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Metformin protects against seizures, learning and memory impairments and oxidative damage induced by pentylenetetrazole-induced kindling in mice

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ABSTRACT

Cognitive impairment, the most common and severe comorbidity of epilepsy, greatly diminishes the quality of life. However, current therapeutic interventions for epilepsy can also cause untoward cognitive effects. Thus, there is an urgent need for new kinds of agents targeting both seizures and cognition deficits. Oxidative stress is considered to play an important role in epileptogenesis and cognitive deficits, and antioxidants have a putative antiepileptic potential. Metformin, the most commonly prescribed anti-diabetic oral drug, has antioxidant properties. This study was designed to evaluate the ameliorative effects of metformin on seizures, cognitive impairment and brain oxidative stress markers observed in pentylenetetrazole-induced kindling animals. Male C57BL/6 mice were administered with subconvulsive dose of pentylenetetrazole (37 mg/kg, i.p.) every other day for 14 injections. Metformin was injected intraperitoneally in dose of 200 mg/kg along with alternate-day PTZ. We found that metformin suppressed the progression of kindling, ameliorated the cognitive impairment and decreased brain oxidative stress. Thus the present study concluded that metformin may be a potential agent for the treatment of epilepsy as well as a protective medicine against cognitive impairment induced by seizures.

1. Introduction

Epilepsy is a common neurological disorder that affects approximately 50 million people worldwide. Meanwhile, cognition impairment, which is the most common and severe comorbidity of epilepsy, greatly diminishes the quality of life [1,2]. "Indeed, many people with epilepsy, and their families, consider cognitive and behavioral consequences of seizures to be at least as troubling as the seizures themselves." Jonathan K reported [1]. Although antiepileptic drug therapy is useful for controlling seizures in many patients, current treatments have untoward impact on cognition rather than restoring it. As the goal of treating epilepsy goes beyond seizure control, there is an urgent need for new compounds with both anticonvulsant and cognition protective properties.

Oxidative stress, which has been reported as an underlying mechanism in the development and progression of epilepsy [3], may be responsible for the cognitive deficits [4]. Therefore antioxidants have been suggested as therapeutic design strategies for the treatment of epilepsy.

Metformin, a widely used medicine for the treatment of type 2 diabetes mellitus, has antioxidant properties which are not fully understood. Metformin has also been demonstrated to confer health and lifespan benefits in laboratory mice, partly by reducing oxidative stress and inflammation [5]. Previous study demonstrated that metformin could reduce reactive oxygen species (ROS) and associated DNA damage by inhibiting mitochondrial respiration [6]. Besides that, metformin also has a protective effect on the antioxidant defense system. It can upregulate uncoupled proteins 2 (UCP2) [7], glutathione and Nrf2 target gene activation [5]. What's more, metformin could enhance spatial learning and memory in C57BL/6 mice [8] and high-fat diet rats [9]. Previous studies demonstrated that metformin can rapidly cross the blood brain barrier (BBB) [10] and has neuroprotective effects [11]. Despite its potential benefits, the effect of metformin on epilepsy has not been investigated. The present study was aimed to

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evaluate if metformin can suppress the progression of pentylenetetrazole-induced kindling, ameliorate the cognitive deficits and oxidative stress induced by epileptic seizure.

2. Materials and methods

2.1. Animals

Adult male C57BL/6 mice weighing 20 ± 2 g (4–6 weeks old) were obtained from the Hebei Medical University and housed in groups of five per cage under standard laboratory conditions. They were kept at constant room temperature (25 ± 1 °C) and humidity (40–60%).The mice were kept on a 12 h light/dark cycle, with lights on at 08:00 AM and with free access to food and water. Animal experiments were performed according to the regulations of laboratory animal management promulgated by the Ministry of Science and Technology of the People's Republic of China [1988] No. 134, which coincides with internationally recognized NIH guidance.

2.2. Drugs and chemicals

All standard chemicals used in this study were of analytical grade. Metformin (MET) and pentylenetetrazole (PTZ) were purchased from Sigma (St. Louis, MO, USA). GSH detection kit and MDA detection kit were obtained from Nanjing Jiancheng Bioengineering Institute (China). Metformin and PTZ were dissolved in physiological saline freshly prior to the injections.

