COMPARISON THE EFFECTS OF TACRINE AND GALANTAMINE ON ACTIVE AVOIDANCE TEST IN RATS WITH DIAZEPAM-AMNESIA MODEL

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ABSTRACT

Tacrine is the first cholinesterase inhibitor approved for treatment of mild to moderate Alzheimer's disease. Galantamine is reversible, competitive acetylcholinesterase inhibitor and positive modulator of nicotinic acetylcholine receptors. Diazepam induced amnesia is well known and used model for studying effects of different compounds on learning and memory processes.

The aim of our study was to compare the effects of Tacrine and Galantamine on learning and memory processes in rats using active avoidance test. The male Wistar rats (9 per group, with body weight 220-240 g) was treated with: 1st - Saline 0.1ml/100g body weight (controls) p.o.; 2nd – Diazepam 2.5 mg/kg i.p. + Saline 0.1ml/100g p.o.(amnesia model), 3rd – Diazepam 2.5 mg/kg i.p. + Tacrine 1 mg/kg p.o.; 4th – Diazepam 2.5 mg/kg i.p. + Galantamine 0.1 mg/kg p.o. All groups of animals were trained in shuttle-box active avoidance test, using original made standard apparatus (Ugo Basile, Italy) with training parameters for rats. In active avoidance test learning session was performed 5 consecutive days and consist 30 trails, memory retention was done 7 days later. The following behavioral parameters were observed: number of correct responses (avoidances), number of escapes from food shocks and number of intertrial crossings. The comparison between groups made by Instat computer program using analysis of variance (ANOVA for repeated measurements).

In active avoidance test control group significantly increased the number of avoidances on learning and on memory retention. The animals with diazepam-amnesia model significantly decreased the number of avoidances on the same days testing in comparison with control group. The rats with Diazepam and Tacrine significantly increased the number of correct responses on second day learning and on memory test, compared to the same day group with diazepam and saline. The animals treated with Diazepam and Galantamine increased the number of avoidances on learning and on memory tests. The group with amnesia model decreased the number of escapes on learning and memory tests compared to saline group. The rats with Diazepam and Tacrine did not change the number of escapes on learning and memory tests compared to the group with amnesia only. The animals with Diazepam and Galantamine significantly increased the number of escapes on all learning and on memory retention. The group with amnesia model decreased the number of intertrial crossings on learning session and memory test. The group with Diazepam and Tacrine did not change the number of intertrial crossings compared to the amnesia model group. The rats with Diazepam and Galantamine increased the number of intertrial crossings on learning, but not kept it on memory retention.

Our results allow us to conclude that Diazepam applied chronically impaired learning capacities and memory function of the animals. Galantamine improved learning ability better than Tacrine on rats with diazepam-amnesia model. Both cholinesterase inhibitors have similar improving effect on long term memory.

Key words: *Tacrine, Galantamine, learning, memory, rats*

INTRODUCTION

Alzheimer's disease (AD) is a degenerative disorder of the brain leads to progressive decline in cognitive behavior. Current treatments for AD aim to correct the biochemical deficits in the brain. Acethylcholinesterase inhibitors restore the central cholinergic deficit and have modest, but significant efficacy on cognitive impairments. They improve the learning and memory processes in AD (Dubois et al., 2008). Tacrine is te first cholinesterase inhibitor used for treating mild to moderate AD (Wilson et al., 2009). Because of infrequent but serious hepatotoxicity after

administration of second-generation cholinesterase inhibitors, which have fewer undesirable side effects, it is rarely used in Europe. Currently tacrine is used mainly in United States of America because of its good therapeutic efficacy. (Aderinwale et al., 2010). Galantamine is alkaloid which inhibitory effect on brain acetylcholinesterase is 10 to 12 times stronger than physostigmine. The inhibition produced by galantamine is long lasting, but reversible (Raskind et al., 2000). Galantamine acts not only by inhibiting cholinesterase, but also induced up regulation of nicotinic receptor expression levels and neuroprotection (Takada-Takatory et al., 2009).

Benzodiazepine induced anterograde amnesia in both humans and animals (Thiebot, 1985). Diazepam induced amnesia is well known and used model for studying effects of different compounds on learning and memory processes (Costa et al., 2010).

The **AIM** of our study was to compare the effects of tacrine and galantamine on learning and memory processes in rats with diazepam-induced amnesia using active avoidance test (Shuttle box).

MATERIAL AND METHOD

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.

Drugs

Diazepam (Sopharma, Bulgaria) is 7-chloro-1, 3-dihydro-1-methyl-5-phenyl-2H-1, 4-benzodiazepin-2-one.

