Estriol - A Ovarian Hormone’s Proconvulsant Outcome on Kainic Acid Induced Seizure in Mice

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Abstract

Background: The estriol which was evaluated for its effect on Kainic acid kindling model of epileptogenesis in mice followed by evaluation on kindling-induced changes in cognitive and motor functions.

Material and methods: Kindling was induced on every alternate day for duration of 45 days and treatment with Kainic acid was given at dose ranging from 15 to 30 mg/kg body weight intraperitoneal (i.p) and estriol was also administered at dosage of 0.005 and 0.1 mg/kg through i.p. After induction of kindling the seizure severity was recorded and further percentage incidence of animals kindled at the end of 45 days was also recorded. Spatial learning and cognitive alterations were assessed by Morris water test (MWT) while motor function was assessed by grip strength meter.

Results: Estriol increased the rate of kindling in both the sexes of mice at great scale. Percentage incidence of seizures was also intensified. A noticeable decline in the grip strength, Morris water was observed following KA-kindling in pre-treated estriol groups of mice both the sexes.

Conclusion: Control animals developed a seizure score of 4 after the end of 5 weeks, mice treated with estriol exhibited kindling in first two weeks only Clomiphene at dose of 0.9 mg/kg i.p. exhibited anticonvulsant effects. The study displayed that estriol has powerful proconvulsant effect.

Keywords: Estriol; Kainic acid; Seizures; Clomiphene; Grip strength meter; Morris water test.

Introduction

Given the heterogeneity and complexity of seizures, its association with estrogens has been difficult to define. Existence of contrasting data on the association between estrogen and epileptic seizures has made it a much more interesting subject. Although it is generally believed that estrogen increase neuronal excitability and mediates proconvulsant effects [1-3]. There are clinical and animal data to show that estrogen also have anticonvulsant effects [4-7]. Estrone (E1), estradiol (E2) and estriol (E3) are the three biologically active estrogen present. Estradiol is the major estrogen in premenopausal women and is the most investigated among all estrogen in various epilepsy models.

Pretreatment with beta-estradiol in female rats showed neuroprotective effects on status epilepticus induced neuronal damage [8]. Estrogen was reported to attenuate clonic seizures induce by kainic acid [9].

Earlier studies of estradiol administration in rodents have revealed proconvulsant effects [3]. Estrogen applied to cortex could increase seizures [10]. Estradiol was shown...
to facilitate kindling [11]. While most of the studies show proconvulsant effects of estradiol, there are also studies which report anticonvulsant and neuroprotective effects.

There are very few studies evaluating the role of estrone and estriol on seizures. Barha and co-workers [12] recently reported a reduced percentage of animals with hippocampal neuronal loss following estrone (E1) [13]. It was also shown to significantly decrease the percentage of animals with clonic seizures and their mortality in kainate-induced seizures [14]. Thus, both anticonvulsant and neuroprotective effects have been reported with estrone.

So far, estriol (E3), the third estrogen has not been investigated for its effects on seizures. There is insufficient information on how it can affect seizures or neuronal excitability. The present study, thus, evaluated the effects of this hormone on Kainic-acid kindling model of epileptogenesis in mice followed by evaluation on kindling induced changes in motor and cognitive functions. Further, since sex can influence the effect of estrogen on seizures, the study would involve both male and female mice.

**Material And Methods**

**Animals**

Swiss Albino mice weighing between 25-35g were used. They were housed in polypropylene cages and maintained at 25-30 °C and 50% relative humidity in a natural light/dark cycle. Animals were given food and water ad libitum. The mice were kept separate from the opposite sex all the time. Institutional Animal Ethics Committee (IAEC) of Pinnacle Biomedical Research Institute (PBRI), Bhopal (Reg. No. 1824/PO/ERe/S/15/CPCSEA) had approved the study and study approval reference number is PBRI/IAEC/PN-16026.

**Experimental design**

**Induction of kindling**

Kainic acid (KA), from Sigma-Aldrich, dissolve in 0.9% saline at 5 mg/ mL to keep the injection volume below 0.5 mL and was prepared fresh on the day of each experiment. Mice were given intraperitoneal (i.p.) injections of KA, at doses ranging from 15 to 30 mg/kg body weight. Behavioural seizure activity was monitored for 2 h and classified according to a modified Racine scale [15]: stage 0, normal behavior; stage 1, immobility; stage 2, repetitive movements, myoclonic twitch, or head bobbing; stage 3, bilateral forelimb clonus and rearing; stage 4, continuous rearing and falling; and stage 5, generalized tonic–clonic seizure. **Grip strength test**

The neuromuscular function was determined with the aid of a grip strength meter. The mouse was allowed to hold the grip with it forepaws. The mouse was then pulled back horizontally until it released its grip. The grip strength reading was read directly read from the digital meter [16].

