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Protective effect of metformin in kainate model of temporal lobe epilepsy in the rat

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Abstract

Background and Objective: Temporal lobe epilepsy (TLE) leads to damage of the hippocampal neurons in the CA3 region. Considering the neuroprotective and anticonvulsant effect of metformin, the present study was conducted to determine the therapeutic effect of this drug in preventing kainate TLE model in the rat.

Materials and Methods: In this study, male Wistar rats were divided into sham, sham under treatment with metformin, epilepsy (induced by the kainate) and epilepsy treated with metformin. Epilepsy model was induced by unilateral intrahippocampal administration of 0.8 µg of kainate/rat. Rats received metformin at 200 mg/kg, i.p. and daily, for 2 weeks after the surgery.

Results: Induction of epilepsy by kainate was followed by a significant seizure and metformin treatment attenuated this. In addition, metformin significantly prevented reduction of density of Nissl-stained neurons in CA3 area of the hippocampus. Metformin treatment also significantly decreased hippocampal MDA and improved glutathione in epileptic group.

Conclusion: Treatment with metformin can prevent seizure intensity in TLE rat model which is due to its reduction of oxidative burden and preservation of hippocampal CA3 neurons.

Keywords: Metformin, Temporal lobe epilepsy, Kainate, Oxidative stress

1. Introduction

Epilepsy which is associated with seizure attacks is characterized by uncontrolled and excessive activity of all or part of the central nervous system. In a person who is prone to epilepsy, attacks occur when the basic level of nervous system excitability (in the part that is prone to epilepsy) reaches a certain critical threshold. As long as the level of irritability is kept below this threshold, no attack will occur. The types of seizures that occur in epilepsy are very diverse, in general, an epileptic seizure is characterized by loss of consciousness, spasms or muscle cramps, and coma (usually associated with a serious injury). The only sign of it is that the person stops what he is doing and stares at a place. Seizures are usually self-limiting phenomena, although in some cases general attacks are not self-limiting and the patient has repeated attacks for 10-20 minutes (recurrent seizures) and during the attacks the patient does not regain consciousness. This condition is known as epilepsy and is a serious emergency threatening the medical team (1, 2). Oxidative stress is a state of imbalance in production of reactive oxygen species (ROS) and reactive nitrogen species (RNS) induced by many different factors. Oxidative stress is a process caused by free radicals, especially ROS. ROS such as superoxide and hydrogen peroxide can have

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nucleophilic reactions with thiols, amines and multi-chain fatty acids found in biological systems. This chemical state is associated with systemic diseases and diseases affecting the CNS. Experimental evidence shows that the problem of oxidative stress in epilepsy causes resistance to drug treatment (3).
Metformin is recommended as the initial treatment for type 2 diabetes (T2D). In addition, it has been shown that it is used as a drug to prevent and delay the onset of diabetes for people prone to diabetes. to be considered in clinical and research communities. Currently, metformin is the only commonly approved drug from the group of biguanides. The mechanism of action of biguanides has not been fully determined. In type 2 diabetic patients, fasting hyperglycemia and postprandial hyperglycemia both decrease after taking biguanides, hypoglycemia has not been observed during treatment with biguanides. Neuroprotective impact of metformin has been shown in different brain disorders (4-7). This study was conducted to unravel protective effect of metformin in kainate model of TLE in the rat.

