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Effects of onopordia, a novel isolated compound from *Onopordon acanthium*, on pentylenetetrazole-induced seizures in mice: Possible involvement of nitric oxide pathway

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ABSTRACT

Epilepsy is identified as a brain disorder and characterized by unpredictable disruption of normal brain function. Due to adverse side effect associated with antiepileptic drugs and also resistance profile, improvement of antiepileptic medications with more beneficial anticonvulsant activity is essential. Natural products have demonstrated their therapeutic properties such as anxiolytic, antidepressant and anticonvulsant activities and a source for identification of novel lead compounds. Therefore, the purpose of this study was to evaluate the effects of *Onopordon acanthium* secondary metabolite, onopordia, on pentylenetetrazole (PTZ)-induced seizure in male mice and investigate the possible role of nitric oxide pathway. Different doses of onopordia (0.1, 1 and 10 mg/kg) and phenobarbital (20 mg/kg) were administered intraperitoneally (i.p., 30, 60 and 120 min) prior to induction of epileptic seizure and compared to control groups. Onopordia demonstrated anticonvulsant effects when administrated at dose of 10 mg/kg, i.p. and optimum time 60 min prior to induction of seizure. Anticonvulsant effect of onopordia was blocked by applying a single dose of a non-selective nitric oxide synthase (NOS) inhibitor, Nω-nitro-L-arginine methyl ester hydrochloride (L-NAME; 10 mg/kg, i.p.), and also a single dose of a selective neuronal NOS (nNOS) inhibitor, 7-nitroindazole (7-NI; 30 mg/kg, i.p.). Administration of ketamine as a N-Methyl-D-aspartic acid (NMDA) receptor antagonist (0.5 mg/kg; i.p.) with onopordia did not change the anticonvulsant effect of onopordia. The results of the present study demonstrated the anticonvulsant effect of onopordia as a new lead compound and also contribution of NO/nNOS pathway on PTZ-induced seizure in mice.

1. Introduction

Epilepsy is a widespread and serious neurological disorder worldwide which is resulted from the unusual neurons firing in the brain.[1–3] Seizure have effect on consciousness, cognition, memory and behavior.[4] Using of current anti-epileptic drugs have some limitations, such as adverse side effects associated with epileptic seizure medications and also occurring anti-seizure drug resistance in some patients.[5,6]

Antiepileptic medications such as barbiturates, benzodiazepines, carbamates and carboxamides demonstrate anticonvulsant activity through interactions with a variety of cellular targets.

Abbreviations: AG, Aminoguanidine hydrochloride; DMSO, dimethyl sulfoxide; eNOS, endothelial NOS; GABA, gamma-aminobutyric acid; iNOS, inducible NOS; ip, intraperitoneally; iv, intravenously; L-Arg, L-arginine; N-Methyl-D-aspartic acid, NMDA; L-NAME, Nω-nitro-L-arginine methyl ester hydrochloride; nNOS, neuronal NOS; NOS, nitric oxide synthase; 7-NI, 7-nitroindazole; PTZ, pentylenetetrazole.

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Modulation of voltage-gated ion channels,[7] enhancement of gamma-aminobutyric acid (GABA) inhibition[8] and decreasing glutamatergic transmission[9] are three major classes of mechanism for antiepileptic drugs. More recently, other neurotransmitters such as nitric oxide has been recognized which modulate synaptic transmission.[10]

Nitric oxide (NO), which is a neurotransmitter in the central and peripheral nervous systems, synthesized from L-arginine by nitric oxide synthase (NOS).[11,12] This acts like an intracellular messenger in physiological reactions.[13] Endothelial NOS (eNOS), inducible NOS (iNOS) and neuronal NOS (nNOS), are three kinds of NOS isoforms.[13]

One of the fundamental roles of NO in the brain, would be seizure induction and progression.[14] In 2009, it was reported that enhancement of anticonvulsant property of lithium chloride would be through L-arginine/NO pathway.[15] Moreover, it has been demonstrated that NO pathway mediates the anticonvulsant effect of tramadol.[16]

More than 80% of drugs are directly taken from natural products or indirectly generated from natural compounds.[17] Therefore, searching for natural anti-seizure medications as alternatives to synthetic ones is of great interest.[18] In this study onopordia, which has been isolated from *O. acanthium* as the active compound[19] was used in order to discover its possibility to manage epileptic seizure. Therefore, the objective of this study was to investigate the probable effect of onopordia as a novel scaffold with neuroprotective features and also involvement of NO/NMDA pathways on pentylenetetrazole induced seizure in mice.

