Implication of Nitroergic System in the Anticonvulsant Effect of Ferulic Acid in Pentylenetetrazole-induced Seizures in Male Mice

Hossein Amini Khoei

Shahrekord University of Medical Science

Shakiba Nasiri Boroujeni

Shahrekord University of Medical Science

Zahra Lorigooini

Shahrekord University of Medical Science

Arash Salehi

Shahrekord University of Medical Science

Reihaneh Sadeghian

Shahrekord University of Medical Science

Mohammad Rahimi- Madiseh (✉ m_rahimi7@yahoo.com)

Shahrekord University of Medical Science <https://orcid.org/0000-0002-3108-4708>

Research

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Abstract

Background and Aim: Given the widespread prevalence of seizures worldwide and the low efficacy of synthetic drugs, studies are needed to find new compounds with appropriate efficacy. Ferulic acid (FA) is a phenolic compound with antioxidant and neuroprotective effects. The aim of present study was to investigate the role of nitrergic system in the anticonvulsant effect of FA in pentylenetetrazol-induced seizures in male mice.

Material and Methods: 64 male NMRI mice weighing 25-29 g were randomly divided into 8 experimental groups (n=8). FA at doses 5, 10 and 40 mg / kg alone and in combination with L-NAME (nitric oxide synthase inhibitor) or L-arg (nitric oxide precursor) was administrated (i.p.). PTZ was injected (i.v. route) 30 min after drugs administration. Seizure onset time was recorded and the nitrite levels of prefrontal cortex and serum were determined by Griess Method.

Results: FA at doses of 10 and 40 mg / kg significantly increased the seizure threshold as well as reduced the serum and brain nitric oxide levels in comparison to the saline- received group. Co-administration of effective dose of FA (10 mg/kg) plus L-arginine significantly decreased the seizure threshold in compared to the effective dose of FA alone. Co-injection of sub-effective dose of FA (5 mg/kg) with L-NAME significantly increased the seizure threshold as well as significantly decreased the brain nitric oxide level in compared to the sub-effective dose of FA alone.

Conclusion: FA partially at least, via modulation of nitrergic system possessed anticonvulsant effect in PTZ-induced seizures in mice.

Introduction

Seizures are abnormal discharge of neurons in the brain that often begins in one area and spreads to another (1, 2). The causatives of seizures development are numerous including trauma, infection, ischemia, thus a drug will not able to cure all types of seizures (3–6). Regardless of the development of many conventional and new antiepileptic drugs, new agents with additional anticonvulsant activity and lower side effects are still an interesting aim in medicine.

Nitric oxide (NO) is a short half-life gas is involved in different biological functions in central and peripheral nervous systems as a neuromodulator (7). NO is synthesized by the nitric oxide synthase (NOS) enzyme from the L-arginine (8). The enzyme is composed of three major isoforms including neural (nNOS), endothelial (eNOS) and inducible (iNOS) NOS (8). NO regulates various processes in the CNS such as seizure (9). It has been determined that blockade of NOS is complicated in the anticonvulsant effect of several of drugs in animal models of seizure (10). Evidence suggests that increase in the NO level decrease the seizure threshold (11). In this regards it has been demonstrated that injection of L-arginine, as a NO precursor, attenuated the anticonvulsant effect of some effective anticonvulsants (12).

Ferulic acid, FA, (4-hydroxy-3-methoxy cinnamic acid) is a phenolic compound found in a number of plants and fruits, as well as in aromatic spices such as coffee (13). In some countries FA has been approved as a flavoring and preservative (14). Previous studies have shown that FA possessed antioxidative stress and antiinflammatory properties (15). Evidence has determined that FA through reducing the oxidative stress and inflammatory markers exerted neuroprotective effects (16–18). It has been well-determined that FA reduced activation of glutamatergic pathway (excitatory pathway) as well as increased the activation of GABAergic pathway (inhibitory pathway) (19). Given that, it seems that FA could exert beneficial effect in increasing the seizure threshold. Therefore, this study aimed to investigate the role of nitric system in the anticonvulsant effects of ferulic acid in PTZ- induced seizure in mice.

Material And Methods

Study design

In this study, 64 male mice weighing 25–30 g were divided into 8 groups (n = 8) based on previous studies (20). Animals were housed in appropriate laboratory conditions (21 ± 2 °C, 12 h light and 12 h dark conditions) and were not restricted in diet and water. The groups are as follows:

Group1: Control mice received normal saline (i.p.); groups 2, 3 and 4: mice received FA at doses of 5, 10 and 40 mg/kg (i.p.), respectively; group 5: mice received L-NAME (nitric oxide synthase inhibitor) at a dose of 10 mg/kg (i.p.); group 6: mice received L-arginine (L-arg) at dose of 100 mg/kg (i.p.); group 7: mice received sub-effective dose of FA (5 mg/kg) plus L-NAME and group 8: mice received effective dose of FA (10 mg/kg) plus L-arg. FA was administrated 30 minutes before and L-NAME and L-arg were administrated 45 min before the PTZ injection. Dose and time of drug administrations were chosen based on previous studies as well as our pilot study (21–23).