2. Materials and Methods

In this study, 40 Albino Wistar male rats (Pasteur Institute, Tehran) were used in the weight range of 210-260 grams. All animals were placed in groups of 3 to 4 in each cage at a temperature of 21-23 degrees Celsius. The animals had free access to tap water and rat food (Pars Feed Company, Karaj) for 4 weeks. In addition, the study was conducted based on the protocols and guidelines recommended by the American National Institute of Health (NIH) for the maintenance and use of laboratory animals and practical solutions available inside the country.
The rats were randomly divided into 4 groups Sham (sham surgery), Sham+metformin, an epileptic group and an epileptic group receiving metformin. To make animals epileptic, kainic acid (Sigma, USA) at 0.8 microgram per rat dissolved in normal saline solution was injected into the CA3 area of the right hippocampus with coordinates: anterior-posterior -4.1 mm, lateral: 4.2 mm, and ventral 4.2 mm below the surface of the skull using a Hamilton syringe (injection volume equal to 5 microliters) and by stereotaxic method and anesthesia with a mixture of ketamine (100 mg/kg) and xylazine (20 mg) per kg. Sham group received only saline solution with the same volume. Metformin was administered intraperitoneally at a dose of 200 mg/kg daily from one hour after surgery till two weeks after that. During the first 24 hours after the surgery and after the animals regained consciousness, the rats were assessed for seizure behavior based on the Racine classification (rating from zero to five) in a four-hour interval using a behavior recording camera and transferring the data to a computer. In this regard, score zero was for no response, score one was for mounting, blinking, or mild facial clonus, score two was for shaking the head or multiple clonus in the head area, and score three was for myoclonic jumps in the limbs. A score of four was considered for clonic convulsions in the front motor limb and standing on two legs, and a score of five was for clonic and whole-body convulsions and loss of balance. At the end of the study, the rats were killed, and after the isolation of the block containing the hippocampus, further experiments were performed.

2.1. Biochemical tests

2.1.1. Malondialdehyde (MDA) measurement

The measurement of malondialdehyde (MDA) level was based on the reaction of thiobarbituric acid (TBA) which was done at a temperature of 90 degrees Celsius. In this experiment, malondialdehyde reacted with malondialdehyde-like substances with thiobarbituric acid and produced a pink color, the maximum absorption of which is at the wavelength of 532 nm. This reaction was carried out at pH=2-3 and at a temperature of 90 degrees Celsius for 45 minutes. After cooling, the absorption was read.

2.1.2. Assay of nitrite

Tissue nitrite concentration measurement is based on Griess reaction. The working solution contained sulfanilamide 1%, naphthylethylene diamine dihydrochloride 0.1%, and phosphoric acid 2.5%. Optical absorption was read at a wavelength of 540 nm and with a known concentration of sodium nitrite.

2.1.3. Measurement of glutathione

For this purpose, the Ellman method was used based on the instructions of the kit and using a plate reader at a wavelength of 412 nm.

2.1.4. Measurement of catalase activity

To measure the catalase activity in the samples, the method described by Beers and Sizer in 1952 was used.

2.3. Cresyl violet staining and quantitative assessment

For histochemical assessment, Nissl (Cresyl violet dye) staining was used. For neuron counting, hippocampal slices were examined. The number of neurons located in the CA3 area was counted.

2.4. Statistical analysis

Statistically, all results were expressed as mean ± standard error (SEM). After determining the distribution of data, one-way ANOVA test was used to analyze the data of biochemical and tissue tests. In all studies, $p<0.05$ was considered as significant.

3. Results

Induction of epilepsy by kainate was followed by a significant seizure and metformin treatment attenuated this. Figure 1 shows the results of measuring malondialdehyde as an index of oxidative stress in the homogenate of hippocampal tissue of animals of different groups. In this regard, the level of malondialdehyde in the epileptic group compared to the sham group was significantly more ($p<0.001$). In addition, the level of this parameter in the epilepsy group treated with metformin at 200 mg/kg was lower than the untreated epilepsy group, which was statistically significant ($p<0.05$).

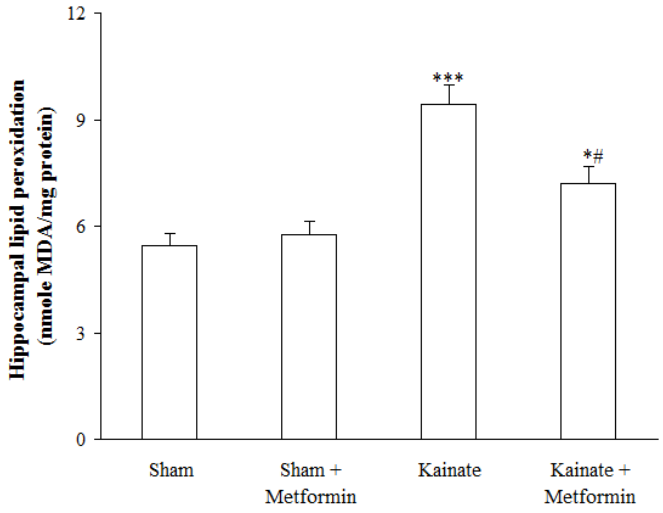

Fig. 1: The level of malondialdehyde in the hippocampus tissue
* $p < 0.05$, *** $p < 0.001$ (in comparison with the sham group), # $p < 0.05$ (in comparison with the untreated epilepsy group). One-way ANOVA and Tukey post-test.

