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Research Article

The effects of treadmill exercise on oxidative stress in Mongolian gerbils with penicillin-induced epilepsy

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ABSTRACT

Aim: To evaluate the role of treadmill exercise on the oxidative stress in Mongolian gerbils with penicillin-induced epilepsy.

Methods: This experimental study included 18 male Mongolian gerbils which were divided into three groups; sham-control group, penicillin group (500 units) and exercise + penicillin (500 units) group. Each animal group was composed of six Mongolian gerbils. The epileptiform activity was verified by electrocorticographic recordings.

Results: The latency of the penicillin+exercise group was longer than the penicillin group, but this difference was not statistically significant. Following the penicillin administration, spike wave frequencies of epileptiform activity in the 10, 30, and 35 minutes were significantly lower in the penicillin+exercise group, compared with the penicillin group. There were generally significant decreases in the spike wave amplitude medians in the penicillin+exercise groups compared with the penicillin group in all time periods between 0 and 5 minutes. The serum superoxide dismutase, catalase and glutathione peroxidase levels increased in the penicillin+exercise group compared with those in the penicillin group.

Conclusion: The results of present study indicate that regular exercise may contribute to the amelioration of epileptic activity by increasing the antioxidant effect.

Keywords: Penicillin-induced epilepsy; treadmill exercise; oxidative stress; Mongolian gerbils.

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Introduction

Epilepsy is a chronic neurological disorder that leads to recurrent spontaneous episodes with an imbalance between the excitatory and inhibitory systems in the relevant parts of the brain [1]. Different scientific explanations have been suggested to explain the reason(s) of

excitability of neurons in the disorder [2]. The etiology of epilepsy is not fully explained in 70% of patients. However, epidemiological studies have revealed that the incidence of epilepsy is increased in various situations, such as, brain trauma, central nervous system (CNS) infections, cerebrovascular diseases, brain tumors, degenerative CNS diseases, physical and mental developmental disorders, and febrile convulsions [3]. With existing antiepileptic drugs, seizures cannot be controlled in some patients, and resistance to these drugs is seen in 20-30% of cases. Thus, alternative treatment methods have been investigated in the light of physiopathological information obtained from experimental studies [4,5].

Oxidative stress, which can lead to irreversible cell damage, is the result of an imbalance between reactive oxygen species (ROS) and antioxidant defense mechanisms [6]. In addition, excessive ROS production also plays a role in the pathogenesis of many neurodegenerative disorders including the onset and progression of epilepsy, as well as Alzheimer's disease and Parkinson's disease [4,7]. However, ROS can be scavenged by enzymatic antioxidants such as superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx) [8].

It has been suggested that a long-term exercise program in resistant epilepsy cases reduces the frequency of seizures [9]. It has also been reported that treadmill exercise promotes learning capacity by influencing hippocampal regenerative sprouting and related gene expression in an epileptic rat model. Furthermore, previous studies showed that exercise increased the antioxidant capacity of brain and reduced the oxidative stress [10-12]. In the present work, we investigated the effect of treadmill exercise on the oxidative stress in Mongolian gerbils with penicillin-induced epilepsy.

Material and methods

Animals

The study protocol was approved by the Experimental Animal Ethics Committee of Abant Izzet Baysal University. Male Mongolian gerbils were used in all experiments. Animals were purchased from Huseyin Aytemiz Animal Research Center of Kirikkale University at a body weight of ~80-90 g (8-12 weeks old, n = 18). Animals were kept under controlled conditions (22 ± 2◦C; 12/12 h reversed light/dark cycle). All procedures in the present study were conducted according to a protocol approved by the ethical committee.

A total of 18 Mongolian gerbils were randomly divided into three groups; sham-control group (2,5 µl saline injected intracortically, n=6), penicillin group (500 IU Penicillin-G in a volume of 2,5 µl injected intracortically, n=6), exercise + penicillin group (exercise + 500 IU Penicillin-G in a volume of 2,5 µl injected intracortically, n=6).