Evaluation of seizure threshold

To inject the PTZ, a needle gauge 30 was attached to the mice tail vein. After fixing the mice's tail, the PTZ was injected at a rate of 1 ml/min by seizure pump. The injection stopped as soon as the clonus of the anterior limb was seen. The minimum dose of PTZ for seizure was considered as the dose of seizure threshold. In this method, the seizure threshold was dependent on the PTZ dose and was related to time (23).

Nitrite assay

The mice were anesthetized and then blood samples were collected from the heart and centrifuged. Mice were then sacrificed and the prefrontal cortex isolated from the brains on ice and immediately immersed in liquid nitrogen. Then, the sample was prepared by homogenizing cortical tissue. Nitrite levels were measured by Griess Method. Afterwards, each well was filled with 100 µl of serum or tissue sample and then mixed with 100 µl of Grease reagent and after 10 minutes at room temperature the absorbance coefficient was read with an automatic spectrophotometer at 540 nm. Nitrite concentration was calculated using a standard reference (Sigma, USA) (11).

Data analysis

Statistical analysis was performed using SPSS 16 software. At first, data were analyzed using Kolmogorov-Smirnov test and then homogeneity of variance was determined by Levene test. One way ANOVA was used to determine the significant differences between treatments and post hoc Tukey test was used to compare the means. Data were recorded as mean $\pm$ standard error mean (SEM) and $p < 0.05$ was considered statistically significant.

Results

Evaluation of the seizure threshold

As shown in Fig. 1, time of seizure onset was significantly increased in the groups received ferulic acid at doses of 5, 10 and 40 mg / kg compared to PTZ group ($P < 0.01$, $P < 0.001$ and $P < 0.05$, respectively). In the L-NAME group, seizure onset was significantly higher than the PTZ group ($P < 0.001$). In the groups received sub-effective dose of ferulic acid plus L-NAME and the effective dose of ferulic acid plus L-arginine the time of seizure onset was significantly increased in compared to the PTZ group ($P < 0.001$). Co-administration of effective dose of ferulic acid (10 mg / kg) plus L-arginine significantly decreased the seizure onset in compared with the group received the effective dose of ferulic acid alone ($P < 0.05$). Mice received sub-effective dose of ferulic acid (5 mg/kg) plus L-NAME, seizure onset was significantly increased in compared to the group received sub-effective dose of ferulic acid alone ($P < 0.05$).

Evaluation of the nitrite level in serum

The results showed that (Fig. 2) the nitrite level in serum was significantly decreased in groups received ferulic acid at doses of 5, 10 and 40 mg/kg in compared to the PTZ group ($P < 0.05$, $P < 0.01$ and $P < 0.001$, respectively).

Evaluation of nitrite level in the brain

Results (Fig. 3) showed a significant decrease in nitrite levels in the brains of mice received ferulic acid at doses of 5, 10 and 40 mg/kg in compared to the PTZ group ($P < 0.001$). In the L-NAME- received group and L-arginine-received group, the level nitrite in the brain was significantly decreased in compared to the PTZ group ($P < 0.001$). In mice received the sub-effective dose of ferulic acid plus L-NAME as well as mice received the effective dose of ferulic acid plus L-arginine, the level of nitrite in the brain was significantly decreased in compared to the PTZ group ($P < 0.001$). Co-injection of sub-effective dose of ferulic acid (5 mg/kg) plus L-NAME significantly decreased the nitrite level in compared with the group received sub-effective dose of ferulic acid alone ($P < 0.05$).

Discussion

In the present study, we observed that treatment with ferulic acid increased the seizure threshold. Our findings demonstrated that nitric oxide pathway (to some extent) mediated the anticonvulsant effect of

ferulic acid. To date, a number of empirical studies have shown the anticonvulsant effects of ferulic acid (21, 24, 25), which are in line with the results of the present study. In the Lin et al. study, ferulic acid showed protective effects against glutamate-induced neuronal stimulation and inhibited glutamate release from cortical synaptosomes by inhibiting voltage-dependent calcium channels in the presynaptic membrane (26).

In the present study, to determine the role of nitroergic system in the anticonvulsant effect of FA in PTZ-induced seizure, first, we showed that administration of NOS inhibitor (L-NAME) increased the onset of seizure. L-NAME also significantly decreased the nitrite levels. Consistent with the above results, it was observed in other studies that intracerebral injection of L-NAME attenuated electroconvulsive therapy (ECT) in mice (27, 28). Another study found that injection of L-NAME to mice exacerbated neural cell death and kainic acid-induced seizures and increased iNOS expression (29). According to the findings, L-NAME, a NOS inhibitor, significantly increased the seizure threshold, indicating the role of nitric oxide and the nitroergic system in the occurrence of seizures.

