Copper sulfate inhibits seizure activity induced by pentylenetetrazole in mice

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A B S T R A C T

Background and Objective: Copper is one of the main micronutrients of body which plays a key role as a cofactor in the function of metabolic enzymes. Previous studies have shown that copper sulfate (CuSO4) inhibits long-term potentiation (LTP) in slices of hippocampal CA1 region. Whereas LTP is involved in learning and epilepsy, it seems that copper effects on LTP could be associated with its effects on epilepsy and seizure. Therefore, the aim of this study was to evaluate the effect of CuSO4 on seizure induced by pentylenetetrazole (PTZ).

Materials and Methods: The effect of various doses of CuSO4 (10, 50 and 100 mg/kg, i.p.), saline (as a control group) or sodium valproate (50, 150 and 100 mg/kg, i.p.) on seizure parameters induced by PTZ (100 mg/kg i.p.) was evaluated in NMRI mice. Twenty minutes after injection of saline or CuSO4, PTZ (100 mg/kg) was injected to induce seizures in animals and seizure parameters were recorded.

Results: Comparison of the effect of CuSO4, saline or sodium valproate on seizure parameters such as stage 2 latency, stage 5 latency and stage 5 duration showed that CuSO4 dose-dependently reduced seizure.

Conclusion: This study showed that CuSO4 significantly inhibits seizure parameters compared with the saline and sodium valproate.

Key Words:
Copper sulfate
Seizure
Pentylenetetrazole
Valproic acid

1. Introduction

Copper is one of the essential micronutrients of the body which plays a key role as a cofactor in the function of metabolic enzymes such as cytochrome oxidase C, superoxide dismutase, metallothionein, dopamine-beta-hydroxylase, lysyl oxidase as well as coagulation factor V and VIII. It is also involved in cellular processes like energy production in mitochondria, detoxification of free radicals, melanin structure, synthesis of neurotransmitters, and stability of connective tissue (1). Previous studies have revealed that disruption in copper homeostasis will cause diseases such as Wilson disease and Menkes syndrome (2).

Seizure is one of the neurological complications in Menkes and Wilson's diseases. Previous studies have shown that oral administration of copper inhibits long-term potentiation in the CA1 region of rat hippocampal slices (3,4). Since long-term potentiation is believed to involve in learning and also seizures, it seems that the effect

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of copper on long-term potentiation could be associated with effects of this element on epilepsy and seizures (5-7). The aim of this study was to assess the influence of intraperitoneal injection of CuSO₄ on seizures induced by pentylenetetrazole in mice, and comparing it with sodium valproate.

2. Materials and Methods

In this experimental study, male NMRI mice, weighing 25 to 30 g, were housed five per cage with free access to food and water. Mice were kept in a vivarium under controlled laboratory conditions (temperature, 22-26°C) with an artificial 12-h light/dark cycle. All animals were allowed to acclimate for ≥5 days before testing with food and water "ad libitum". All experimental procedures were approved by the Ethics Committee of the Arak University of Medical Sciences. Animals were randomly divided into seven groups with eight mice per group. Animals received saline, copper sulfate (10, 50 and 100 mg/kg, i.p.) and sodium valproate (50, 150 and 300 mg/kg, i.p.). Twenty minutes after injection, all mice received 100 mg/kg of pentylenetetrazole (PTZ) intraperitoneally (8). Mice behaviors were monitored for seizures immediately after PTZ injection for a period of 30 min and seizure responses were assessed. Seizure stages were classified into four phases; stage 1: hypoactivity, stage 2: partial clonus (clonic seizure activity affecting face, head, and/or forelimb or forelimbs), stage 3: generalized clonus (sudden loss of upright posture, whole-body clonus involving all four limbs and tail, rearing, and autonomic signs), and stage 4: tonic-clonic (maximal) seizure (generalized seizure characterized by tonic hindlimb extension) (9). Tonic-clonic maximal seizures were associated with death. Some mice recovered spontaneously. It was not unusual for mice to exhibit multiple episodes of tonic hindlimb extension within the 30 min observation period. In this research, latency to stage 2 seizure, stage 3 seizure and from the onset of stage 3 to death were recorded.

