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Effects of Cholinesterase Inhibitors Tacrine and Galantamine in Pentilentetrazole induced Cognitive Dysfunction in Mice

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Abstract

The aim was to study the effects of tacrine and galantamine on learning and memory processes in pentilentetrazole-induced model of cognitive dysfunction in mice. Thirty-two male albino mice divided into four groups were treated as follows: Group 1: saline (controls); Group 2: saline+pentilentetrazole (PTZ) (model group); Group 3: tacrine+PTZ; Group 4: galantamine+PTZ. The effects of tacrine and galantamine were studied in automatic set-up for active learning with negative reinforcement "Shuttle-box" (Ugo Basile, Italy). The following parameters were recorded: number of conditioned stimulus responses (avoidances), number of unconditioned stimulus responses (escapes) and number of inter-trial crossings. The comparison between groups was made by Instat computer program using analysis of variance (ANOVA for repeated measurements). The pentilentetrazole (PTZ) significantly decreased

the number of conditioned and unconditioned stimulus responses and exploratory activity in mice. Tacrine removed the suppressing effect of PTZ on the central nervous system and improved learning. Galantamine showed a better effect on long term memory. Both cholinesterase inhibitors improved the locomotor activity of the experimental animals.
***Keywords*:** tacrine, galantamine, pentilentetrazole, learning, memory, mice

Introduction

The cognitive deficit is characteristic not only of dementias, but also of other neurological disorders such as depressions, schizophrenia, epilepsy etc. [1]. Epilepsy is the most predominant neurological disorder characterized by recurrent seizures [2]. It affects more than 50 million people worldwide [3, 4]. Cognitive impairments are not only the result of seizures. Antiepileptic drugs suppressing the central nervous system also induce memory deficits, learning disabilities and behavioral problems in patients [5].

Pentilentetrazole (PTZ) is a widely used experimental chemical model of human epilepsy. The mechanism of action includes enhancement of the glutamatergic system. Seizures induce transient cerebral ischemia and neuronal death [6, 7]. The median effective dose of antiepilepric drugs such as phenobarbital and sodium valproate on the modification of Lorke's method in male CD1 mice is determined by PTZ and maximal electroshock [8]. The different doses of PTZ, typically in the dose range of 35 to 100 mg/kg, induce clonic and tonic seizures. A kindling model of epilepsy develops within 5 to 9 weeks as a gradual reduction in the seizure threshold and increases the intensity of seizures. PTZ is applied every 48 hours subcutaneously at a dose of 40 mg/kg. This model is appropriate to test drugs with potential antiepileptic action.

The PTZ exercise convulsive effect on rodents at the same time causes cognitive deficits [9]. When used as a model of retrograde amnesia, PTZ is injected in the same or similar dose immediately following the behavioral test [10]. The PTZ-induced convulsion model may also be used to establish a possible anticonvulsant action of drugs from other pharmacological groups. New research has established the anticonvulsant effect of the

antidepressant tianeptine against pentylentetrazole-induced seizures along with amelioration of seizure-induced cognitive impairment [11]. Pentylentetrazole-induced seizure behavior has been studied in rat offspring prenatally exposed to co-administration of alcohol and restraint stress [12].

There are data that galanthamine and tacrine have prominent protective effects against glutamate neurotoxicity on primary cultures from the cerebral cortex of fetal rats [13]. The mechanisms of neuroprotection include acetylcholinesterase inhibition and a special role of alpha-4 and alpha-7 receptors [14]. The main aim of cholinesterase inhibitor treatment is to inhibit the enzymatic degradation of the neurotransmitter acethylcholine, resulting in an increased amount of acetylcholine in the synaptic terminals [15]. Tacrine is a monoaminoacridine derivate, first generation cholinesterase inhibitor, used for treating mild to moderate Alzheimer's Disease and other forms of dementias [16, 17]. Because of infrequent but serious hepatotoxicity, it is rarely used in Europe. Currently, tacrine is used mainly in the USA because of its good therapeutic efficacy [18]. Galantamine belongs to second-generation cholinesterase inhibitors, which have fewer undesirable side effects. It is an alkaloid, whose unique mechanism of action also includes allosteric modulation of nicotinic receptors [19]. Galantamine has been discovered by the Bulgarian scientist Dimitar Paskov. He isolated alkaloid from the common snowdrop (Galantus nivalis - now a protected plant species). Perhaps this is the reason why the trade name of the preparation in Bulgaria is Nivalin. The clinical administration of the preparation includes not only the treatment of dementias, but also diseases of the peripheral nerves, disruption of neuromuscular blockade when non-depolarizing muscle blockers are used, to stimulate motility in postoperative intestinal atony and others clinical indications.