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

Male Wistar rats weighting 220-240 g were divided into 4 groups of 9. Rats were kept under standard laboratory conditions in a 08:00-20:00 h light/dark cycle and were provided with food and water *ad libitum*. The drugs were administered 60 minutes before testing. The following experimental groups were used: A: saline (0.1 ml/100 g body weight) p.o. (control); B: Diazepam 2.5mg/kg i.p. + saline 0.1ml/100g body weigh p.o. (amnesia model group); C: Diazepam 2.5 mg/kg i.p. + Tacrine 1.0 mg/kg p.o. and D: Diazepam 2.5 mg/kg i.p. + Galantamine 0.1 mg/kg p.o.

Behavioral 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 30 trials (6 sec light and buzzer, 670 Hz and 70 dB, followed within 3 sec by random 0.4 mA foot electrical stimulation and 12 sec pause). Seven days later a 1-day memory retention test was performed using the same parameters without foot stimulation.

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

Statistical evaluation

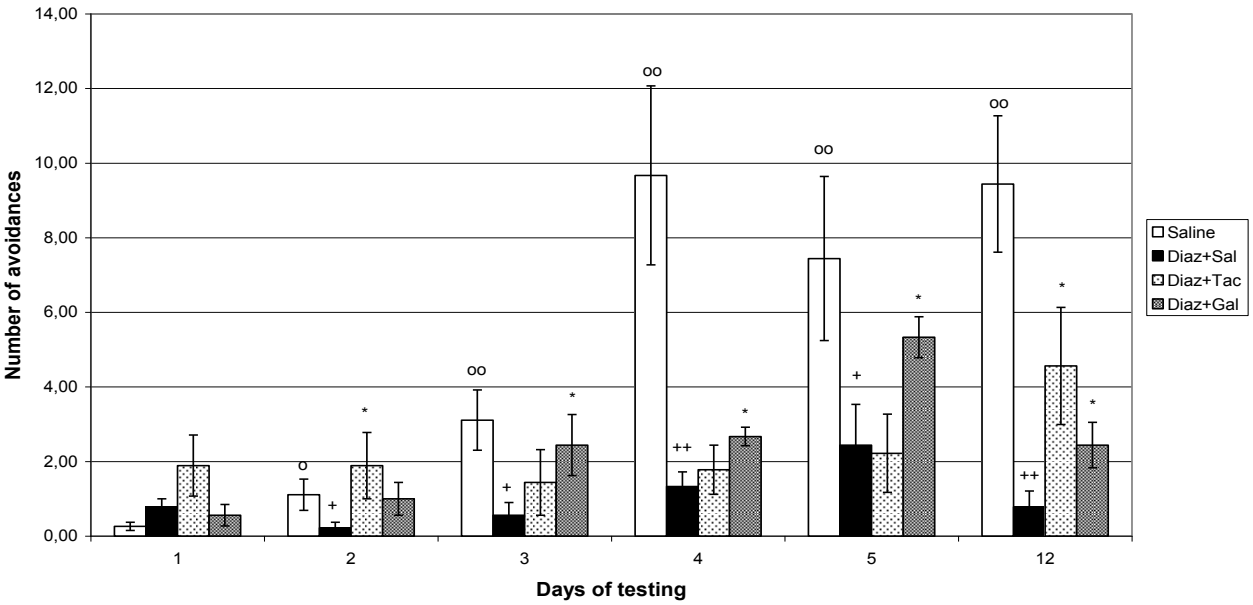
The means ± SEM for each group of rats were calculated using Instat computer program. A two-way ANOVA for repeated measurements was used to compare different groups with the respective controls with the Turkey-Kramer multiple comparison test.

RESULTS

In active avoidance test the control group showed significantly increased the number of conditioned stimuli responses (avoidances) on 2^{nd} ($p<0.05$), 3^{rd}, 4^{th} and 5^{th} ($p<0.01$) day learning and on memory retention test ($p<0.01$), in comparison with 1^{st} day (Figure 1). The animals with diazepam-amnesia model significantly decreased the number of avoidances on 2^{nd}, 3^{rd} ($p<0.05$), 4^{th}

