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Full Length Research Paper

Sub-chronic treatment of calcineurin inhibitor averts impairment of cognitive function in animal model of amnesia

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Although the actions of tacrolimus-calcineurin inhibitor on cognitive function have been studied; its impact on scopolamine-induced memory impairment has not been explored. Therefore, this study examined the effects of one-week tacrolimus treatment on memory deficit caused by scopolamine, which is an established pharmacological model of Alzheimer's disease induction. The evaluation of hippocampus-dependent learning and memory deficits was carried out in two phases, namely, behavioral performance using the elevated plus maze model, and measurement of oxidative stress markers in brain samples. Findings showed that administration of tacrolimus prevented the decline of hippocampus-dependent long-term memory. In correlation, tacrolimus pre-treatment prevented scopolamine associated increase in the activity thiobarbituric acid reactive substances and decrease of the reduced glutathione. The results suggest that sub-chronic use of calcineurin inhibitor averts impairment of learning and memory through restoring the oxidative stress markers levels.

Key words: Calcineurin inhibitor, impairment, learning, memory.

INTRODUCTION

The use of pharmacological models for Alzheimer's disease is widely accepted as an approach to develop new medicines. One of the most common models to study Alzheimer's disease is scopolamine induced amnesia. It is a non-selective muscarinic receptor antagonist (Ebert and Kirch, 1998; Van Dam and De Deyn, 2006; Klinkenberg and Blokland, 2010). Although, the effectiveness of scopolamine amnesia as a pharmacological model for Alzheimer's disease is well documented, there is no model that is exactly similar to Alzheimer's disease (Gilles and Ertlé, 2000). Scopolamine produces cholinergic deficit similar to the deficit observed in Alzheimer's disease and has been employed as a reference to develop dementia-related disorders in animals as well as humans (Bartus et al., 1982; Hasselmo, 2006; Winters et al., 2006; Young et al., 1995; Klinkenberg and Blokland, 2010). Accumulated evidence demonstrates a clear correlation between scopolamine administration and impairment of memory. For instance, a dose of scopolamine harms short-term and procedural recall classes in individuals (Oh et al., 2011). Evidence suggests that scopolamine triggers a marked reduction in the activity of neuronal networks within the hippocampus (Anagnostaras et al., 1999; Yaguchi et al., 2009). Additionally, scopolamine impairs hippocampus-dependent acquisition and consolidation of information in rats (Sun et al., 2007; Wang et al., 2010).

Oxidative damage has been attributed to the disproportion between antioxidant defense and reactive oxygen molecules. It plays a crucial role in the development of neurodegenerative disorders, including Alzheimer's disease (Golechha et al., 2012). Experimental evidence suggests that enhanced oxidative stress mediates neuronal degeneration and cell death

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associated with Alzheimer's disease (Markesbery et al., 1997). For example, scopolamine model of Alzheimer's disease shows an increase in malondialdehyde activity and a decrease in glutathione levels and consequently leading to memory loss (Ishola et al., 2013).

The stimulation of the neuronal networks within the hippocampus and cortex can be regulated by calcineurin, a signaling molecule hinders memory (Wang and Kelly, 1997; Winder et al., 1998). Earlier studies showed that the increase in the levels of calcineurin might have negative impact on learning and memory, synaptic plasticity, and neurogenesis (Dineley et al., 2007). Furthermore, it has been found that significant increase in the expression of calcineurin weakens long-term potentiation and that pharmacological inhibitors of calcineurin facilitate NMDA-dependent long-term potentiation in hippocampal slices (Cavallucci et al., 2013). The action of calcineurin on memory is most probably through dephosphorylating phosporylated-Ca^{2+}/calmodulin-dependent protein kinase II (P-CaMKII). Tacrolimus is one of the available calcineurin inhibitors and their effect on the normal physiological process has been investigated.