The aim of the present study was to compare the effects of the cholinesterase inhibitors tactine and galantamine on learning and memory processes in a pentelentetrazole-induced retrograde amnesia model in mice using shuttle-box active avoidance test.

Materials and Methods

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 the Bulgarian Food Safety Agency No49/30.06.2011 and the Ethics Committee of the Medical University Plovdiv No3/05.07.2012. All efforts were made to minimize animal suffering and to use only the number of animals necessary to produce reliable scientific data.

Pentilentetrazol (Sigma) is 1,5-pentamethylentetrazole,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.

Fresh drug solutions were prepared fresh each morning in distilled water.

Animals

The 32 male albino mice weighing 21-26 g were divided into 4 groups of 8. The animals were kept under standard laboratory conditions in a 07:00-19:00 h light/dark cycle in a quiet room at 22ºC-24ºC and were provided with food and purified drinking water ad libitum. The following experimental groups were used: Group 1: saline 0.1 ml/10 g body weight (controls); Group 2: saline + PTZ 45 mg/kg (model group); Group 3: tacrine 1 mg/kg + PTZ 45 mg/kg; Group 4: galantamine 0.1 mg/kg + PTZ 45 mg/kg. The mice were treated subcutaneously with PTZ immediately after testing in a shuttle-box apparatus. The cholinesterase inhibitors were applied intraperitoneally 30 minutes before testing. The selected doses of tacrine and galantamine showed the best results in our previous experiments on naïve aminals.

Behavioral test

Shuttle-box active avoidance test

The active avoidance test with negative reinforcement was performed in a shuttle box. A conventional shuttle-box was used, originally made as an automatic reflex conditioner (Ugo Basile, Italy). Learning sessions were held for 5 days and consisted of 50 trials (6 sec light

and buzzer, 350 Hz and 35 dB, followed by 3 sec random 0.2 mA foot electrical stimulation and a 12 sec pause). Seven days later, the memory retention test was performed using the same parameters without foot stimulation.

The following behavioral signs were observed: number of correct responses on conditioned stimulus, i.e. avoidances; number of escapes from foot stimulation (unconditioned stimulus responses, i.e. escapes), and number of intertrial crossings.

The method of retrograde amnesia with PTZ was conducted according to the method of V. Petkov [20].

Statistical evaluation

The means ± SEM for each group of mice 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 and Discussion

In shuttle-box active avoidance test, controls significantly increased the number of conditioned stimulus responses (avoidances) on the 4^{th} and 5^{th} days of learning ($p<0.05$) and on memory retention test ($p<0.01$) compared to the first day (Fig. 1).

The mice treated with PTZ and saline decreased the number of avoidances on 2^{nd}, 3^{rd}, 4^{th} and 5^{th} days of learning ($p<0.05$) and in the memory test ($p<0.01$), compared to the saline group on the same days. The animals with PTZ-induced amnesia and treated with tacrine significantly increased the number of avoidances on 3^{rd} and 5^{th} days of learning ($p<0.05$) compared to the same days group with amnesia model only. The experimental group with PTZ and galantamine increased the avoidances on 2^{nd} day of learning and in the memory test ($p<0.05$), compared to the group with model of impaired memory on the same days (Fig 1).

