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Aromatase inhibition by letrozole attenuates kainic acid-induced seizures but not neurotoxicity in mice

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ABSTRACT

Evidence shows neurosteroids play a key role in regulating epileptogenesis. Neurosteroids such as testosterone modulate seizure susceptibility through its transformation to metabolites which show proconvulsant and anticonvulsant effects, respectively. Reduction of testosterone by aromatase generates proconvulsant 17-β estradiol. Alternatively, testosterone is metabolized into 5α-dihydrotestosterone (5α-DHT) by 5α-reductase, which is then reduced by 3α-hydroxysteroid oxidoreductase enzyme (3α-HSOR) to form anticonvulsant metabolite 3α-androstanediol (3α-Diol), a potent $GABA_A$ receptor modulating neurosteroid. The present study evaluated whether inhibition of aromatase inhibitor letrozole protects against seizures and neuronal degeneration induced by kainic acid (KA) (10 mg/kg, i.p.) in Swiss albino mice. Letrozole (1 mg/kg, i.p.) administered one hour prior to KA significantly increased the onset time of seizures and reduced the% incidence of seizures. Pretreatment with finasteride, a selective inhibitor of 5α-reductase and indomethacin, a selective inhibitor of 3α-hydroxysteroid oxidoreductase enzyme (3α-HSOR), reversed the protective effects of letrozole in KA-induced seizures in mice. Microscopic examination using cresyl violet staining revealed that letrozole did not modify KA-induced neurotoxicity in the CA1, CA3 and DG region of the hippocampus. Letrozole treatment resulted in the reduced levels of 17-β estradiol and elevated the levels of 5α-dihydrotestosterone (DHT) and 3α-Diol in the hippocampus. Finasteride and indomethacin attenuated letrozole-induced elevations of 5α-DHT and 3α-Diol. Our results indicate the potential anticonvulsant effects of letrozole against KA-induced seizures in mice that might be mediated by inhibiting aromatization of testosterone to 17β-estradiol, a proconvulsant hormone and by redirecting the synthesis to anticonvulsant metabolites, 5α-DHT and 3α-Diol. Acute aromatase inhibition, thus, might be used as an adjuvant in the treatment of status epilepticus and can be pursued further.

1. Introduction

Epilepsy is the most common serious neurological disease and is defined as “a disease of the brain characterized by an enduring predisposition to generate epileptic seizures” (Fisher et al., 2014). According to World Health Organization (WHO), around 50 million people currently suffer from epilepsy worldwide, and approximately 2.4 million people are diagnosed with epilepsy each year. Despite the advent of newer antiepileptic drugs, over 30% of people suffering from epilepsy have a refractory seizure (WHO, 2015). Hence, it is important to elucidate novel targets/drugs for this disease.

Neuroactive steroids or neurosteroids are a group of steroids produced peripherally by exocrine glands such as ovaries and adrenal gland or synthesized directly within the brain by converting cholesterol into pregnenolone, which is converted to progesterone, deoxycortisone, testosterone (Biagini et al., 2010; Reddy and Mohan, 2011). Steroid hormones synthesized in the periphery should cross the blood-brain barrier to influence neuronal signaling. These neurosteroids play a major role in the neuroendocrine control of brain excitability and seizure susceptibility due to their conversion into different metabolites such as allopregnanolone, allotetrahydrodeoxycorticosterone, estradiol and androstenediol (Gangisetty and Reddy, 2010; Pereira et al., 2009; Reddy and Mohan, 2011; Ryan and Frye, 2008). Testosterone has a prominent role in seizure susceptibility, and it shows both proconvulsant and anticonvulsant effects based on the animal model employed and type of seizures (Edwards et al., 1999; Frye et al., 2001a;

Abbreviations: 5α-DHT, 5α-dihydrotestosterone; 3α-HSOR, 3α-hydroxysteroid oxidoreductase; 3α-Diol, 3α-androstanediol; WHO, World Health Organisation; CYP, cytochrome; KA, kainic acid; PTZ, pentylenetetrazole; TLE, temporal lobe epilepsy; DG, dentate gyrus; i.p., intraperitoneal; CV, cresyl violet; AEDs, antiepileptic drugs; 2MEOHE2, 2-methoxyestradiol

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Herzog et al., 1998; Reddy, 2004b). Testosterone is metabolized to different neurosteroids through individual pathways by distinct enzymes. Testosterone reduced to intermediate 5α-dihydrotestosterone (5α-DHT) by the 5α-reductase enzyme, which is then further reduced by 3α-hydroxysteroid oxidoreductase (3α-HSOR) to form 3α-androstanediol (3α-Diol). 3α-Diol, structurally corresponding to allopregnanolone, reduces brain excitability via $GABA_A$ receptors (Reddy and Kulkarni, 2000). On the other hand, aromatase, a cytochrome P450 enzyme (CYP19A1), converts testosterone into 17β-estradiol, which enhances brain excitability (Martini et al., 1993). The enzyme is highly expressed in areas that are involved in the pathophysiology of epilepsy such as hippocampus and neocortex.