Figure 2 shows the results of measuring nitrite as another indicator of oxidative stress in hippocampal tissue homogenate of different groups. In this regard, the nitrite level in the epileptic group was higher than the sham group, and this difference was statistically significant ($p<0.05$). Also, the epileptic group treated with metformin at 200 mg/kg had a lower nitrite level than the epilepsy group and more than the sham surgery group, although these differences were not statistically significant.

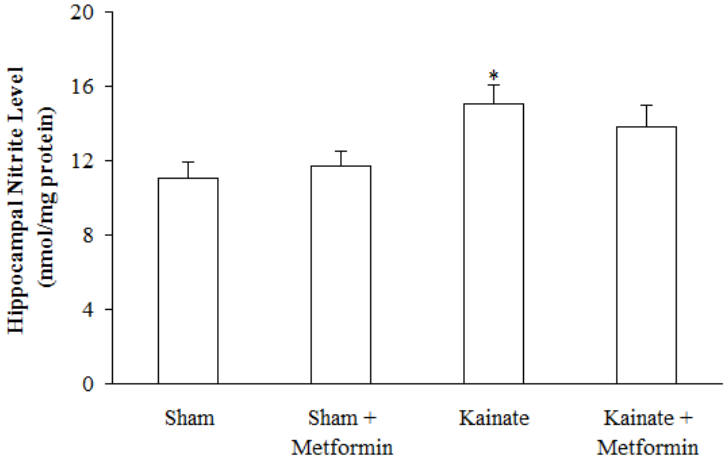

Fig 2: The level of nitrite in the hippocampus tissue in rats.
*$p<0.05$ (in comparison with the sham group). One-way ANOVA and Tukey post-test.

Figure 3 shows the results of measuring glutathione as an index of oxidative stress in hippocampal tissue homogenate of different groups. In this regard, the level of glutathione in the epileptic group was lower

than the sham group, and this difference was statistically significant ($p<0.01$). In addition, the level of glutathione in the epileptic group treated with metformin at 200 mg/kg was higher than the untreated epileptic group, which was statistically significant ($p<0.05$).

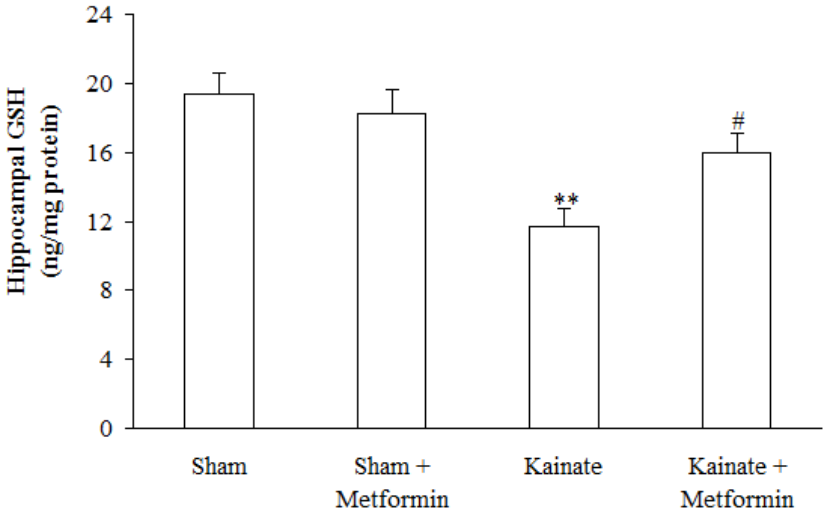

Fig 3: Glutathione level in hippocampal tissue.
**p<0.01 (in comparison with the sham group), #p<0.05 (in comparison with the untreated epilepsy group). One-way ANOVA and Tukey post-test.

