures, tonic hindlimb extension, and death. Although all of these stages were observed and their times were recorded using a chronometer, only the clonic seizure threshold (i.e., the time until the start of clonic seizure) was used to compare between different groups.

3.5. Ethical Considerations

The institutional review board and the ethics committee of Kashan University of Medical Sciences approved this study. Food and water were provided to the animals ad libitum. All ethical considerations regarding the use of laboratory animals were observed in light of the requirements of the Helsinki declaration.

3.6. Statistical Analyses

We analyzed the data using SPSS statistical software, version 13.0. All data were expressed as mean ± SD. The Kolmogorov-Smirnov test was used to assess the normality of the data in each group. Differences between the onset of seizures were analyzed using two-way analysis of variance and Tukey post-hoc tests, and the difference was considered statistically significant when $P < 0.05$.

4. Results

As shown in Table 1, no significant difference was found between the SHAM group and the CON group in terms of the onset of clonic seizure. In other words, laparotomy did not affect the onset of seizure. In addition, the onset of clonic seizures in the VEH group was not significantly different to that in the OVX group. In other words, use of the sesame oil vehicle had no effect on the onset of seizure. However, the onset of clonic seizures in the OVX group significantly decreased compared to that in the SHAM group ($P < 0.002$).

Moreover, the onset of clonic seizures increased significantly in the estradiol group compared to the VEH group ($P < 0.019$) (Table 2). In addition, the results of treatment with the soy extract did not show significant changes compared to the VEH group (Table 2).

Table 1. Mean and Standard Deviation in the CON, VEH, SHAM, and OVX Rat Groups in Terms of the Onset of Clonic Seizures Induced by PTZ in Ovariectomized Rats^a

Groups	N	The Onset Of Clonic Seizures (Sec)
CON	10	71.70 ± 10.85
SHAM	10	64.69 ± 3.23
OVX	10	51.30 ± 6.84 ^b
VEH	10	52.7 ± 7.18

Abbreviations: CON (intact female rats); OVX (laparotomy in the female rats accompanied by the removal of the ovaries: ovariectomized rats); SHAM (laparotomy in the female rats without removing the ovaries); VEH (ovariectomized rats that received sesame oil vehicle).

^aValues are expressed as mean ± SD.

^b $P < 0.002$, as compared to the SHAM group.

Table 2. Mean and Standard Deviation After 28 Days' Administration of EST and SOY on the Onset of Clonic Seizures Induced by PTZ in Ovariectomized Rats^a

Groups	N	The onset of clonic seizures (sec)
EST	10	61.84 ± 8.53 ^b
SOY	10	59.2 ± 4.91

Abbreviations: EST (ovariectomized rats that received 100 µg/kg estradiol) and SOY (ovariectomized rats that received 20 mg/kg alcoholic extract of soy).

^aValues are expressed as mean ± SD.

^b $P < 0.019$, as compared to the VEH group

5. Discussion

The present study showed that ovariectomy decreased the onset of PTZ-induced seizure. On the other hand, estradiol displayed anticonvulsant effects. The protective effects of estrogen against epileptic activities have also been reported in previous studies. Pereno et al. represented that

a Kainic acid-induced seizure model after an injection of 0.1 mg/kg of estradiol benzoate within 24 hours is neuroprotective in the stria terminalis (18). Moreover, the anticonvulsant effects of estradiol were shown in ovariectomized rats in models of picrotoxin- (19) and cyclosporine-induced seizures (20). In the current study, the prolonged use of estradiol was found to delay the onset of seizures in ovariectomized rats. This finding was in line with a previous study in which the prolonged usage of estrogen had neuroprotective effects against neuronal damage-induced seizures (21). However, another study reported that the acute administration of estrogen significantly increases the risk of seizures (22).

The pharmacological levels of estradiol can reversibly decrease the N-Methyl-D-aspartic acid-induced flow, excitation, and cell death of nerve cells (23). The activation of glutamate or the inactivation of GABA plays an important role in the process of creating epilepsy, (24). In another study, subcutaneously planted estradiol capsules were used to treat PTZ-induced seizures and it was suggested that estradiol may play the role of an anticonvulsant through 3α-5α-TPH, which increases in the hippocampus and functions as a GABAA receptor agonist (25). The effects of estrogen on seizure depend on various factors such as duration of treatment, methods of administration, or the neurotransmitter in the nervous system that is involved in seizure (26). The anticonvulsant effects of estrogen could possibly be attributed to substances such as neuropeptide Y (NPY) (27), which is a strong inhibitory neurotransmitter in the central region of the hippocampus (28). Sex hormones might act through different mechanisms such as changing the gene expression, releasing neurotransmitters, and a direct effect on neurotransmitter receptors (26).

In this study, 20 mg/kg of alcoholic extract of soy had no effect on the onset of seizures. However, Mohammadpour et al. showed that the proconvulsion effect of soy extract is dependent on the presence or absence of ovarian hormones (29). Moreover, previous studies have shown that soy extract can mimic the effects of estrogen and so reduce the latency of myoclonic and generalized tonic clonic seizures in ovariectomized rats (30). However, in the present study we did not observe this effect. Nonetheless, earlier studies reported that phytoestrogens play either estrogen or estrogen antagonist roles (13). Soy contains the isoflavones genistein and daidzein (31). Westmark et al. showed that the isoflavones in soybeans are effective in decreasing the threshold of seizure in mouse models and, therefore, they may increase the risk of epilepsy (32). Westmark's hypothesis declares that the soy phytoestrogens interact with the metabotropic glutamate receptor and stimulate the production of the synaptic proteins that play a key role in reducing the seizure threshold (33).

In this study, we demonstrated that the chronic administration of soy extract, a rich source of phytoestrogens, does not have a significant effect on the onset of PTZ-induced convulsions in ovariectomized rats, while the chronic administration of estradiol showed an anticonvulsant effect. The types of soy and the treatment period may exert an influence in this regard. Yet, our results were inconsistent with those of other studies (29, 30). Considering the various effects of this herb, we recommend that midwives and nurses assess the diet of women suffering with seizures during different periods of the ovarian cycle, pregnancy, and menopause. However, it is not possible to generalize the results of this study to all humans.

Due to the controversy concerning the effects of soy extract, we recommend that its molecular effects on seizure be further investigated. Moreover, nurses and midwives are recommended to conduct a well-designed clinical trial to assess the effects of soy extract on women with convulsion.

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Footnotes

Authors' Contribution: In this study, all authors were involved in all areas of the research.

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