Aminoglutethimide, first generation aromatase inhibitor, has been tried as an antiepileptic drug in combination with other standard drugs (Aguilar et al., 1961). Letrozole is a third generation reversible non-steroidal aromatase inhibitor, approved by US-FDA, for the treatment of postmenopausal women with hormone receptor-positive or hormone receptor unknown locally advanced or metastatic breast cancer (NIH, 2013). A recent preclinical study in our laboratory demonstrated the protective effect of letrozole in the prevention of kindling induced by pentylenetetrazole (PTZ) in mice (Rashid et al., 2015). Also, letrozole was previously reported inhibiting the testosterone-induced increase in PTZ seizure activity in mice (Reddy, 2004b). Clinically, letrozole has been shown to improve seizure control in a 61-year-old man with temporal lobe epilepsy (Harden and MacLusky, 2004, 2005). It suggests that letrozole should be further investigated as a beneficial treatment for male patients with epilepsy. Therefore, in the present work, we examined the effect of letrozole against kainic acid-induced seizures and neurotoxicity in mice. Kainic acid (KA) is an excitotoxic analog of glutamate and an agonist of ionotropic KA receptors. KA-induced status epilepticus model is the most accepted model of temporal lobe epilepsy (TLE) (Levesque and Avoli, 2013; Loscher and Brandt, 2010). To explore the mechanisms involved, we utilized pharmacological tools including finasteride, a selective inhibitor of 5α-reductase and indomethacin, a selective inhibitor of the 3α-HSOR enzyme, to ascertain whether the effects are mediated by increased synthesis of 5α-DHT or 3α-Diol respectively. Further, since systemic administration of KA induces epileptic seizures, behavioral changes, and consequent neuropathological changes bilaterally in the CA1, CA3 and dentate gyrus (DG) regions of the hippocampus (Haas et al., 2001; Hellier et al., 1998), we evaluated the histopathological changes in the hippocampus and measured the levels of the hormones involved in the testosterone metabolic pathway. We hypothesize that aromatase inhibitors will reduce local estradiol levels by inhibiting the conversion of testosterone to 17β-estradiol, and will reduce brain excitability as estrogens are excitatory and potentiate seizures. 3α-Diol is a structural analog of allopregnanolone and plays a significant part in mediating the anticonvulsant effects. Thus, it is possible that aromatase inhibition could be a potential approach for reducing brain excitability.

2. Material and methods

2.1. Animals

All the experimental procedures were performed in compliance with the guidelines of the Institutional Animal Ethics Committee of Jamia Hamdard, New Delhi under a protocol approved (Project no.1266, year, 2016) by the committee. Swiss albino male mice (30–40 g, 8–10 weeks old) were procured from Central animal house facility, Jamia Hamdard, New Delhi. Animals were acclimatized for 7 days in the laboratory and were housed in polypropylene cages. All animals were maintained under controlled conditions of temperature and humidity (25 ± 2 °C, 55–65%) and the light-dark cycle of 12:12 h and maintained on pellet feed with food and water *ad libitum*.

2.2. Study design

The study design is represented in (Fig. 1). The experiments were performed in the morning and the drugs treatments were given between 10 and 11 am in the soundproof room of neurobehavioral

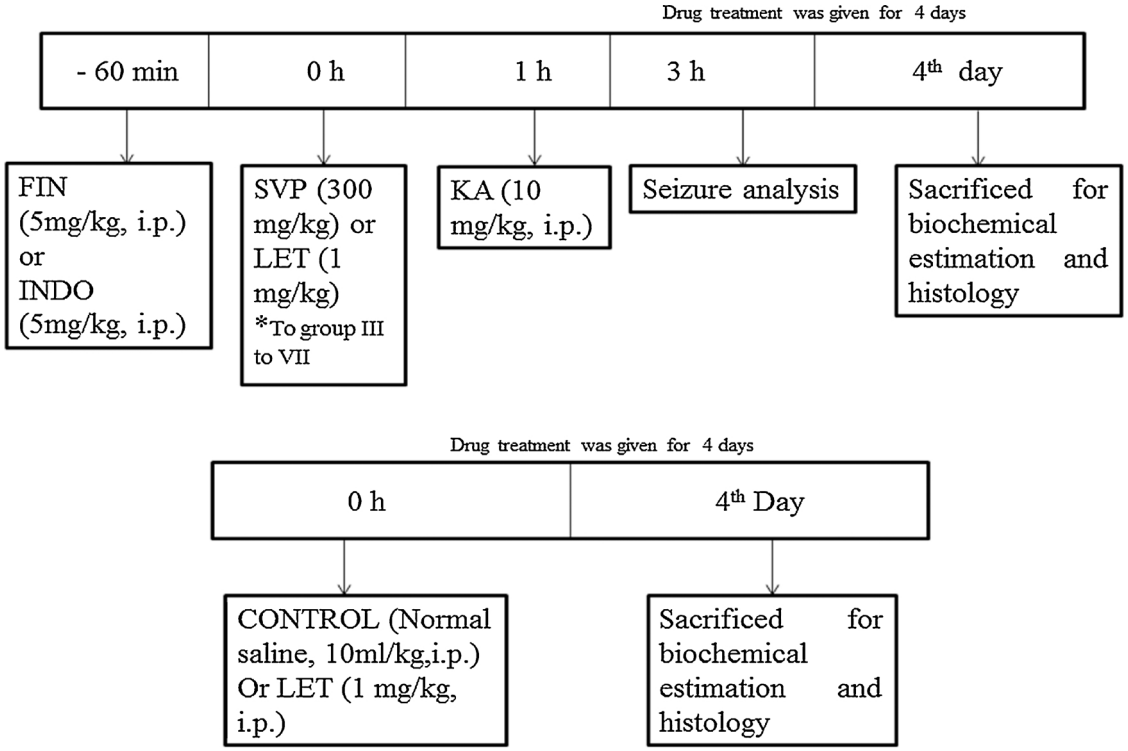

Fig. 1. Schematic depiction of dosing schedule. KA-Kainic acid (10 mg/kg); LET-Letrozole (1 mg/kg); FIN-Finasteride (5 mg/kg); INDO-Indomethacin (5 mg/kg); SVP-Sodium valproate (300 mg/kg).