2. Materials and methods

2.1. Experimental animals

Male adult NMRI mice weighing 25–30 g, were used before acute administration. The animals were maintained in standard polycarbonate cages, in a group of four-five mice in each cage. The cages were kept in a temperature-controlled room (24 ± 1 °C) on a 12-h light/dark cycle with free access to food and water. The behavioral experiments were carried out between 09:00 and 13:00 and each animal was used just once and the number of animals in each group was 8–12. All the animal studies were performed according to Tehran University of Medical Sciences guidelines for animal care. Furthermore, efforts were made to reduce animal suffering and using only the number of animals necessary to produce reliable scientific data.

2.2. Chemicals

The following drugs and chemicals were used throughout this study. Pentylenetetrazole (PTZ), Nω-nitro-L-arginine methyl ester hydrochloride (L-NAME), 7-nitroindazole (7-NI), aminoguanidine hydrochloride (AG), L-arginine (L-Arg), phenobarbital, ketamine hydrochloride and dimethyl sulfoxide (DMSO) were purchased from Sigma Co. (St. Louis, MO, USA). Onopordia was extracted from medicinal plant, *O. acanthium* and confirmed by experimental data.[19]

Onopordia solution was prepared in saline (0.9%)/DMSO (90:10, v/v), to provide the appropriate concentrations. PTZ was dissolved in saline (0.9%) in order to provide the 5 mg/ml (0.5%) concentration. Except PTZ which was administered intravenously (i.v.), all other drugs were administered intraperitoneally (i.p.) at required doses in a volume of 10 ml/kg of the mice body weight. Appropriate controls were prepared for each experiment.

2.3. Determination of seizure threshold

A 30-gauge butterfly needle was applied in order to insert into the tail vein of mice, while the mice was restrained in a mice restrainer. An infusion pump (Harvard, USA) was employed in order to infuse PTZ at the constant rate of 1 ml/min.[20] Infusion was paused after observation of forelimb clonus followed by full clonus of the body. The dose of PTZ (mg/kg of mice weight) needed to induce clonic seizure was applied as an index of seizure threshold.[21]

2.4. Treatment

2.4.1. Experiment 1

Animals received acute i.p. injections of phenobarbital (20 mg/kg, i.p.) and different doses of onopordia (0.1, 1 and 10 mg/kg) at different times (30, 60 and 120 min) before determination of PTZ-induced seizure threshold. Control animals received saline/DMSO solution at the same procedure.

2.4.2. Experiments 2 & 3

The nonspecific NOS inhibitor, L-NAME (10 mg/kg) and also NO precursor, L-Arg (60 mg/kg), were acutely administered 15 min before saline and onopordia (10 mg/kg, the dose which induced the highest seizure threshold in experiment 1 and 75 min before induction of seizure by PTZ.

2.4.3. Experiments 4 & 5

In experiments 4 & 5, in order to assess the nNOS role, the specific nNOS inhibitor 7-NI (30 mg/kg, i.p.) was administered 15 min before saline and onopordia (10 mg/kg, i.p.) as well as the iNOS inhibitor, AG (100 mg/kg, i.p.). The results were compared to the control group.

2.4.4. Experiment 6

In experiment 6, mice received a single dose of the N-Methyl-D-aspartic acid (NMDA) receptor antagonist, ketamine (0.5 mg/kg, i.p.) alone and 15 min before the effective doses of onopordia (10 mg/kg, i.p.). The PTZ-induced seizure threshold was determined 60 min after the injection of onopordia. The doses and times of injection of L-NAME, L-Arg, AG, 7-NI, and ketamine were chosen based on previously published study.[15] Control animals received the same volume of the saline/DMSO solution in all experiments.