Exercise procedure

For experiments, an animal treadmill (May Time 0804, Animal Treadmill) was used. Primarily to avoid any stress, all animals were subjected to conditioning exercise at the lowest treadmill speed lasting 5 min over a period of 14 days. To prevent avoidance and force the animals, incremental electrical shock were given (1–6 mA) to continue running exercise. After adaptation stage, control group rats were put on hold in the cages with the same conditions until the surgery, and experimental groups continued to exercise for 8 weeks

according to the treadmill exercise protocol [13]. For chronic exercise application, 3 phases were given 5 days a week (Monday to Friday) and 2 months on each exercise day. First stage; 5 min 2m / min, Second stage; the next 5 min 5 m / min and the third phase; last 20 min 8 m / min.

Experimental Procedure

For intracortical injections, the animals were anesthetized with Xylazine (10 mg/kg, i.p.) and Ketamine (100 mg/kg, Ketalar, Eczacıbasi, Turkey). Saline or penicillin was injected to the right cerebral cortex intracortically by using a stereotaxic device (Stoelting, Wood Dale, IL, USA). The coordinates (mm) applied were relative to the skull surface, with the upper incisor bar 3.4 mm below the level of the interaural line, according to the rat brain atlas: posterior to the bregma AP=−2; right to the midsagittal line, L=2 mm, and dorsoventral, DV=2 [14].

After completing 120 min electrocorticographic (ECoG) recording of animals, blood samples were collected for serum separation. Blood samples were stored overnight at 4˚C and centrifuged at 4000 x g for 15 min to separate the serum. Serum samples were stored at -70˚C until further analysis.

Measurements of serum antioxidant enzyme activities

Measurement of serum antioxidant enzymes: For analyzing the levels of glutathione peroxidase (Gpx), catalase (CAT) and superoxide dismutase (SOD), ELISA method was used. Amount of Gpx was measured by Gpx kit (ER0274, FineTest, China) and expressed as nmol/ml, CAT enzyme was determined by CAT Kit (ER0264, FineTest, China) and the results were reported as U/mI. Finally the amount of serum SOD was analyzed by SOD kit (ER0332, FineTest, China) and the levels of the enzyme were reported as nmol/ml. These assays employ the quantitative sandwich enzyme immunoassay technique.

Statistical analysis

All results are presented as the means ± standard error of the mean (SEM). The normality of the data was tested with the one-sample Kolmogorov-Smirnov test before analyses. After verifying that data from electrophysiological recordings and serum antioxidant enzyme levels were normally distributed, one-way analysis of variance (ANOVA) and Tukey post hoc tests for multiple comparisons were performed. For all statistical tests, $p < 0.05$ was considered statistically significant.

Results

No epileptiform activity was observed during recordings in the sham group.

Latency of the first epileptiform activity. After penicillin injection, spike waves of epileptiform activity were first seen between 5 and 10 minutes). The latency of the penicillin+exercise group (270±28) was longer than the penicillin group (224±19), but this difference was not statistically significant.

The effects of thymoquinone on spike wave frequency. Following the penicillin administration, spike wave frequencies of epileptiform activity in the 10 (81,28), 30 (124), and 35(142) minutes were significantly lower in the penicillin+exercise group compared with the penicillin group. For the same minutes, in the penicillin group was found 110, 165 and 178 values, respectively.

The effects of thymoquinone on spike wave amplitude. There were generally significant decreases in the spike wave amplitude medians in the penicillin+exercise groups compared with the penicillin group in all time periods between 0 and 5 minutes. There was no significant difference among groups between 5 and 120 minutes, in terms of amplitude.