**Spatial memory: Morris water maze test**

Spatial learning and cognitive alterations were assessed using the water maze test described by Morris [17] and further tested by Charles [18]. It consists of a circular plastic pool of 73 cm in diameter and filled with water, kept at 27 ± 2°C, at a depth of 56 cm. The pool was divided conceptually into four quadrants and a platform (6 × 6 cm) was placed 1 cm below the water surface in the center of one of the four quadrants. A mouse was released into the water at one of four randomly selected positions near and facing the wall. Mice were trained with 4 trials per day for 5 days (at 1 min intervals). In probe trails, mice were allowed to swim for 360 s. The pool remained in the same position inside of the room, due to the fact that an animal’s ability to locate the platform depends on the use of visible keys available around the pool. A record should be made of latency, defined as the time elapsed from the moment of release to the moment it climbed on the platform. It was considered that an animal has found the platform when it stays on it for 5 s.

**Statistical analysis**

The data obtained were analysed by analysis of variance (ANOVA) followed by Dunnett's multiple comparison test. P<0.05 was significant.
Results

Consequence of estriol and clomiphene in kainic acid model of mice on incidence of kindling. Kindling was induced chemically in both the sexes by using repetitive treatment of KA at a subconvulsant dose (25 mg/kg i.p) every alternate day (Figure 1 Effect of estriol and clomiphene on the incidence of animals kindled following repeated treatments).

Figure 2a: Drug therapy (Estriol and Clomiphene) their consequences on seizure severity in 35 days (male mice) through the course of induction of kindling by KA.
Group of animals both the sexes who were given clomiphene and diazepam followed by KA illustrated very significant reduction in percentage of incidence (Figure 1). They illustrated the similar performance in severity of seizure scale too (Figure 2a,b).

When group of animals both the sexes were pre-treated with estriol at dosage of (0.005 and 0.01 mg/kg i.p.), it was observed that it reduces the kindling induction time from 5 weeks to 3-2 weeks for male and female mice respectively. Furthermore, it also elevates the percentage incidence of seizures (Figure 1), on the other hand Clomiphene (0.9 mg/kg i.p) exhibited significant (P<0.01) reduction of percentage incidence of KA-induced kindling and postpones the development of kindling (Figure 1, 2).

Reciprocated dosage with KA at a subconvulsive dose (25 mg/kg i.p.) every alternate day, induced chemical kindling in both the sexes (Figure 1). We observed that animals treated with Estriol (0.005 and 0.01 mg/kg i.p.) showed significant (P<0.01) increase in incidence of KA-induced rapid kindling.

Estriol at both the doses has produced maximum rate of incidence of kindling before the completion of duration of treatment in both the treatment groups that is in male and female. Whereas treatment with Clomiphene (0.9 mg/kg i.p.) showed significant (P<0.01) reduction of incidence of KA-induced kindling. While combination of Clomiphene with lowest dose of Estriol (0.005 mg/kg i.p.) has also significantly (P< 0.01) increased incidence of KA-induced kindling irrespective of the sexes. Further, treatment with diazepam (3 mg/kg i.p.) exhibited significant (P<0.01) reduction on incidence of KA-induced kindling in both the sexes (Figure 4.1). Beside we detected in Figure 2a and 2b while observing Effect of Estriol and Clomiphene on seizure severity in 35 days during development of KA-induced kindling in mice, that administration of subconvulsive dose (25 mg/kg i.p.) of KA on alternate day induced seizure severity of 4.0 in male while 4.3 in female mice on 35th day (Figure 2a; Figure 2b). There was significant (P<0.01) increase in mean seizure score as compared to control group (Group 1) in both the sexes but more in female (Figure 2a; Figure 2b). Animals treated with Estriol (0.005 mg/kg i.p.) showed seizure severity of 4 in male while 4.2 in female mice on 14th day but higher dose (0.01 mg/kg i.p.) showed seizure severity of 4 in male while 5 in female on 7th day i.e. it reduces the time of induction of kindling. There was significant (P<0.01) increase in
mean seizure score (4 on day 14th; 4 on 7th day) in male at lower and higher doses respectively. While in case of female there was also significant (P<0.01) increase in mean seizure score (4.2 on day 14th; 5 on 7th day) in female at lower and higher doses respectively.

Addition of Clomiphene (0.9 mg/kg i.p.) to estriol (0.005 mg/kg i.p.) has shown seizure severity of 3.8 and 4.2 on day 21st in male and female respectively. There was significant (P<0.01) increase in mean seizure score in male and female respectively. Clomiphene at dose (0.9 mg/kg i.p.) showed seizure severity of 1.4 in male and 1.6 in female on 35th day while there were significant (P<0.01) changes in mean seizure score in both the sexes as compared to group 2. Diazepam at dose (3 mg/kg i.p.) showed seizure severity 0.91 in both the sexes on 35th day.