2.5. Statistical analysis

Values are presented as the mean ± S.E.M. of seizure threshold in mice for each experimental group. Independent sample *t*-test and one-way ANOVA followed by Tukey's multiple comparisons of variances were used to analyze data where appropriate. SPSS statistical software package (version 22) was used for all the data analysis.

3. Results

3.1. Effect of time and dose in onopordia treatment on the seizure threshold

Fig. 1, shows the time course effect of different doses of onopordia on the seizure threshold. As shown, intraperitoneal administration of onopordia (10 mg/kg, i.p.) significantly increased the PTZ-induced seizure threshold ($P < 0.01$) 30 and 60 min after administration, compared with saline-treated control animals. However, administration of onopordia (10 mg/kg, i.p.) did not affect the seizure threshold 120 min prior to seizure induction. On the other hand, PTZ-induced seizure threshold were not changed with

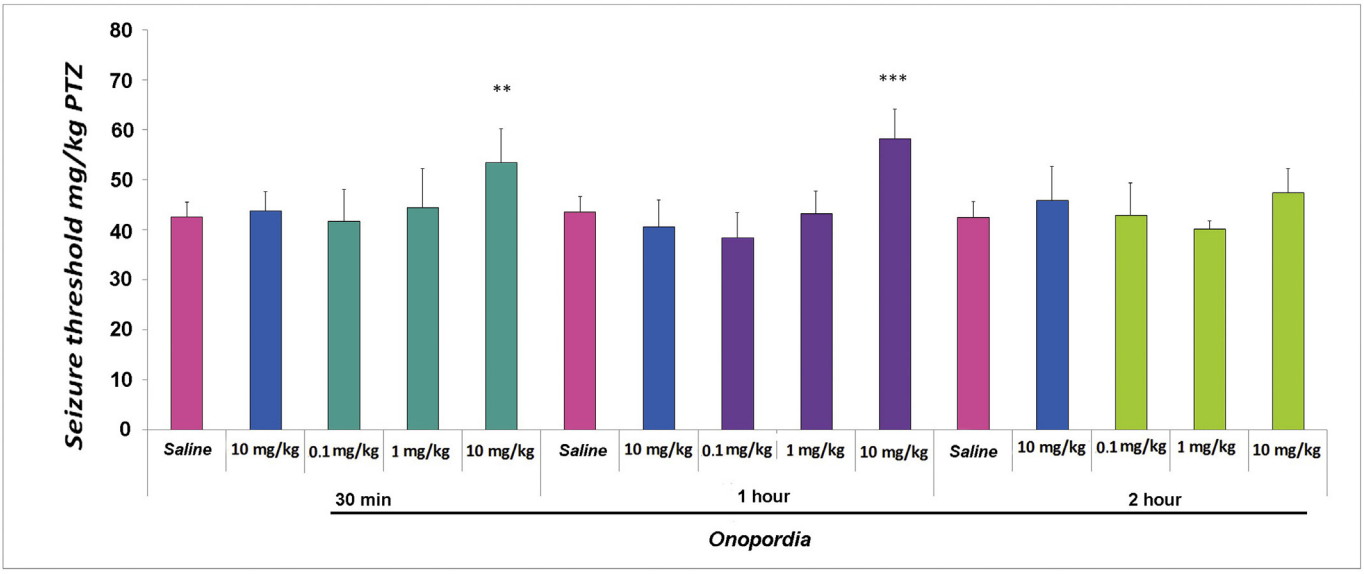

Fig. 1. Time course effect of different doses of onopordia (0.1, 1 and 10 mg/kg) on the PTZ-induced clonic seizure threshold in mice. Different doses of onopordia were administered 30, 60 and 120 min with (ocean green, violet and light green columns respectively) before PTZ and the effects were compared with those of a control sample and also phenobarbital at the same time course (blue columns). Data are expressed as means ± SEM of the seizure threshold in each group. *P < 0.05 and ***P < 0.001 compared with the saline group.

administration of lower doses of onopordia (1 and 0.1 mg/kg) after 30, 60 and 120 min ($F(11,95) = 11.969$, $P < 0.001$). Interestingly, the PTZ-induced seizure threshold increment for onopordia was slightly higher than that of phenobarbital (20 mg/kg, i.p.; $P < 0.001$).