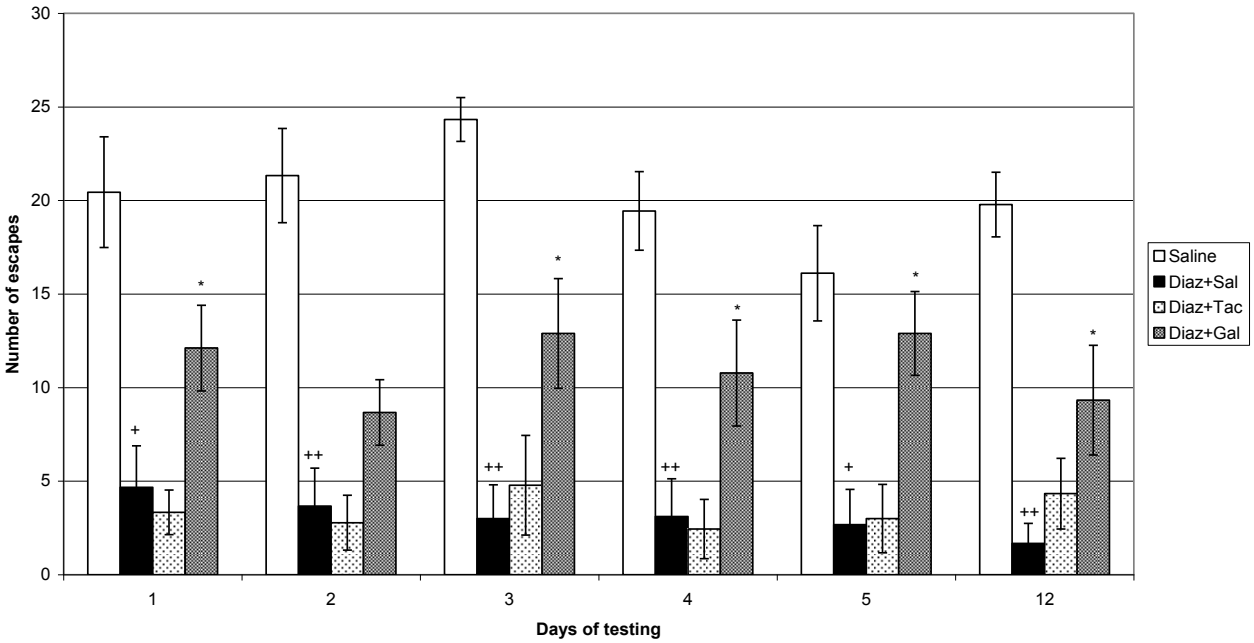
($p<0.01$) and 5th ($p<0.05$) day learning and on memory test ($p<0.01$), in comparison with respective day control group. The rats with Diazepam 2.5 mg/kg and Tacrine 1.0 mg/kg significantly increased the number of conditioned stimuly responses on 2nd day learning ($p<0.05$) and on memory test ($p<0.05$), compared to the same day group with diazepam and saline. The animals treated with Diazepam 2.5 mg/kg and Galantamine 0.1 mg/kg statistically significant increased the number of avoidances on 3rd, 4th and 5th day of learning session ($p<0.05$) and on 12th day ($p<0.05$) in memory test (Figure 1).

Figure 1. Effects of Tacrine and Galantamine on diazepam-amnesia model in rats
Number of conditioned stimuli responces (avoidances)



^{o} $p<0,05$ and ^{oo} $p<0,01$ compared to the 1st day control group;
$^{+}$ $p<0,05$ and $^{++}p<0,01$ compared to the same day control group;
* $p<0,05$ and ** $p<0,01$ compared to the same day group with diazepam and saline.

Figure 2. Effects of Tacrine and galantamine on diazepam-induced amnesia in rats. Number of unconditioned stimuli responces (escapes)



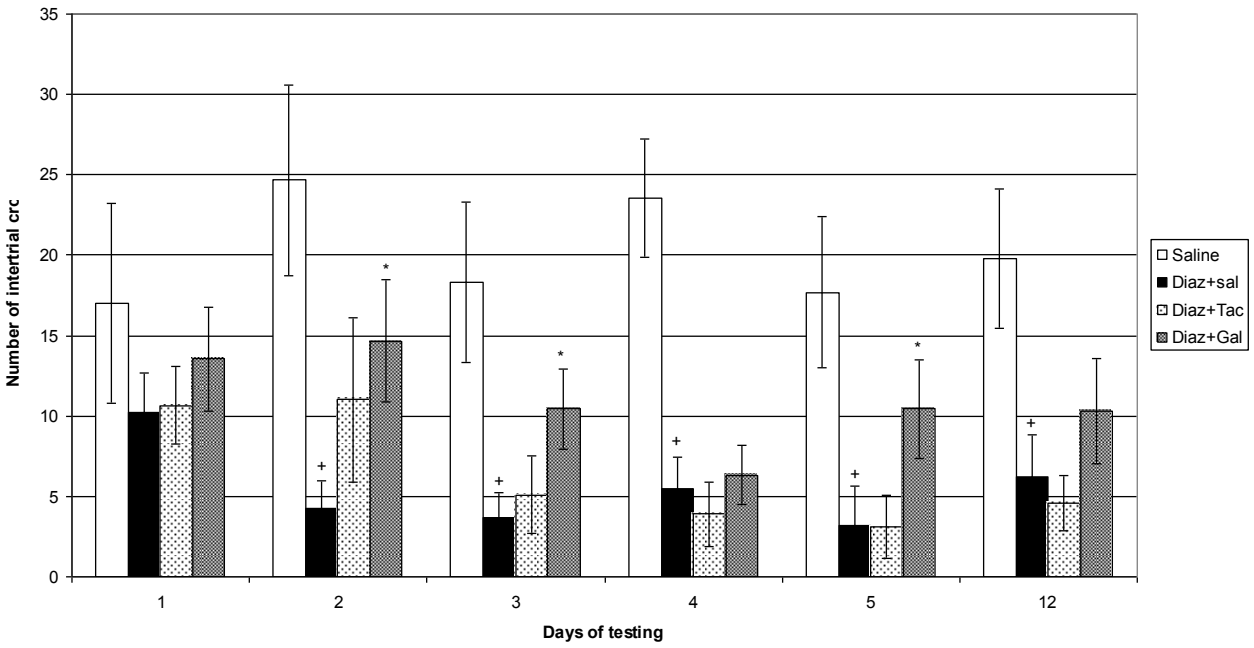
$^{+}$ $p<0,05$ and $^{++}$ $p<0,01$ compared to the same day saline group (control);

