

Research Article

Effects of Cholinesterase Inhibitors on Experimental Model of Retrograde Amnesia

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Abstract: The Tacrine and Galantamine are cholinesterase inhibitors for treatment of Alzheimer's disease. The pentylentetrazole model of retrograde amnesia is suitable for studying of learning and memory impairments. The aim of present study was to compare the effects of the cholinesterase inhibitors Tacrine and Galantamine on pentylentetrazole amnesia model in mice using passive avoidance test. The 32 male albino mice 21-26g was used, n=8. The groups were: Saline 0.1ml/10g body weight; Saline + Pentylentetrazole (PTZ) 45mg/kg; Tacrine 1mg/kg + PTZ 45 mg/kg; Galantamine 0.1mg/kg + PTZ 45 mg/kg. The mice were treated subcutaneously with PTZ immediately after testing in Step-down apparatus (Ugo Basile, Italy). The cholinesterase inhibitors were applied intraperitoneally 30 minutes before testing. The latency of reaction (60 seconds) was a criterion for learning and memory. A two-way ANOVA for repeated measurements was used to compare different groups with the respective controls. In step-down passive avoidance test controls significantly increased the latency of reactions on learning session and on short and long memory tests. The mice with PTZ decreased the latency of reaction compared to the same day controls. The group with Tacrine and PTZ lowery increased the latency in comparison with PTZ group. The animals with Galantamine and PTZ significantly increased the latency of reactions on lerning and memory tests. Our results allow us to conclude that PTZ had small impairing effect on cognitive functions in mice. The Galantamine in low dose antagonizes inhibitory effect of PTZ on brain functions better than Tacrine.

Keywords: retrograde amnesia, mice, step-down, Pentylentetrazol, Tacrine, Galantamine.

INTRODUCTION

Retrograde amnesia (RA) is one of the central lobe features of temporal lobe amnesia in humans [1]. A retrograde amnesia interval is longer with more extensive hippocampal damage or damage extending beyond the hippocampal system is responsible for loss of more remote memories [2]. A typical example of this type of amnesia is senile dementia.

Cognitive impairment is frequently observed in epileptic patients. It has been seen that not only epilepsy but antiepileptic drugs also impair cognitive functions. A large number of the epileptic patients suffer from memory deficits, learning disabilities and behavioral problems [3].

Pentylentetrazole (PTZ) has been used most frequently for establish the kindling model of epilepsy, on which the test drugs of potential antiepileptic action. Kindling develop within most 5 to 12 weeks as a gradual reduction in seizure threshold and increase the intensity of seizures. The PTZ applied subcutaneously

three times per week in a subconvulsant dose. When used as a model of retrograde amnesia PTZ is injected into the same or similar dose immediately following the passive avoidance acquisition trail [4]. Experiment completed within one or two weeks. The pentylentetrazol exercise convulsive effects on rodents at the same time causes and cognitive deficits [5].

Cholinergic inhibitors are used to treat the learning and memory disturbances. Their chronic administration stabilized the cognitive and functional status of the patient and slows disease progression [6]. Tacrine is monoaminoacridine derivate, first generation cholinesterase inhibitor for treatment of mild to moderate Alzheimer's disease and other form of dementias [7]. Galantamine is alkaloid which unique mechanism of action included also allosteric modulation of nicotinic receptors [8]. It is suggested that the reduction of nicotinic receptors is associated with the degree of cognitive impairment in the adults whose illnesses start with dementia such as Alzheimer's and Parkinson's diseases [9].

The aim of present study was to compare the effects of the cholinesterase inhibitors tacrine and galantamine on pentylentetrazole amnesia model in mice using step-down passive avoidance test.

MATERIAL AND METHOD

Ethical statement

All experiments were carried out according to the guidelines for the use of laboratory animals in EU and Bulgaria. Official permission for the study was obtained by Bulgarian Food Safety Agency №49/30.06.2011 and Ethics Committee of the Medical University Plovdiv №3/05.07.2012.