Figure 4 shows the results of measuring catalase activity as another antioxidant index in the homogenate of hippocampal tissue of animals of different groups, which confirms the destructive effect of kainic acid on endogenous antioxidant indices. In this regard, the level of catalase in the epileptic group was lower than the sham group, and this difference was statistically significant ($p<0.05$). Also, the epileptic group treated with metformin at 200 mg/kg had a higher catalase level than the untreated epileptic group, however, these differences were not statistically significant.

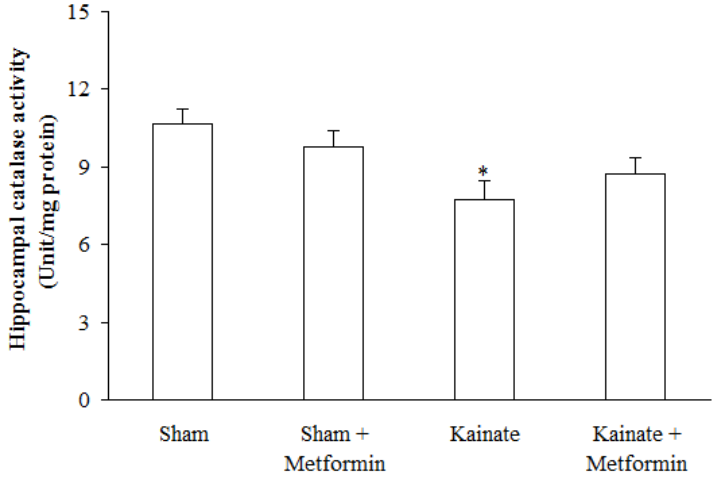

Fig 4: The level of catalase enzyme activity in the hippocampal tissue .
*p<0.05 (in comparison with the sham group). One-way ANOVA and Tukey post-test.

Figure 5 shows the results of histopathological examinations with Nissl staining and the use of Cresyl violet dye in different groups. In this regard, in the epileptic group, a noticeable decrease in neurons was observed compared to the sham surgery group ($p<0.01$). In the epileptic group treated with metformin, there was a significant increase of neurons compared to the epileptic group and treatment with metformin significantly reduced the destruction of neurons ($p<0.05$).

