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Effect of Diltiazem on Retention and Retrieval of Memory in Young and Aged Mice

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Abstract: Diltiazem (DTZ) is widely used in the prophylaxis of hypertension and treatment of angina. The effects of DTZ and other calcium channel blockers on memory have been discussed with several procedures and different theories have been suggested. In the present study, the effect of DTZ on retention and retrieval of memory in young and aged mice was investigated by using the passive avoidance apparatus. For this purpose, after weighting, coding and classifying the mice, they were grouped as follow: test group received electric shock plus DTZ (10 and 30 mg kg^{-1}, i.p.), blank group received electric shock plus normal saline and control group received only electric shock. In all three groups delay time of leaving the platform for both retention and retrieval test of memory was measured. DTZ was administered immediately after receiving electric shock in the retention test, but in retrieval test DTZ was administered 24 h after receiving electric shock. The results indicated that 30 mg kg^{-1} of DTZ impaired retention and retrieval of young mice memory. The 30 mg kg^{-1} of DTZ enhanced retention while 10 and 30 mg kg^{-1} of it improved retrieval of aged mice memory.

Key words: Diltiazem, calcium channel blockers, passive avoidance, retention, retrieval glutamatergic system, NMDA receptors

INTRODUCTION

Calcium channel blockers are a group of drugs that block calcium entry at specific L-type channels on neuronal cell bodies, in cardiac and vascular smooth muscles and cerebral vasculature (Golden *et al*., 1996). Diltiazem (DTZ) is a benzothiazepine calcium antagonist, causes a does-dependent inhibition of the transmembran influx of calcium ion into muscle cells via the L-channel (Rigat and Mahuran, 2009). DTZ is used in the treatment of stable and hypertension unstable angina pectoris, myocardial infarction, coronary artery spasm, cardiac arrhythmias, raynaud's phenomenon, primary pulmonary hypertension, esophageal motility disorders and migraine (Quartermain *et al*., 2001). Some studies have suggested that CCAs could be useful as cognitive enhancers on the basis of their ability to improve memory in young adult animals. For example, CCAs reverse experimentally induced amnesias (Genkova-Papazova *et al*., 1997; Zupan *et al*., 1993, 1996), improve reversal learning and spatial working memory (Levy *et al*., 1991; McMonagle-Strucko and Fanelli, 1993) and ameliorate brain lesions effect on learning (Popovic *et al*., 1997; Finger *et al*., 1990). Although, a number of studies have suggested that treatment with Calcium Channel Antagonist (CCAs) can ameliorate impairments in learning and memory in aged animals, evidence for a nootropic effect of CCAs in neurological normal young adult animals is ambiguous (Quartermain *et al*., 2001). This study attempts to resolve some of this ambiguity by the effect of DTZ on retention and retrieval of memory.

MATERIALS AND METHODS

Young (aged 3 months) and old male and female (aged 15 months) Wistar albino mice were used throughout the study. The animals were obtained from Razi Inistute, Karadje, Iran. The animals were kept in an animal house with a 12-h light/12-h dark cycle and controlled temperature (23±2°C) with relative humidity of 45-55%. They were housed in groups of 8 in Plexiglas animal cages and were given free access to food (Pars Khorakdam, Shushtar, Iran) and top water. All experiments were performed between 9 and 12 h from

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February to August, 2009 in Ahwaz Jondishapour University of Medical Sciences, School of pharmacy and each animal was used once. All procedures were carried out in accordance with the International Guidelines for Animal Care and Use.

The experiments: Two groups of young adult and aged mice were used. Each group subsequently divided into four groups (each of 8 animals). The first and second groups received DTZ 10 and 30 mg kg^{-1}, respectively. The third group received normal saline (1 mL/100 g) and the fourth group was untreated. The step-down apparatus used to test passive avoidance, consisted of a box measuring 25×25×20 cm with an electrifiable grid floor. There was a round plastic platform 1 cm high and 9 cm in diameter which could be enclosed by a 20 cm long hollow plastic cylinder with an inner diameter of 10 cm. On 1st day, groups of 4 animals were given access to learning apparatus for 3 min to familiarize them with the new environment. On second day, the mice were individually placed on the platform inside the cylinder. After 10 sec the cylinder was removed and the step-down latency was measured. Animals that had latencies longer than 30 sec were discarded. On 3rd day, the same procedure was followed as on day 2, except that a 1 sec foot shock (1 mA) was administered as soon as the animals left the platform with all 4 legs. The animals were injected with drugs immediately after foot shock, to study the effects on retention of memory or 24 h after the foot shock, to study the effect on retrieval of memory. On 4th day, the step-down latency of the mice was recorded. Each animal was used only once. All drugs were administered intraperitoneally.

RESULTS AND DISCUSSION

The results of this study indicate that the mean of step-down latency on day 4 in comparison with day 2 become longer ($p< 0.05$) in retention and retrieval of memory in all young and aged groups of mice (Fig. 1-4).