2.3. Induction of kindling and design of the experiment

A subconvulsive dose of PTZ (37 mg/kg, i.p.) was injected on alternating days for a total of 14 times [11]. The animals were observed for 30 min after each PTZ administration. Seizure stage was evaluated using the following scale [12]: stage 0, no response; stage 1, mouth and facial jerks; stage 2, convulsive waves axially through the body; stage 3, myoclonic jerks and rearing; stage4, clonic convulsions with the animal falling on its side; and stage 5, repeated severe tonic-clonic convulsions or lethal convulsions. The seizure severity during induction of kindling was recorded.

Animals were randomly divided into four groups with eight in each group. The control group received 0.9% saline i.p. every other day (10 ml/kg, 14 injections total). The PTZ group received saline pretreatment along with PTZ (37 mg/kg) every other day. The PTZ + MET group received MET pretreatment in dose of 200 mg/kg in addition to alternate day treatment of PTZ for 14 injections. Metformin was given 30 min before PTZ. The MET group received 200 mg/kg of metformin alone to study any effect of metformin on the cognitive functions and the biochemical parameters.

2.4. Learning and memory assessment

The Morris water maze (MWM) test was used for learning and memory behavior assessment [13]. The MWM test was done 24 h after the last administration of PTZ. Learning and memory behavior evaluations were performed in a 120-cm diameter water pool and virtually divided into four quadrants. The pool was filled with water (22 ± 1 °C) and was made opaque with wheat. A colorless escape platform (10 cm in diameter) was submerged 1 cm beneath the water surface, located in a designated target quadrant. The maze was located in a quiet test room, surrounded by many visual cues outsides of the maze which were visible from within the pool and could be used by the mice for spatial orientation.

Each test consisted two parts, learning trials (existed platform) and probe trials (non-existed platform). The acquisition test was performed for five consecutive days of training with four trials per day. Animals were given 60 s to locate the hidden platform, and any animals that did not find the platform within 60-second period were placed on the platform for 15 s. The acquisition time was recorded at the time the animal got into the water and ending at the time the animal reached the submerged platform. On the sixth day, probe trials without platform were assessed with only one starting point, and the time spent in the target quadrant where the platform had been located was recorded.

2.5. Tissue dissection

Following the behavioral tests, the animals were sacrificed and the brains were quickly harvested and frozen in liquid nitrogen and stored at −80 °C until further utilization.

The brain tissue samples were thawed and 10% (w/v) homogenates were made with ice-cold 0.1 M phosphate buffer (pH 7.4). The homogenates were used to determine the content of lipid peroxidation product and reduced glutathione.

2.6. Malondialdehyde (MDA) determination

MDA, an index of lipid peroxidation, was measured based on the reaction with thiobarbituric-acid (TBA) reaction described by Okhawa et al. [14]. MDA reacts with TBA as a thiobarbituric acid reactive substance to produce a pink complex with a peak absorbance at 532 nm. The quantification of MDA was determined by comparing the absorption to the standard curve of MDA equivalents.

2.7. Glutathione (GSH) estimations

Assay of GSH was performed in tissue homogenates by the method of Ellman [15]. The brain homogenates were precipitated in cooled trichloroacetic acid 10% and centrifuged at 2000×*g* for 15 min. Supernatants were incubated with DTNB in a 1 M phosphate buffer, PH 7.4. The colored complex formed by DTNB and GSH was measured spectrophotometrically at 412 nm. A standard curve of GSH was used to calculate GSH levels.

2.8. Statistic analysis

Data were expressed as mean ± SD. Significance of seizure stage was analyzed using Kruskal–Wallis one-way analysis of variance on ranks. Analysis of variance (ANOVA) for repeated measures was used to analyze the escape latencies in Morris water maze test among the groups over a period of 5 days. Other data were analyzed by one-way ANOVA. All statistical analysis was performed with SPSS 13.0 software and $p < 0.05$ was considered statistically significant.

3. Results

3.1. Protective effect of metformin against pentylenetetrazole-induced kindling

In the PTZ group, repeated administration of subconvulsive PTZ (37 mg/kg) on every second day (for 28 days, 14 injections) resulted in a gradual increase in seizure score culminating in generalized clonic-tonic seizures. As shown in Fig. 1, pretreatment with metformin (200 mg/kg, i.p.) significantly suppressed the progression of kindling as evidenced by the decrease in seizure score as compared to the PTZ group. There were significant differences in seizure score between the PTZ and PTZ + MET groups from the 8th to the 14th PTZ administration ($p < 0.05$ from the 8th injection