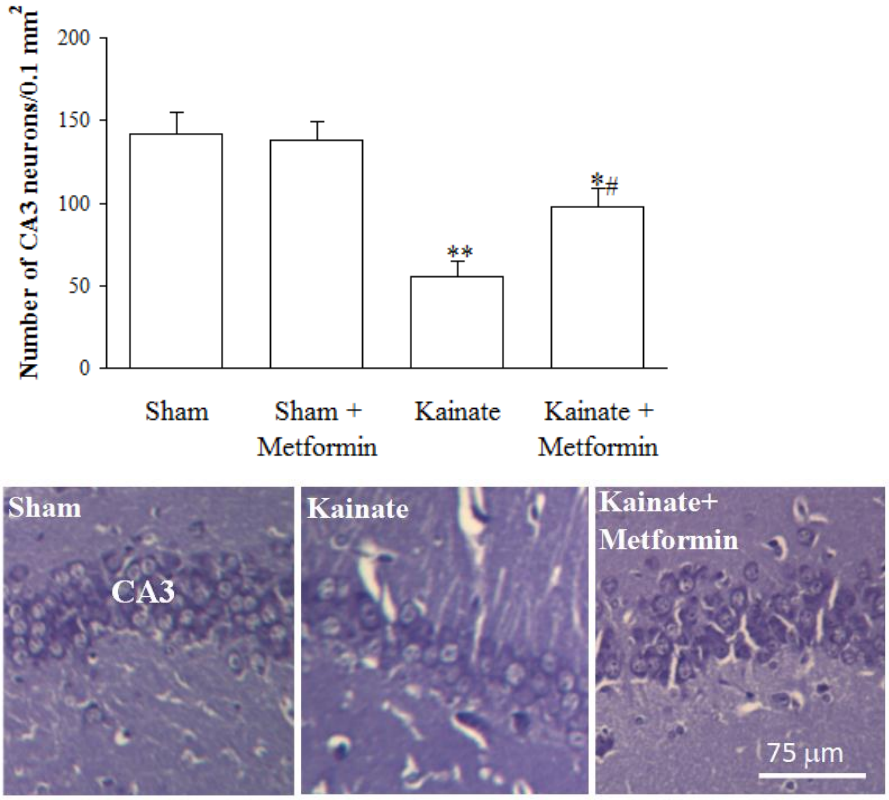

Fig 5: Density of Nissl-stained neurons in the CA3 area of the hippocampus in different groups and comparable photomicrographs .
*p < 0.05, ** p < 0.01 (in comparison with the sham group), # p < 0.05 (in comparison with the untreated epilepsy group). One-way ANOVA and Tukey post-test.

4. Discussion

Epilepsy is one of the most severe and common brain disorders. About 50 million people worldwide suffer from its effects. This is while approximately one hundred million people experience at least one epileptic seizure during their lifetime. The brain is significantly sensitive to oxidative stress because it is the organ that uses the most amount of oxygen in the body. Also, because there is a large amount of polyunsaturated fatty acids in the brain, it is very sensitive to lipid peroxidation. Epilepsy is a chronic neurological disorder that requires long-term treatment. Studies on epilepsy have shown that oxidative stress is manifested by an imbalance between oxidative and antioxidant mechanisms, and oxidative stress plays a central role in this disease. This process also occurs due to insufficient amount of antioxidant factors or excessive accumulation of reactive oxygen species (ROS), which plays a very important role in the emergence of multiple diseases. The collected evidences support the theory that oxidative stress is the mediator of epilepsy induction and it also leads to abnormal structural changes in cellular proteins, membrane lipids, and DNA and RNA (8, 9).

After induction of seizure attacks by chemicals or electrical kindling, it has been known that animals show behavioral alterations. Moreover, metformin treatment can reduce seizure severity including mortality, seizure score, and even duration of seizures. These evidences suggest the potential role of anti-diabetic metformin to prevent epilepsy symptoms (10).

Currently, there is no effective drug with disease-modifying action for epilepsy. Hence, the current therapeutic approach is mostly symptomatic and supportive in order to improve the life quality of the patients (10). Epileptogenesis process entails various molecular and cellular changes which can be targeted as the potential treatment and/or prevention of epilepsy. Metformin has been found to prevent some of the cellular changes that underlie the destructive epileptogenesis process including neuronal loss, gliosis, and even apoptosis which are amongst the

well-known changes in epilepsy (10-12). Metformin is also capable to attenuate oxidative stress which is an important factor in the initiation and progression of epileptogenesis (13-15).

In this study, it was found that following the induction of temporal lobe epilepsy by hippocampal injection of kainic acid, marked convulsive behavior was observed in rats and treatment with metformin led to its reduction. In addition, density of pyramidal neurons in the CA3 region of the hippocampus in the epileptic group showed a significant decrease compared to the sham group, and metformin treatment also led to an increase in the density in the CA3 region. Emerging reports strongly indicate neuroprotective, anti-inflammatory, anti-oxidant, anti-epileptogenic, and anti-epileptic effects of metformin in animal models of epilepsy and seizure-like phenotype (16-20). Part of such beneficial effect of metformin has been attributed to its modulatory effects on protein kinase activation, mammalian target of rapamycin (mTOR), brain-derived neurotrophic factor (BDNF), tyrosine protein kinase B (TrkB) (brain-derived neurotrophic Factor-) besides enhancing neuronal progranulin expression (16, 19, 21). On this basis, metformin has the potential as an adjuvant drug to exert anti-epileptic effect which may be promising in managing drug-resistant kinds of epilepsy. In addition, research evidence indicated that since microglial cells and astrocytes are involved in neuroinflammation during epileptic seizures (20, 21), another part of metformin may be due to its down-regulation of such cells in the brain, which may have occurred in our study. However, further studies in this field are strongly required.

Conclusion

To conclude, the results of this study showed that metformin treatment has an anticonvulsant effect and also through the reduction of the oxidative factors malondialdehyde and NO and the increase of antioxidants such as glutathione and catalase, it preserves the neuronal density in the CA3 and hippocampus regions in the experimental model of induced epilepsy.

Conflict of interest statement

No conflict of interest is declared by the authors.

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