* $p<0,05$ compared to the same day group with diazepam and saline (amnesia model).

Control rats did not change the number of escapes on learning sessions and memory test (Figure 2). The rats with Diazepam 2.5 mg/kg and saline decreased the number of unconditioned stimuli responses (escapes) on the 1st and 5th day ($p<0.05$), 2nd, 3rd and 4th day ($p<0.01$) learning and on 12th day ($p<0.01$) memory retention, compared to the same day control group. The animals with Diazepam 2.5 mg/kg and Tacrine 1.0 mg/kg did not changed the number of escapes on learning session and on memory retention compared to the group with amnesia model only. The rats with Diazepam 2.5 mg/kg and Galantamine 0.1 mg/kg significantly increased the number of escapes on all days learning ($p<0.05$) and on memory test ($p<0.05$), in comparison with the same days group with Diazepam and saline (Figure 2).

There were no clear differences between the number of intertrial crossings made by control rats during the 5-days learning sessions and memory test (Figure 3). The rats with amnesia model only significantly decreased the number of intertrial crossings on 2nd, 3rd, 4th and 5th days ($p<0.05$) learning and on memory retention ($p<0.05$), in compareson with the same day control group. The group with Diazepam and Tacrine made the number of intertrial crossings similar to the group treated with diazepam and saline on lerning and memory tests. The rats with Diazepam and Galantamine increased the number of intertrial crossings on 2nd, 3rd and 5th days learning session ($p<0.05$) but not kept it on memory retention, compared to the same day amnesia model group (Figure 3).

Figure 3. Effects of Tacrine and Galantamine on diazepam-amnesia model in rats.
Number of intertrial crossings



$^{+}$ $p<0,05$ compared to the same day saline group (control);
* $p<0,05$ compared to the same day group with diazepam and saline (amnesia model).

DISCUSSION

In active avoidance test (Shuttle-box) the control rats learned the task andresults from the memory retention test showed that they had retained the knowledge.

We observed that diazepam applied chronically impaired learning capacities and memory function of the animals and decreased the locomotor activity (number of intertrial crossings). Similar results were obtained by Raffa et al. (1990) in passive avoidance tests in rats, intraperitoneally injected with diazepam in doses of 2 to 16 mg/kg prior to training. According to Viana et al. (1994), the 2 mg/kg dose causes full amnesia in rats with elevated T-maze, a method introduced in the 1990s to study the effects of drugs on long-term memory and fear. According to these authors, the amnesic effect is associated not only with the dose of benzodiazepines but also with the time of drug delivery – before or after trainings, or just before testing in the equipment. The model of intraperitoneal administration before training used in this study is considered to be the best.

Our results allow us to conclude that galantamine improve learning ability better than tacrine on diazepam impaired memory in active avoidance test. Both cholinesterase inhibitors have similar improving effects on memory function.

There are data that galanthamine and tacrine have prominent protective effects against glutamate neurotoxicity on primary cultures from the cerebral cortex of fetal rats (Takada-Takatory Y. et al., 2006). The mechanisms of neuroprotection include acetylcholinesterase inhibition and special role of alpha4 and alpha7 receptors (Akaike A. et al., 2010).

Galanthamine (Nivalin) is possible to be a preferable drug in its oral form for the patients with Alzheimer's disease due to its good efficacy and low toxicity for long term therapy.

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