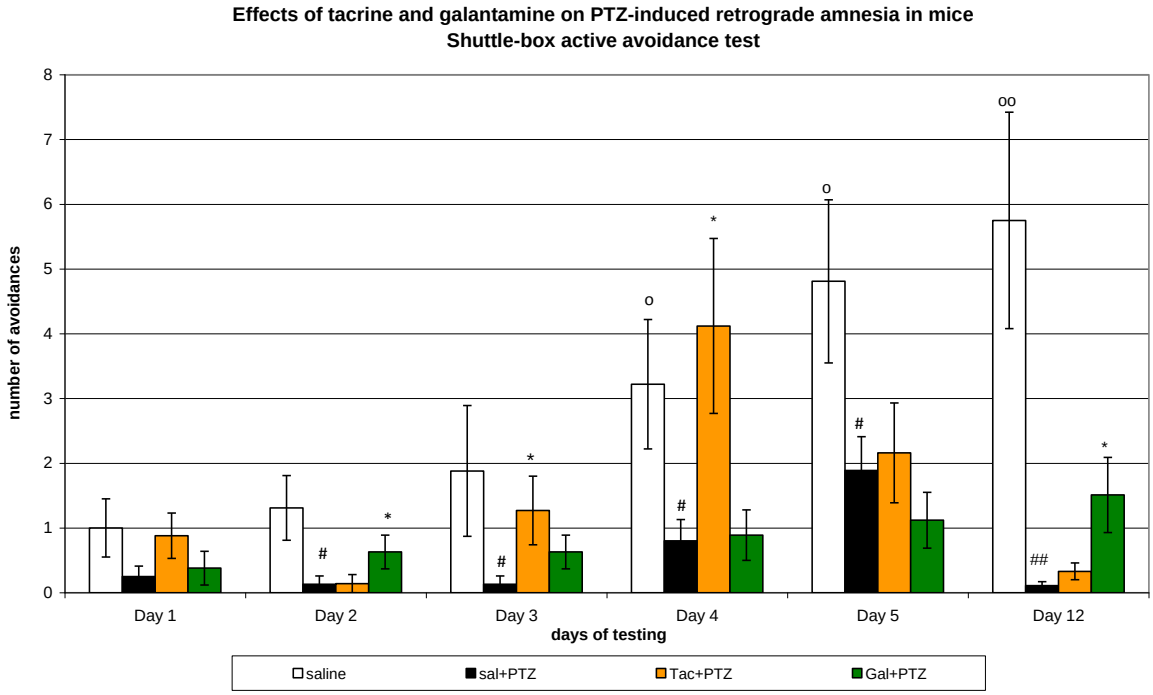

Figure 1. Effects of tacrine and galantamine on learning and memory processes in PTZ-induced model of retrograde amnesia in mice. Shuttle-box active avoidance test. Number of avoidances.

$^{o}p<0.05$ and and $^{oo}p<0.01$compared to the first day control group;

$^{\#}p<0.05$ compared to the same day control group;

*p<0.05 compared to the group with PTZ-induced amnesia model in same day.

The controls did not change the number of unconditioned stimulus responses (escapes) on learning sessions and on memory retention test, compared to the 1st day of learning (Fig. 2). The mice with PTZ-retrograde amnesia model significantly decreased the number of escapes on the 4th and 5th days of learning ($p<0.05$) and in the memory test (12th day) ($p<0.05$) in comparison with the control group on the same days. The two experimental groups with PTZ and tacrine or galantamine increased the number of escapes on the 3rd, 4th and 5th days of learning ($p<0.05$) compared to the group treated with PTZ and saline (model group) on the same days. Only animals with PTZ and galantamine significantly increased the escapes on memory retention ($p<0.05$) compared to the PTZ-amnesia model group (Fig. 2).