3.2. Effect of L-NAME and L-Arg on the anticonvulsant effect of onopordia

Acute administration of the nonspecific NOS inhibitor, L-NAME (10 mg/kg, i.p.), had no significant effect on modifying seizure threshold. However, it significantly reversed the anticonvulsant effect of onopordia (10 mg/kg, i.p.), when administered 15 min before onopordia (10 mg/kg, i.p.) ($F(3,31) = 32.038$, $P < 0.001$). Saline and L-NAME was administered 15 min prior to onopordia. Treatment with L-Arg, the NO precursor (60 mg/kg, i.p.), 15 min prior to onopordia (10 mg/kg, i.p.) did not modify the anticonvulsant effect of onopordia ($F(3,31) = 10.412$, $P < 0.001$; Fig. 2).

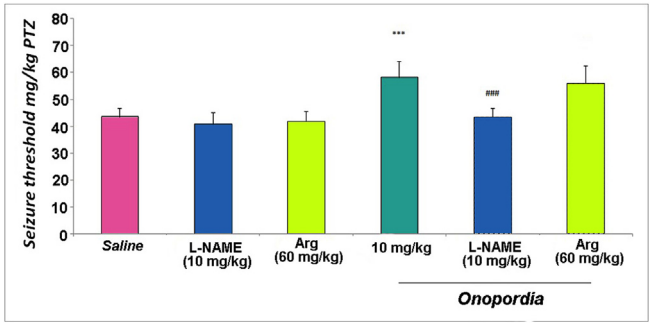

Fig. 2. Effect of L-NAME and L-Arg on anticonvulsant effect of onopordia. L-NAME (10 mg/kg, i.p.) and L-Arg (60 mg/kg, i.p.) were administered 15 min before onopordia (10 mg/kg, i.p.). Data are expressed as means ± SEM of the seizure threshold in each group. ***P < 0.001 compared with the saline group and # # #P < 0.001 vs onopordia (10 mg/kg; i.p.).

3.3. Effect of 7-NI and AG on the effective doses of onopordia

As depicted in Fig. 3, pretreatment with 7-NI (specific nNOS inhibitor; 30 mg/kg, i.p.) before onopordia (10 mg/kg, i.p.) significantly reversed the anticonvulsant effect of onopordia ($F(3,31) = 33.45$; $P < 0.001$). While, selective inhibitor of iNOS, AG (100 mg/kg, i.p.) had no effect on anticonvulsant effect of onopordia ($F(3,31) = 36.522$, $P < 0.001$; Fig. 3).

3.4. Effect of the NMDA receptor antagonist on the effective dose of onopordia

In order to investigate the role of NMDA receptors in the anticonvulsant effect of onopordia, we examined the effect of the NMDA receptor antagonist, ketamine, on the anticonvulsant effect of onopordia. Administration of ketamine (0.5 mg/kg, i.p.) 15 min before onopordia (10 mg/kg, i.p.) did not modify the anticonvulsant effect of onopordia ($F(3,31) = 33.317$ $P < 0.001$; Fig. 4).

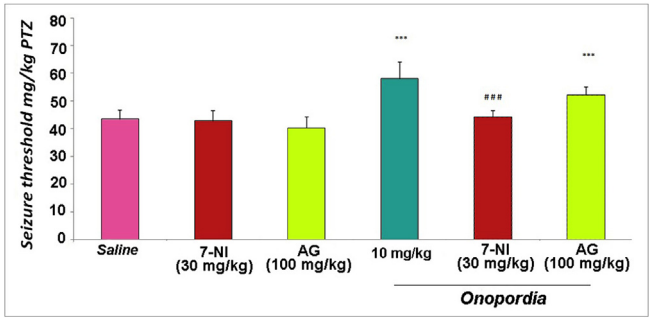

Fig. 3. 7-NI (30 mg/kg, i.p.) and AG were administered 15 min before onopordia (10 mg/kg, i.p.). Data are expressed as means ± SEM of the seizure threshold in each group. ***P < 0.001 compared with the saline group. # # #P < 0.001 vs onopordia 10 mg/kg, i.p.).