Serum antioxidant enzyme activities. The serum values of CAT and Gpx decreased in the penicillin group ($p < 0.001$, $p < 0.001$, respectively) compared with those in the sham-control group. Moreover, the SOD, CAT and Gpx, levels increased in the penicillin+exercise group compared with those in the penicillin group ($p < 0.001$, $p < 0.001$, and $p < 0.001$, respectively) (Table 1).

Groups	SOD (nmol/ml)	CAT (U/ml)	Gpx (nmol/ml)
Sham-control	0.70±0.17	41.6±0.78	86.8±8.27
Penicillin	0.25±0.08	30.1±2.04¶	53.7±12.2¶
Penicillin+ exercise	1.95±0.66*†	51.6±4.29*†	124.2±20.1*†

SOD: Superoxide dismutase. CAT: Catalase. Gpx: Glutathione peroxidase.

¶.*P < 0.05 (Penicillin vs Sham-control).*

**†P < 0.05 (Penicillin+ exercise vs. Penicillin and Sham-control).*

Discussion

Antiepileptic drugs have limited clinical effects as well as some undesirable side-effects such as central nervous system, liver failure and bone health disorders [15]. Thus, as nonpharmacologic therapies have been investigated by epilepsy patients, an increasing number of reports in recent years have shown physical activity as a complementary therapy for epilepsy [16-18]. There are some characteristics such as low oxygen uptake and cardiorespiratory compliance, low power and flexibility that trigger seizures of epileptic events and lead to a sedentary life [9,19,20]. Gotze et al [21] were the first to investigate the positive effect of physical exercise on seizure threshold. After that, some clinical and experimental studies have investigated the effects of various exercise models on behavioral, electrophysiological, cellular, molecular and biochemical outcomes in the epileptic condition [9,19,22,23]. In addition, regular exercise can relieve psychological and cardiovascular adverse events in epileptic patients and reduce the number of seizures [17]. It has been suggested that regular exercise in the adolescent period may modulate neuronal susceptibility to epileptogenic insults in later stages of life [24,25]. Long-term exercise in benign epileptic children may contribute to the development and regulation of neurocognitive and psychological function [26]. Experimental studies have demonstrated that an aerobic training program in animals with epilepsy may reduction seizure frequency and susceptibility to subsequently evoked seizures [16,17,24,27]. Unsaturated fatty acids and free radicals have been reported in large amounts compared to other organs due to active oxidative metabolism in the brain. The antioxidant levels were also reported to be low. For all these reasons, the nervous system is very sensitive to the destructive effects of ROS [28,29]. Additionally, oxidative stress has been shown to induce neuronal degeneration in experimental models of epilepsy and many other neurodegenerative disorders including Alzheimer's disease and Parkinson's disease [7,30-33]. In contrast, ROS is scavenged by defense mechanisms such as enzymatic antioxidants such as SOD and GPx, and thus reducing the deleterious effects of oxidative

stress [8]. In the course of this knowledge and experience, it has been shown that regularly exercising animals and people are receiving responses to improving endurance capacity by reducing oxidant production and increasing antioxidant defense mechanisms and mitochondrial biogenesis [34,35]. Therefore, they are the neuroprotective effects of aerobic exercise training [36,37]. In addition, exercise also increases the expression and levels of neurotrophic and growth factors such as Insulin-like growth factor 1, fibroblast growth factor 2 and brain-derived neurotrophic factor [38-40].

In this experimental study in Mongolian gerbils, following the penicillin administration, spike wave frequencies of epileptiform activity in the 10, 30, and 35 minutes were significantly lower in the penicillin+exercise group, compared with the penicillin group. There were generally significant decreases in the spike wave amplitude medians in the penicillin+exercise groups compared with the penicillin group in all time periods between 0 and 5 minutes. Additionally, the SOD, CAT and Gpx levels increased in the penicillin+exercise group compared with those in the penicillin group. The results of present study indicate that regular exercise may contribute to the amelioration of epileptic activity by increasing the antioxidant effect.

Compliance with ethical statements

Conflicts of Interest: None.

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