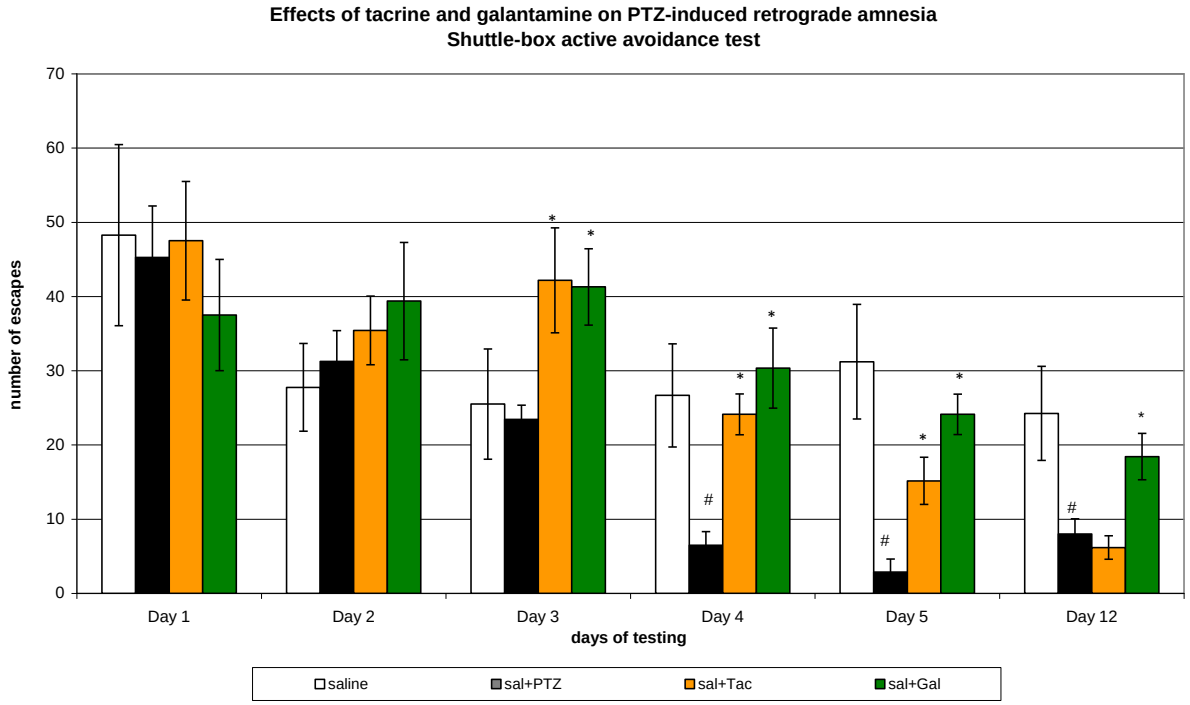

Figure 2. Effects of tacrine and galantamine on learning and memory processes in PTZ-induced model of retrograde amnesia in mice. Shuttle-box active avoidance test. Number of escapes.

#p<0.05 compared to the same day control group;

*p<0.05 compared to group with PTZ-induced amnesia model in the same day.

The saline group did not change the number of inter-trial crossings in learning and memory sessions, compared to the 1st day (Fig. 3). The mice with PTZ-retrograde amnesia model decreased the inter-trial crossings on the 2nd, 3rd, 4th and 5th days of learning ($p<0.05$) and in the memory test ($p<0.05$) compared to the controls on the same days. The groups with PTZ and tacrine or galantamine significantly increased the number of inter-trial crossings on 3rd ($p<0.01$), 4th and 5th days of learning ($p<0.05$) in comparison with the same days amnesia model group. In the memory retention test, the groups with PTZ and cholinesterase inhibitors did not change the number of intertrial crossings, compared to the same day group with amnesia model (Fig. 3).

The PTZ significantly decreased the number of conditioned and unconditioned stimulus responses and exploratory activity in mice. Our results showed that PTZ had strong