In the next step, mice were treated with nitric oxide precursor (L-arginine) and observed that L-arginine did not significantly change the seizure threshold. Given that L-arginine is a precursor to nitric oxide, it was expected to decrease the onset of seizures. Various studies have reported contradictory results regarding the effectiveness of L-arginine against chemical or electrical seizures. In a study by De Sarro et al, it was observed that intracerebral injection of L-arginine exacerbated seizure-induced seizures (27). In the study of Kaputlu et al., L-arginine inhibited the anticonvulsant activity of L-NAME in PTZ-treated mice (28). Another study reported that L-arginine exacerbates NMDA-induced seizures and this effect is inhibited by L-NAME (30). However, some researchers have suggested that L-arginine possessed anticonvulsant effects (31, 32). It could be concluded that the inconsistencies regarding the effects of L-arginine can be due to differences in seizure patterns, drug dosage and route of administration (intraperitoneal or intracerebral) in various researches.

In the present study, ferulic acid caused a significant decrease in the nitric oxide levels, indicating that the anticonvulsant effects of ferulic acid, partially at least, mediated through nitroergic system. Researchers have shown that ferulic acid in ischemic mice has neuroprotective effects by reducing the activity of iNOS, eNOS and nNOS; indicating that modulation of the three NOS isoforms have critical role in neuroprotective activity of this agent (18, 33). In the study of Xu et al., ferulic acid significantly decreased nitric oxide levels in reserpine-treated rats (16). In the study of Sadar et al. (2016), administration of ferulic acid (20 and 40 mg / kg) to rats with ulcerative colitis decreased nitrosative stress parameters and decreased iNOS mRNA expression (34). In another study by Ogiwara et al., it was observed that ferulic acid inhibits the production of RNS free radicals in vitro and significantly inhibits lipopolysaccharide NO production in mouse macrophage cells (35).

In the present study, in the group received sub-effective dose of ferulic acid plus L-NAME, the seizure threshold was significantly increased as well as the nitrite level in the brain significantly decreased,

indicating a strengthening effect of ferulic acid in the presence of L-NAME due to L-NAME inhibition activity.

Conclusion

According to the findings of the present study, administration of ferulic acid increased the seizure threshold, which is associated with a significant decrease in serum and brain nitrite levels. We showed that anticonvulsant effect of ferulic acid, partially at least, mediate nitrenergic system.

Declarations

Ethics approval and consent to participate

All procedures were carried out in accordance with the regulations of the University and the Guide for the Care and Use of Laboratory Animals of National Institutes of Health (Ethics code: IR.SKUMS.REC.1398.031) and Guide for the Care and Use of Laboratory Animals (8th edition, National Academies Press). Full efforts were made to reduce the use of animals and to advance their welfare.

Consent for publication

All authors are agreed to publish this manuscript.

Availability of data and materials

Data regarding the present study are available at Medical Plants Research Center, Shahrekord University of Medical Sciences.

Competing interests

The authors have no conflicts of interest to declare regarding the study described in this article and preparation of the article.

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Authors' contributions

HAK: study design, performed experiments and writing manuscript, SNB: performed experiment and collecting data, ZL: data analysis, AS: performed experiments, RS: edited manuscript and MRM: study design, writing manuscript.

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Figures

Figure 1

Comparison of the seizure threshold in the experimental groups. Data are expressed as mean $\pm$ SEM and was analysis using one-way ANOVA followed by Tukey's post-hoc test. * $P < 0.05$ and *** $P < 0.001$ compared to the control group (PTZ received mice). \$ $P < 0.05$ compared to the group received the effective dose of ferulic acid (10 mg / kg). ### $P < 0.001$ in compared to the group received sub-effective dose of ferulic acid (5 mg / kg). C: Control group (received PTZ) and FA: ferulic acid.

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Figure 2

Comparison of serum nitrite levels in the study groups. Data are expressed as mean $\pm$ SEM and was analysis using one-way ANOVA following Tukey's post-hoc test.* P <0.05, ** P <0.01, *** P <0.001 in compared to the control group (PTZ –received mice). C: Control group received PTZ and FA: ferulic acid.

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Figure 3

Comparison of the nitrite levels in the brain samples in the experimental groups. Data are expressed as mean $\pm$ SEM and was analysis using one-way ANOVA following Tukey's post-hoc test. * P <0.05, ** P <0.01, *** P <0.001 in compared to the control group (PTZ-received mice). # P <0.05 in compared to the group received sub-effective dose of ferulic acid (5 mg / kg). C: Control group received PTZ and FA: ferulic acid.

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