**Figure 3:** Drug therapy (Estriol and Clomiphene) their consequences on grip strength of mice with reciprocated dose of KA.

**Figure 4:** Drug therapy (Estriol and Clomiphene) their consequences on the Morris water test of mice with reciprocated dose of KA.
Consequence of estriol and clomiphene on grip strength (GS) and Morris water maze following repeated treatment with a subconvulsant dose of KA for 5 weeks in mice. A noticeable decline in the grip strength and morris water was observed following KA-kindling in pre-treated estriol groups of mice both the sexes (p<0.01, Figure. 3,4). Clomiphene and diazepam were found to be unsuccessful in reversing the estriol effects in KA-kindled mice for grip strength (GS) and Morris water maze tests.

Discussion

It has been commonly accepted that estrogens are excitatory to the central nervous system (CNS) and mediate proconvulsant effects [1-3]. However, recently it has become clear that they also have anticonvulsant [4,6,7] and neuroprotective [9] effects. These opposite effects of estrogens on seizures depends upon treatment duration, latency prior to seizure testing, dose, hormonal status and/or seizure type and model used etc. In the present study, we endeavoured to determine how Estriol (E3), the third estrogen, affects seizure susceptibility in a rodent model of epileptogenesis. We selected estriol due to two reasons: firstly, this is the hormone which has virtually been neglected in epilepsy research even though it is one of the three principle estrogens produced by the body [11] and secondly, it has been recently been considered to be one of the safest hormone in postmenopausal women undergoing hormone replacement therapy (HRT) [19,20] as well as offered neuroprotection in patients with multiple sclerosis [21,14].

From the result we concluded that estriol at both the doses significantly reduced the time for induction of kindling and moreover when control animals developed a seizure score of 4 after the end of 5 weeks, mice treated with estriol exhibited kindling in first two weeks only (Figure 1 and 2). The estriol treated mice also showed significant increase in the % incidence of animals kindled (Figure 1) and a higher seizure severity. Human dose of estriol was used in our study and surprisingly this dose produced such a marked proconvulsant effect, which is ironic and thought for concern regarding the patients who are at risk for seizure disorders. Moving forward the behaviour indicative of more kindled females than males’ mice in the above study, the explanation for this could be related to changes in the estrous cycle and exacerbation of catamenial seizures in females [22]. Their estrous cycle dates were not included in our study. Wahnschaffe [23] reported, however, that natural changes in the sex hormone levels during the estrous cycle doesn’t affect seizure susceptibility in the amygdala kindling model of epilepsy. Henceforth, as we have used another model, it is indicative that the seizure susceptibility was affected by changes in hormone levels in female mice.

The role of Estradiol, the most extensively investigated estrogen, on seizure activity is thought to be partly mediated through estrogen receptors (ERS) [24] [25]. Pretreatment with Clomiphene Citrate, an antagonist of ERS, couldn’t reverse the marked proconvulsant effects of Estradiol. However, it demonstrated significant antiepileptogenic effects against development of PTZ-kindling. The antiepileptogenic effects of Clomiphene observed in our study were even comparable to diazepam. Though not many studies have investigated the effects of Clomiphene on seizures, our findings are in agreement with a case report [26] where Clomiphene benefited a 36 year old man from developing seizures. Other clinical evidence also shows anticonvulsant effects of Clomiphene in both epileptic men and women [27,28]. Nicoletti and co-workers [3] demonstrated a mild anticonvulsant effect of Clomiphene against kainic acid (KA)-induced seizures in rats and a proconvulsant effect when Clomiphene was used at higher doses. It has been postulated that Clomiphene may improve seizures by either normalizing the serum testosterone levels, by raising the serum levels of other anticonvulsant drugs or by an action at a cerebral level [26].

AED therapy and epilepsy is associated with motor and cognitive dysfunction, for this reason we also evaluated the effects on grip strength, rotarod and Morris water test following KA- induced kindling in mice. A convincing decline in the strength rotarod and Morris water test was noted after KA-induced kindling indicating a decline in motor function and spatial memory, clomiphene and diazepam were unsuccessful in reversing their effect. Our study suggests that estriol may have distinct effect on cognitive functions that the other two estrogen, estrone and estradiol. Clomiphene has recently been speculated to treat cognitive impairment [29].

Conclusion

From our study we concluded that estriol has contrasting powerful proconvulsant effect from other two estrogens (estrone, estradiol). It enhances the development of KA-induced kindling which was not reversed by clomiphene and diazepam. Its administration in patients who has history or susceptible to seizures is not advisable.

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Conflict of Interest

None declared.

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