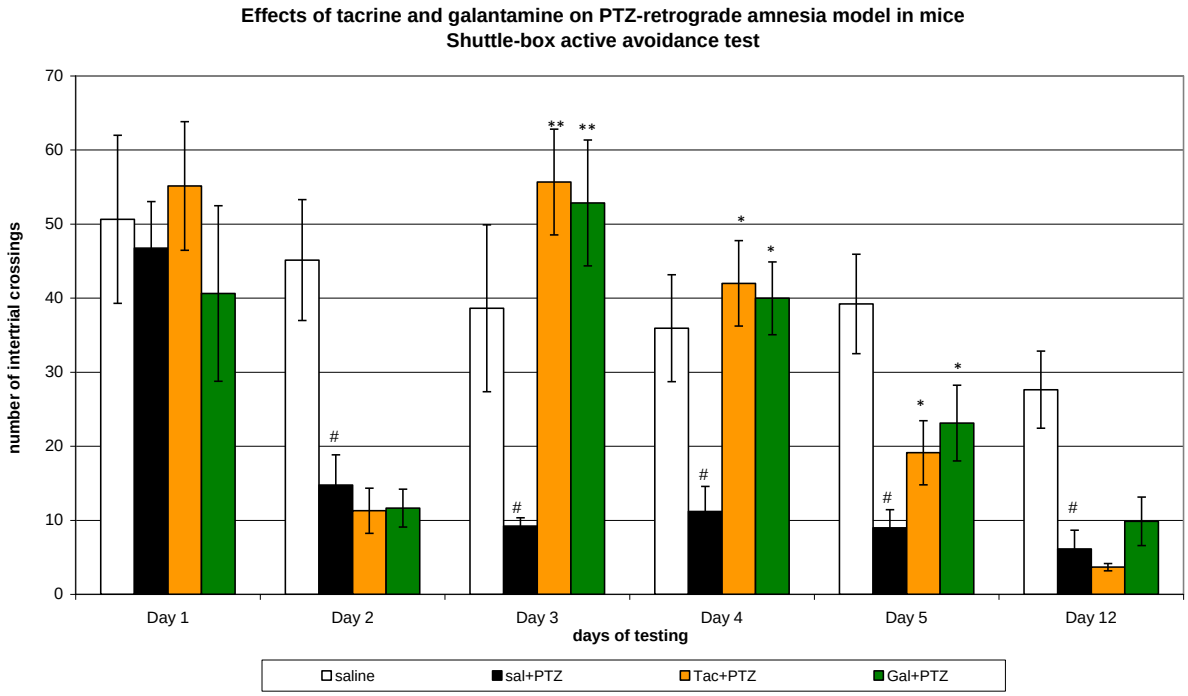

Figure 3. Effects of tacrine and galantamine on learning and memory processes with PTZ-induced model of retrograde amnesia in mice. Shuttle-box active avoidance test. Number of intertrial crossings.

$^{\#}p<0.05$ compared to the same day control group;

*p<0.05 and **p<0.01 compared to group with PTZ-induced amnesia model in the same day.

impairing effect on learning and memory in mice with retrograde amnesia model in active avoidance test. In our previous experiments in step-down passive avoidance test PTZ manifested a weak impairing effect on the cognitive functions in mice with same amnesia model [21]. On the kindling model of epilepsy, the damaging effect of PTZ on learning and memory is much more pronounced [22, 23].

Senile dementia is a typical example of retrograde amnesia. It is one of the central lobe features of temporal lobe amnesia in humans [24]. Febrile seizures in childhood exist among predisposing factors of temporale lob epilepsy. The molecular pathways include increased IL-1B activities and nuclear factor kappa B, thereby leading to calcium-dependent glutamate release [3, 25]. A retrograde amnesia interval is longer with more extensive hippocampal damage or damage extending beyond the hippocampal system, responsible for loss of more

remote memories [26]. The cholinergic system plays a pivotal role in the processes of learning and memory, therefore it underlies the pathogenesis of diseases connected with cognitive deficits [27]. Cholinesterase inhibitors have a stimulating effect on the CNS and can antagonize the depressant effects of PTZ by increasing acetylcholine levels in the brain. Our results partially confirmed this assumption.

Conclusion

Having in mind the suppressing effect of pentilentetrazole on the central nervous system (CNS), it can be assumed that it not only suppresses cognitive functions, but also reduces the motor activity of the experimental animals. Our experiments confirmed this suggestion. Comparing tacrine (first generation cholinesterase inhibitor for the treatment of mild to moderate Alzheimer's disease) with galantamine (second generation), we found that both of them remove the suppressing effect of PTZ on learning and memory processes in mice. Tacrine antagonized the effects of PTZ on the central nervous system and improved learning. Galantamine showed a better effect on long term memory. Both cholineasterase inhibitors increased locomotor activity of experimental animals.

We expect that the resulting experimental data can be used in clinical practice due to the fact that galantamine can be administered at very small doses to improve cognition without disturbing the physical activity of the patient. Therefore, in these doses there is very little probability of occurrence of side effects such as cholinergic nausea, vomiting and diarrhea. In addition, galantamine does not cause liver injury, which is characteristic of tacrine.

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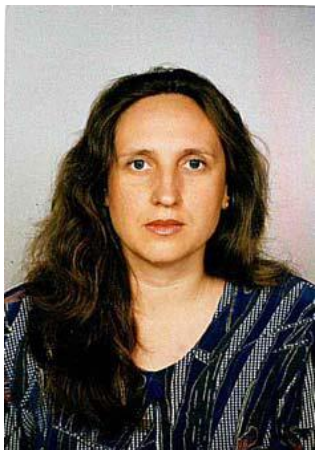

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