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Evaluation of neuroprotective, anticonvulsant, sedative and anxiolytic activity of citicoline in rats

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ABSTRACT

Citicoline (cytidine-5′-diphosphocholine) is a neuroprotective agent that is administered following ischemic and traumatic brain injuries. There is little information about the antiseizure and anxiolytic effects of citicoline, which are therefore addressed in the present study. For evaluating the anticonvulsant effect of citicoline in the pentylentetrazole seizure model, a single intraperitoneal dose of citicoline was administered at 50, 100 or 150 mg/kg. Sedative and anxiolytic effects of citicoline were examined via elevated plus maze and pentobarbital induced sleep tests. Results show that citicoline at the doses of 100 and 150 mg/kg significantly delayed the latent period compared with the control ($P < 0.05$). Citicoline at the doses of 100 and 150 mg/kg significantly decreased total locomotion compared with the control ($P < 0.05$). Additionally, citicoline at the doses of 100 and 150 mg/kg significantly increased both percentage of entry and time spent in the open arms in the elevated plus maze test ($P < 0.05$). The pentobarbital induced sleep test showed that citicoline significantly reduced the latency to sleep ($P < 0.05$). Our results suggest that acute administration of citicoline has anticonvulsant activity and sedative effect.

1. Introduction

Epilepsy is a chronic neurological disorder with high prevalence that is caused by abnormal electrical activity in the brain (Sander, 2003). Normal brain function depends on the proper balance between excitatory and inhibitory currents, and their imbalance can induce abnormalities such as epileptic seizures. This is the case of temporal lobe epilepsy, which is frequently associated with hippocampal sclerosis, possibly caused by a primary brain injury (Avoli et al., 2002; Curia et al., 2014; Ziburkus et al., 2013). Traumatic brain injury is a common source of post-traumatic epilepsy, and accounts for approximately 5% of all epilepsy cases (Hauser et al., 1991; Hunt et al., 2013).

Following traumatic brain injury, repair mechanisms such as axonal sprouting and reorganization of neural networks may disrupt the balance between excitation and inhibition, which increases the possibility of developing spontaneous seizures and epilepsy (Dudek and Spitz, 1997; McCormick and Contreras, 2001; Benardo, 2003). After head trauma, neuroprotective agents such as citicoline (cytidine-5′-diphosphocholine) are administrated as part of medical intervention to limit secondary damage and enhance recovery (Graham et al., 2006; Secades, 2011). Citicoline has neuroprotective properties when administered for a transient ischemic attack, traumatic brain injury, or other neurodegenerative and cognitive disorders (Davalos et al., 2002; Graham et al., 2006; Saver, 2008). The neuroprotective activity of citicoline has been shown repeatedly in preclinical models of brain ischemia and trauma (Adibhatla and Hatcher, 2005; Grieb, 2014).

Citicoline stabilizes the cell membranes via stimulation of structural phospholipids synthesis, particularly of phosphatidylcholine, which is a key component for cell membrane integrity and repair (Zweifler, 2002; Saver, 2008). Other neuroprotective mechanisms of citicoline are believed to be the attenuation of phospholipase A2 activity (Adibhatla and Hatcher, 2003), restoration of membranous Na+/K+ -ATPase activity (Secades and Frontera, 1995), prevention of glutamate-mediated neurotoxicity (Hurtado et al., 2005), reinforcement of the intracellular antioxidative system, and reduction of the generation of free radicals (Adibhatla et al., 2001). Following administration parenterally or orally, citicoline quickly converts to cytidine and choline, which cross the blood-brain barrier separately and are reconverted to citicoline inside brain cells (Galletti et al., 1985, 1991).

Karpova et al. (2015) investigated the inhibitory effects of citicoline on acute pentylentetrazole-induced generalized epileptiform activity using a mouse model. Their results show that citicoline at higher doses has an anticonvulsive effect on acute generalized seizure activity (Karpova et al., 2015).

Despite the clinical regular use of citicoline following traumatic brain injury and brain ischemia (Graham et al., 2006), there is little scientific information about its effects on seizures and convulsions.

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Moreover, the effects of citicoline on pentylentetrazole-induced seizures have not been evaluated previously in rat models. Additionally, there is a need to evaluate the sedative and anxiolytic activities of citicoline in regard to comorbid disorders (Vazquez and Devinsky, 2003).

In the present study, we addressed the anticonvulsant activity of citicoline on pentylentetrazole-induced behavioral seizure activity in the Wistar rat. Additionally, we examined the sedative and anxiolytic effects of citicoline using open-field tests, elevated plus mazes, and pentobarbital-induced sleep tests.

2. Materials and methods

2.1. Animals

All experiments were performed on adult male Wistar rats (200–250 g) that were purchased from the Razi Research Institute (Mashhad, Iran). Animals were maintained under standardized housing conditions (temperature, 22 ± 2 °C, 12 h light/dark cycle with light on from 7 a.m., and 60 ± 5% humidity) in Plexiglas cages with free access to food (standard laboratory rodent chow) and tap water *ad libitum*. Experiments were carried out between 9 a.m. and 12 p.m. and each animal was used only once. In each type of test, 50 rats were randomly allocated into five experimental groups (n = 10).