Figure 1 and 2 show comparison of step-down latency between day 4 and 2 in young mice received Diltiazem (10, 30 mg kg^{-1}), normal saline and untreated in memory retention (Fig. 1) and memory retrieval (Fig. 2) test. In both figs the mean step-down latency become longer in 4th day. On the other hand Fig. 3 and 4 reveals comparison of step-down latency between 2nd and 4th day in aged mice received Diltiazem (10 and 30 mg kg^{-1}) or normal saline and an untreated group in memory retention (Fig. 3) and memory retrieval (Fig. 4) test.

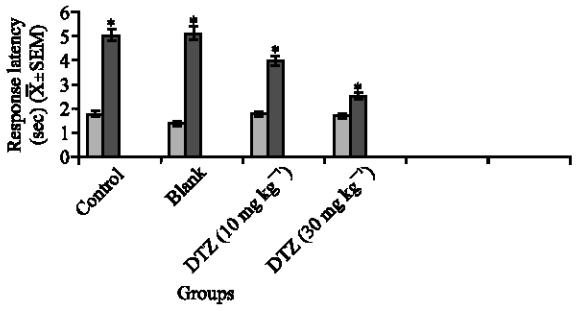

Fig. 1: Comparison of step-down latency between day 4 and 2 in young mice received Diltiazem, normal saline and untreated in memory retention test. Significant difference between day 4 and day 2 is shown as *p<0.05. Control: No injection, Blank: Normal saline (1 mL/100 g)

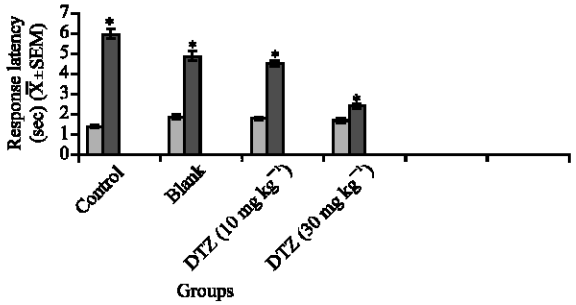

Fig. 2: Comparison of step-down latency between day 4 and 2 in young mice received diltiazem, normal saline and untreated in memory retrieval test. Significant difference between day 4 and 2 is shown as *p<0.05. Control: No injection, Blank: Normal saline (1 mL/100 g)

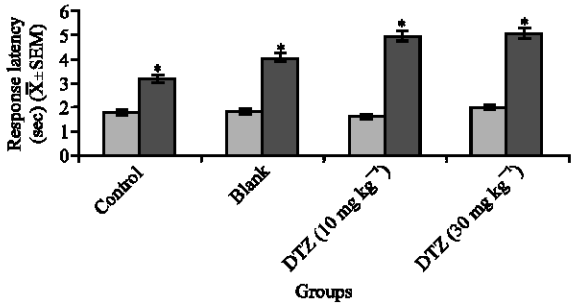

Fig. 3: Comparison of step-down latency between day 4 and 2 in aged mice received Diltiazem, normal saline and untreated in memory retention test. Significant difference between day 4 and 2 is shown as *p<0.05. Control: No injection, Blank: Normal saline (1 mL/100 g)

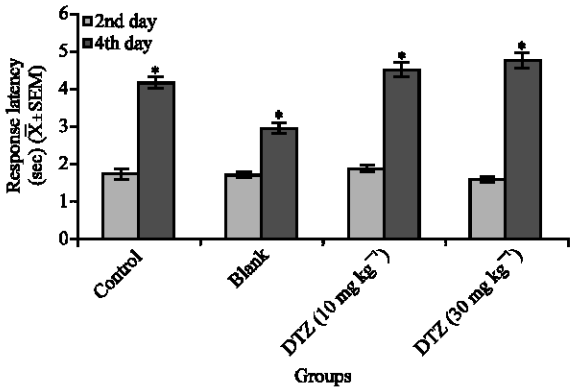

Fig. 4: Comparison of step-down latency between day 4 and 2 in aged mice received Diltiazem, normal saline and untreated in memory retrieval test. Significant difference between day 4 and 2 is shown as *p<0.05. Control: No injection, Blank: Normal saline (1 mL/100 g)

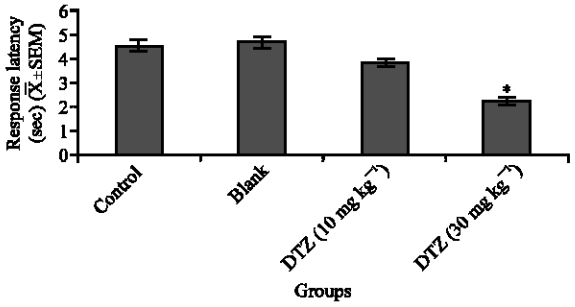

Fig. 5: Comparison of step-down latency between days 4 in young mice received diltiazem, normal saline and untreated in memory retention test. Significant difference between Diltiazem (30 mg kg^{-1}) and groups received Normal saline and untreated is shown as *p<0.05. Control: No injection, Blank: Normal saline (1 mL/100 g)

DTZ treated young mice with 30 mg kg^{-1} dose in comparison to normal saline and untreated groups showed impairment in retention of memory (Fig. 5), while 30 mg kg^{-1} of DTZ enhanced retention and retrieval of memory (Fig. 6, 7) and 10 mg kg^{-1} of this agent improved retrieval of aged mice (Fig. 7). Present findings are in agreement with results of Forette *et al.* (1998), which revealed that CCB therapy enhances the cognitive function in aged patients (Forette *et al.*, 1998) and an European study which showed that CCB treatment reduces the incidence of Alzheimer's disease (Fagard and Staessen, 1999). Also, the other study in 2010 showed that CCB regimen improves cognitive abilities in aged patients (Paran *et al.*, 2010).