Chemical compounds

Pentylentetrazole (Sigma) is 6,7,8,9-Tetrahydro-5H-tetrazolo[1,5-a]azepine. Tacrine (Sigma) is 1,2,3,4-tetrahydro-5-aminoacridine. Galantamine (Sopharma, Bulgaria) is 4a,5,9,10,11,12-hexahydro-3-methoxy-11-methyl-6H-benzofuro [3a,3,2ef][2]benzazepin-6-ol,hydrobromide.

Animals

The 32 male albino mice 21-26g was used, n=8. The groups were: A: Saline 0.1ml/10g body weight; B: Saline + Pentylentetrazole (PTZ) 45mg/kg (amnesia model group); C: Tacrine 1mg/kg + PTZ 45 mg/kg; D: Galantamine 0.1mg/kg + PTZ 45 mg/kg. The mice were treated subcutaneously with PTZ immediately after testing in Step-down apparatus. The cholinesterase inhibitors were applied intraperitoneally 30 minutes before testing. The latency of reaction (60 seconds) was a criterion for learning and memory.

Behavioral test

Step-down passive avoidance test

An automatic set-up for a passive avoidance "Step-down" test (Ugo Basile, Italy) was used in a wire-floor cage with round central plastic platform. Learning sessions consisted of 2 trials separated by a 60 minute interval. During each trial, electronic stimulation (0.2mA) was delivered to the wire floor for duration of 10s. Learning sessions were performed on 1st day, a short memory retention session was performed 24 hours later (2nd day), and a long memory retention session was performed on the 7th day. The memory retention tests were performed using the same parameters, but with the absence of a foot shock. A latency of reaction of 60 seconds (the rat was required to remain on the platform for more than 60 seconds) was used as a criterion for learning and retention.

The method of retrograde amnesia with PTZ was made by V. Petkov [10].

Statistical evaluation

The means \pm SEM for each group of rats were calculated using Instat computer program. A two-way ANOVA for repeated measurements was used to compare different groups with the respective controls

with the Turkey-Kramer multiple comparison test. A p-value of $P < 0.05$ was considered representative of a significant difference.

RESULTS

Control group significantly increased the latency of reaction on learning ($p < 0.05$), short and long memory tests ($p < 0.05$), compared to the first day (Figure 1). The mice with saline and PTZ slightly decreased the latency of reactions on learning and short memory retention, compared to the respective days control group (Figure 1).

The group of mice with amnesia model and Tacrine 1 mg/kg significantly increased the latency of reactions ($p < 0.05$) only in first day learning, compared to the amnesia model group. The group of animals with PTZ and 0.1 mg/kg Galantamine significantly increased the latency of reactions on learning ($p < 0.05$), short and long memory tests ($p < 0.05$), compared to the respective day group with saline and PTZ (Figure 1).

DISCUSSION

Our results showed that PTZ had small impairing effect on cognitive functions in mice with retrograde amnesia model. We think that the degree of damage depends on the duration of the impact of harmful agent. This demonstrates our experience with PTZ used to create a kindling model for several weeks, whereby the damaging effect on learning and memory is much more pronounced [11, 12]. The present data suggest that the central effects of post-training PTZ treatment account for amnesia, rather than the convulsion per se [13]. It was found that PTZ impair working memory and long-term spatial memory of old mice (6 and 12 months) in passive avoidance test and in elevated plus maze [14].

Kindling effect is a relatively permanent alteration of brain functions leading to increased excitability to ineffective stimuli [15]. Several reports indicate that free radical generation due to the increased activity of glutamateergic transmitter plays a crucial role in neuronal cell death of the PTZ-kindled animal [16, 17]. Hippocampus plays the role of a determinant structure in the development of chronic brain hyperexcitation at a PTZ kindling, and it well known that in the inhibitory synapses of the hippocampus the neurotransmission is realized by GABA [18]. PTZ is a stimulant of the CNS with pro conflict and convulsant effects in rats and mice. These pharmacological effects are mediated through a specific interaction with the GABA-gated chloride ionophore [5].

Hippocampus is anatomic structure connected with learning and memory, rich with cholinergic neurons and target for cholinesterase inhibitors [19]. In our experiments the improving effect of tacrine is better expressed on learning and short-term memory. Galantamine has improving effect on learning, short-

term and long-term memory in mice with pentylentetrazol-induced retrograde amnesia in step-down passive avoidance test.