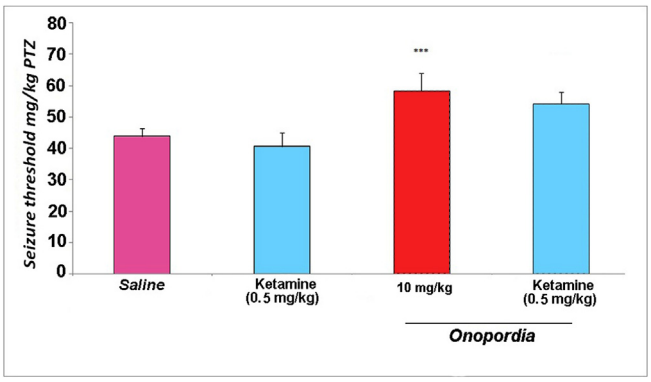

Fig. 4. Ketamine (0.5 mg/kg, i.p.) was administered 15 min before onopordia (10 mg/kg, i.p.). Data are expressed as means ± SEM of the seizure threshold in each group. ***P < 0.001 compared with saline group.

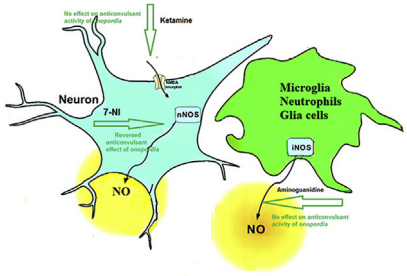

Fig. 5. Anticonvulsant activity of onopordia in PTZ-induced seizure in mice, proposing the involvement of NO pathway.

4. Discussion

Epileptic seizure, a worldwide illness, affects a large population in the world. One of the most effective medications for the treatment of epileptic seizure is via NO pathway. Meanwhile, medicinal plants have been used for treating human diseases and traditional medicine has recently drawn a rather increasing attention to itself as the most affordable source of treatment. Therefore, they can be great resources to develop new drug candidates.[22]

Recently, the benefits of herbal treatments for epilepsy have been prominent. Anticonvulsant activity of aqueous extract of *Feretia apodanthera* Del.,[23] ethanolic extract of *Psidium guajava* (guava) leaves[24] hydroalcoholic extract of *Anacyclus pyrethrum* root[25] and also plant secondary metabolites such as curcumin[26] and quercetin[27] in the PTZ model of clonic seizure in mice has been reported. In addition, it is obvious that isolation of active compounds from medicinal plants in order to identify chemical structures and also performing further optimization is of great interest.

In the current study, we have demonstrated the anticonvulsant effect of onopordia which was isolated from the medicinal plant *O. acanthium*. We also described that the anticonvulsant effect of onopordia was significantly reversed by acute administration of a nonselective NOS inhibitor (L-NAME) and a selective nNOS inhibitor (7-NI), while the iNOS inhibitor, AG, did not contribute to the anticonvulsant effect of onopordia (Fig. 5). L-Arg administration (60 mg/kg, i.p.) in combination with onopordia (10 mg/kg, i.p.) did not alter the effect of onopordia on seizure threshold.

Furthermore, acute administration of ketamine (the NMDA receptor antagonist) did not mutate the effects of onopordia on the seizure threshold (Fig. 5). Therefore, we suggest that nNOS but not iNOS route, plays an important role in the modulatory effect of onopordia. Based on the effects of L-NAME and 7-NI on the anticonvulsant activity of onopordia, this study proposed that the effect of onopordia on seizure threshold may be mediated via a NO-dependent mechanism.

5. Conclusions

In conclusion, the current findings demonstrate that onopordia had anticonvulsant activity in PTZ-induced seizure. The role of NOS inhibitors on these findings, proposes the involvement of NO pathway in the anticonvulsant activity of onopordia in the PTZ model of clonic seizure in mice. In addition, this study indicated that onopordia only slightly more potent anticonvulsant agent than phenobarbital (the standard anticonvulsant medication; $P < 0.001$). Consequently, this compound could be subjected for more optimization in order to generate a more potent anticonvulsant agent with lower toxicity and fewer side effects.

Declaration of competing interest

No conflict of interest exists in relation to the submitted manuscript.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jtcme.2019.11.005.

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