2.1. Data analysis

Data of seizure stages are expressed as means± standard error of the mean (S.E.M.). Data were analyzed by one-way analysis of variance (ANOVA) and followed by Tukey's post hoc test. In all experiments, a P-value <0.05 was considered as the significance level between the groups.

3. Results

Figure 1 shows the effect of intraperitoneal administration of different doses of CuSO₄ (10, 50, 100 mg/kg) on PTZ-induced seizure. One-way ANOVA (F₃, ₃₁=37.184, p<0.0001) and Post-hoc analysis revealed a significant increase

![Figure 1. Comparison of the effect of copper sulfate and sodium valproate on stage 2 latency. Statistical analysis (analysis of variance) revealed that both copper sulfate and sodium valproate dose-dependently increase stage 2 latency.](image-url)
in latency to stage two seizures (S2L) for CuSO4 at doses of 50 and 100 mg compared with saline-treated control animals. Intraperitoneal injections of sodium valproate (50, 150 and 300 mg/kg) also increased S2L in valproate-treated animals as compared to controls (Figure 1). Statistical analysis with ANOVA revealed no significant difference between two groups (F6, 58=41.667, p<0.0001).

All doses of sodium valproate and copper sulfate significantly decrease the reverse of time that need to reach to stage 3 seizure multiplied to 100 as compared with the control group (F6,58 = 153.736, p <0.001) (Figure 2). The reverse of time between onset of stage 3 seizure to animal death multiplied to 100 showed that this parameter significantly decreases in all groups compared with control group (F6,58 =13.656, p<0.001). It means that time of animal death after the fifth stage of seizures increases in all groups as compared to controls (Figure 3).

**Figure 2.** Comparison of effect of copper sulfate and sodium valproate on stage 3 latency. Statistical analysis (analysis of variance) revealed that both copper sulfate and sodium valproate dose-dependently increase stage 3 latency.

**Figure 3.** Comparison of the effect of copper sulfate and sodium valproate on revers of time from stage 3 to death. Statistical analysis (analysis of variance) revealed that both copper sulfate and sodium valproate dose-dependently decrease this parameter.
4. Discussion

These results showed that intraperitoneal injection of copper sulfate has an inhibitory effect on seizure parameters in mice. Many studies have been conducted on the relationship between copper and epilepsy, and the identification of toxic effects of copper on the body systems (10). In line with results obtained in this study, it has been reported that seizures and epilepsy are neurological symptoms in patients with Menkes disease. In this x-linked disease, copper transport is impaired and serum levels of copper and ceruloplasmin protein decrease (11). Also, in animal models of Menkes disease, there is increased risk of seizures and neuronal damage that can be removed with copper-containing supplements. Many articles attribute the seizures in this disease to a reduction of serum copper concentration (12-14). Routine injection of copper to mice also eliminates the seizure potentials created by the combination of microwaves and chlorpromazine and convulsive seizures by adding penicillin to sensorimotor cortex of the brain (15). Brunia et al in 1972 reported that plasma copper concentrations increase in patients with different types of epilepsy (16).

On the other hand, Kuzuya and colleagues in 1993 acclaimed that although serum copper increases in epileptic patients who use anticonvulsant drugs, this increase is not significantly different from control group (17). Verroti and his colleagues in 2002 also showed that before and after treatment with anticonvulsant drugs, there is no relationship between plasma copper and epilepsy (18). However, Doretto et al in 2002 reported that serum copper concentrations in mice with audiogenic seizures is more than normal mice (19). The results of our study showed inhibitory effects of copper sulfate injection on seizures induced by PTZ.

Conclusion

The results of this study showed that intraperitoneal injection of copper sulfate has inhibitory effect on seizures induced by intraperitoneal injection of PTZ in NMRI strain mice. These results can help to investigate the chronic effects of copper sulfate on the seizure and anticonvulsant effects of copper and other metals used in enzymes building.

References


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