The improving effect of tacrine on cognitive processes in mice treated with PTZ is confirmed by

other authors [20]. Neuroprotective effect of galantamine has been demonstrated in various pharmacological models of brain injury of the experimental animals [21].

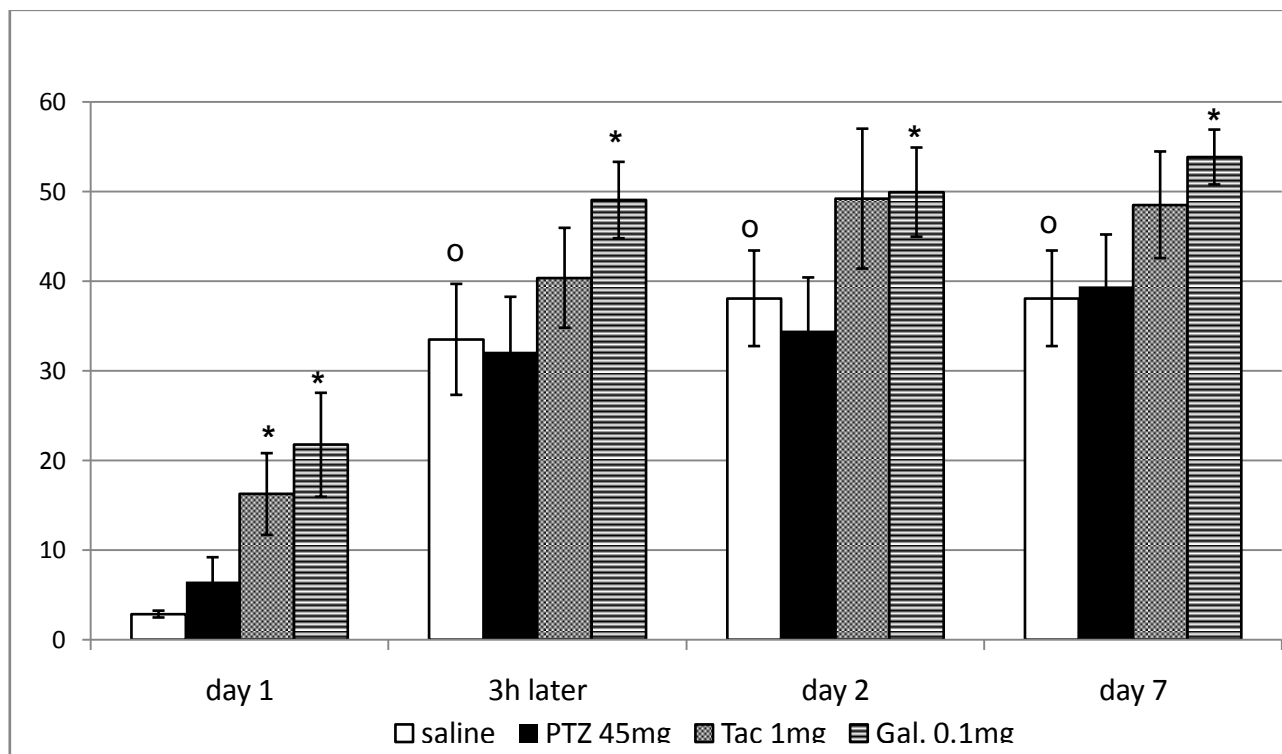


Fig-1: Effects of Tacrine and Calantamine on retrograde amnesia model in mice (Step-down passive avoidance test).

X-axis - days of testing; Y-axis - latency of reactions in seconds

^oP<0.05 compared to the first day control group

*P<0.05 compared to the respective day PTZ-amnesia model group

CONCLUSION

Our results allow us to conclude that galantamine in low dose antagonizes inhibitory effect of PTZ on brain functions better than tacrine. We think that good experimental and clinical results, the lack of hepatotoxicity and good tolerance make galantamine preferably for the treatment of mild to moderate cognitive impairment.

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