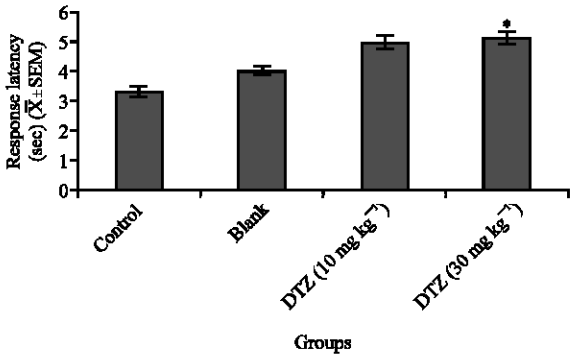

Fig. 6: Comparison of step-down latency between days 4 in aged mice received Diltiazem, normal saline and untreated in memory retention test. Significant difference between diltiazem (30 mg kg^{-1}) and groups received normal saline and untreated are shown as *p<0.05. Control: No injection, Blank: Normal saline (1 mL/100 g)

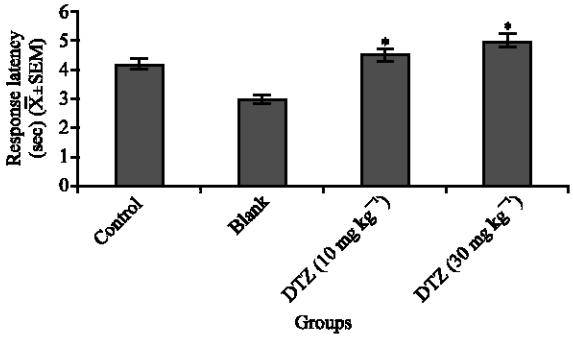

Fig. 7: Comparison of step-down latency between days 4 in aged mice received Diltiazem, normal saline and untreated in memory retrieval test. Significant differences between Diltiazem (10 and 30 mg kg^{-1}) and groups received normal saline and untreated is shown as *p<0.05. Control: No injection, Blank: Normal saline (1 mL/100 g)

Regarding to widespread use of anti-hypertensive drugs and extensive discussions on the effect of these drugs, the effect of DTZ on retention and retrieval of memory on young and aged mice was studied. DTZ according to its mechanism is used to treat hypertension, angina pectoris, cardiac arrhythmias and migraine (Quartermain *et al.*, 2001). As in most cases, DTZ should be used chronically or for whole life period, this knowledge helps the physician to properly choose the drug and the dosage; on the other hand helps patient to be aware which is being used. Some studies showed that Calcium Channel Blockers (CCBs) therapy can improve learning and memory disturbances in aged animals

(Clements *et al*., 1995; Quartermain *et al*., 2001; Rigat and Mahuran, 2009). Using CCBs don't induce dementia in very young animals. This may be because of the fact that in young animals the N-type CCs are crucial in memory function, whereas the CCBs exclusively block L-type CCs (Clements *et al*., 1995; Voglis and Tavernarakis, 2006; Seoane *et al*., 2009). Also, the study on CCBs, including DTZ on passive avoidance behavior showed that DTZ can improve memory retention and retrieval of memory in aged mice (Quartermain *et al*., 2001). It seems that by increasing the age, neurons will be damaged and their electrical conductivity decrease. There are some evidences for the role of glutamatergic, including NMDA receptors in perirhinal plasticity and recognition memory (Ziakopoulos *et al*., 1999; Voglis and Tavernarakis, 2006; Barker and Warburton, 2008). It has been showed that glutamate releases during neuronal injury can extend the existing damage and cause neuronal death (Gouix *et al*., 2009); this event is called toxic oxidative cascade. NMDA receptor blocker can decrease the anoxia-induced neuronal damage. It is probable that these results cannot be extended in human models. Also alteration in aged brain could because of changes in calcium dynamics (Chen *et al*., 2000), loss of cholinergic neurons (Stemmelin *et al*., 2000), DNA oxidative damage (Wu *et al*., 2002) or serotonergic system (Birthelmer *et al*., 2003). Electroshock induced memory impairment may not be improved by CCBs therapy. It should be considered that CCBs can potentiate the memory in mice, while they are not able to produce such effect in human beings (Maxwell *et al*., 1999). The N-type CCBs are important in neuron transmitter release; it was shown those agents can decrease Ach release which may result in learning and memory weakness (Schmahmann, 2003). The current study showed that 30 mg kg^{-1} of DTZ impaired retention and retrieval of young mice memory. The 30 mg kg^{-1} of DTZ enhanced retention and retrieval while 10 mg kg^{-1} of it improved retrieval of aged mice memory.

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