Ten rats were used for each treatment group. All animal experiments were carried out in accordance with the European Communities Council directive of 24 November 1986 (86/609/EEC) and in accordance with local FUM committee for Human and Animal ethics.

2.2. Chemicals

Pentylentetrazole and pentobarbital were purchased from Sigma Aldrich (USA). Diazepam and citicoline were obtained from Tolid Daru Pharmaceutical Co. (Tehran-Iran) in injectable form. Pentylentetrazole and pentobarbital were dissolved in physiological saline solution. All drugs were injected intraperitoneally (i.p.) in a volume of 0.002 ml/g to avoid overhydration and hypertension. Fresh drug solutions were prepared each day during the experiment.

2.3. Assessment of pentylentetrazole induced acute seizure

Fifty rats were randomly allocated into five groups (n = 10). In three experimental groups, the rats were allocated to receive citicoline at the doses of 50, 100 or 150 mg/kg 30 min before the administration of pentylentetrazole (60 mg/kg). In the remaining two groups, rats were injected with normal saline or diazepam (2 mg/kg) as controls. Following injection of pentylentetrazole, each animal was placed separately into a transparent plexiglas cage and observed for at least 30 min to record the occurrence of generalized seizure behaviors as described by Racine (1972). Generalized seizure behaviors were defined as generalized clonic convulsions (rearing and bilateral forelimb clonus) with preservation of righting reflex (stage 4 of Racine's scale) and generalized tonic-clonic convulsions with loss of righting reflex seizures (stage 5 of Racine's scale). The time lapse before the onset of generalized seizures and the percentage of seizures were recorded. To evaluate the protective effects of citicoline against mortality due to seizures induced by the injection of a lethal dose of pentylentetrazole (90 mg/kg), animals were pretreated with citicoline in doses of 50, 100 or 150 mg/kg 30 min before pentylentetrazole injection. The animal mortality rate was recorded up to 24 h.

2.4. Homogenization of animals behavioral seizure activity

Due to individual variation in responses, animals with similar behavioral seizure responses to the single dose of pentylentetrazole (40 mg/kg) injection were selected two weeks prior final experimental test. For this purpose, only animals that showed stage 3 of Racine's scale were selected for major test.

2.5. Open field test

Locomotor activity was measured in an apparatus comprised of a wooden platform enclosed by four white wooden walls (100 cm × 100 cm × 50 cm). The floor was divided by red lines into 25 equal squares (20 cm × 20 cm) and a central square drawn with black marker. The open field was placed inside a light- and sound-attenuated room. Rats were routinely tested during the first half of the dark phase of their light/dark cycle. In three experimental groups, animals were given a single i.p. dose of citicoline at the doses of 50, 100 or 150 mg/kg 30 min before starting the test. In two control groups, animal were treated with diazepam at the dose of 2 mg/kg, or normal saline 30 min before starting the experiments. The test was performed following previously described procedures (Biagini et al., 1993). Briefly, each rat was placed in a corner square of the open field apparatus and its behavior was recorded for 5 min using a video camera (Panasonic, Japan) placed above the apparatus.

2.6. Elevated plus maze

The apparatus was comprised of two opposite open metal arms (50 cm × 10 cm) without side walls and two enclosed arms (50 cm × 10 cm × 20 cm) with sides and end walls that extended from a central square (10 cm × 10 cm), all painted black. The maze was elevated to the height of 50 cm and placed in a lighted room. The illumination level at the center of maze was maintained at 50 lx on the floor of the apparatus.

Rats were routinely tested during the first half of the dark phase of their light/dark cycle. At the start of the test, rats were individually placed on the central part of elevated plus maze, always facing towards the same open arm, and allowed to explore the maze freely for 5 min. The behavior of the animals was recorded continuously using a video camera placed above the apparatus and then was scored using the Image EP software. After each test, the maze was carefully cleaned with 70% ethanol and dried with tissue paper (Hogg, 1996).

Experimental animals were given a single i.p. dose of citicoline at the dose of 50, 100 or 150 mg/kg 30 min before behavioral tests. Control animals were given diazepam at the dose of 2 mg/kg or normal saline 30 min before behavioral tests.

Entry into an arm was defined as the animal placing all four paws over the line marking that area. The number of entries and the time spent in the open and closed arms were recorded during a 5 min test period. The percentage of open arm entries (100 × open/total entries) was calculated for each animal.

2.7. Pentobarbital-induced sleeping time

To evaluate the effects of citicoline on pentobarbital-induced sleeping time, 30 min after pretreatment with the citicoline (50, 100 or 150 mg/kg), diazepam (2 mg/kg) and normal saline (0.002 ml/g), animals were injected with pentobarbital (40 mg/kg, i.p.). The time interval between the administrations of pentobarbital until the loss of the righting reflex was recorded as onset of sleep and the interval between the loss and regaining of righting reflex was considered as the sleeping time (Dandiya and Collumbine, 1959).