Even though the pharmacological actions of tacrolimus have been significantly tested, their impact on scopolamine-induced amnesia in the hippocampus and cerebral cortex has not been fully studied. In this paper, behavioral and molecular experiments have been used to study the effect of calcineurin inhibitor on cognitive deficit associated with scopolamine.

MATERIALS AND METHODS

Animals

All animal experiments were carried out according to the Animal Care and Use Committee in King Faisal University. Adult albino Wistar rats with a weight of 175 to 200 g, at the beginning of the test, were distributed randomly into three experimental groups. A group of six rats was housed in a cage in a climate-controlled room temperature on a 12/12-h light/dark schedule (lights on at 7 am) with free access to standard rodent chow and water. They were acclimatized for one week after dividing them into three experimental groups, namely, control, scopolamine, and tacrolimus/scopolamine.

Drugs and chemicals

Tacrolimus and scopolamine were purchased from M/s. Sigma Chemicals, India.

Experimental protocol

Experimental group one was given 0.1% DMSO for seven days as a control. The rats in the second group were administered 1 mg/kg (0.1% DMSO) scopolamine on day seven through intra-peritoneal injection. The animals in the third group were given tacrolimus for seven days consecutively and on the 7th day, they also received 1 mg/kg (0.1% DMSO) scopolamine.

Elevated plus maze model

The cognitive function of all three groups were assessed through the elevated plus maze task. Several lines of evidence have shown that elevated plus-maze model can be used to test the hippocampus-dependent memory using spatial cues. The elevated plus-maze technique was composed of a central platform linked into four arms in a plus sign shape and the height of maze was 50 cm from the ground. The end of the two arms was closed (50 × 40 ×10 cm) and the other two were opened (50 × 10 cm) (Itoh et al., 1990). During the learning phase and long-term memory tests (that is, seven days after the beginning of tacrolimus administration), rats were placed individually into the end of one of the open arms and the time needed for each rat to reach the covered arm with all of its legs was called transfer latency. The decrease in the transfer latency suggests memory improvement, whereas the increase in the transfer latency indicates memory impairment.

Lipid peroxidation assay

Thiobarbituric acid reactive substances (TBARS) were estimated as a measure of lipid peroxidation (Ohkawa et al., 1979; Mattson, 2009). The supernatant from the brain tissue samples collected in a test-tube. To this was added to 0.2 ml of 8.1% sodium dodecyl sulphate, 1.5 ml of 30% acetic acid (pH 3.5), 1.5 ml of 0.8% of thiobarbituric acid. The mixture was then kept for 1 h at 95°C. Subsequently 1 ml of distilled water was added followed by 5 ml of n-butanol-pyridine mixture (15:1 v/v) was added. The mixture was then centrifuged at 4000 g for 10 min. Thereafter, absorbance was determined at 532 nm using spectrophotometer.

Anti-oxidant level

The level of reduced glutathione (GSH) in the brain tissue samples was determined according to the method described by Beutler et al. (1963). Trichloroacetic acid (10% w/v) was added to supernatant collected from brain tissue homogenate in a ratio of 1:1. The mixture was then centrifuged at 1000 g for 10 min at 40°C. 0.5 ml was taken out of the mixture and to it was added 2 ml of 0.3 M disodium hydrogen phosphate and 0.25 ml of 0.001 M freshly prepared [5, 5`-dithiobis (2-nitrobenzoic acid) which was dissolved in 1% w/v citric acid]. The absorbance was measured at 412 nm through spectrophotometer.

Statistical comparisons

Values were represented as mean ± standard error of the mean (SEM). Comparisons amongst the three experimental groups were carried using one-way analysis of variance (ANOVA), followed by Tukey's multiple range using GraphPad Prism. P values < 0.05 were considered significant.

RESULTS

Behavioral tests

Sub-chronic tacrolimus treatment prevents cognitive dysfunction associated with Alzheimer's disease

It is commonly believed that spatial learning and memory is a hippocampus and cerebral cortex-dependent function (Anagnostaras et al., 2003). In this study, the effect of

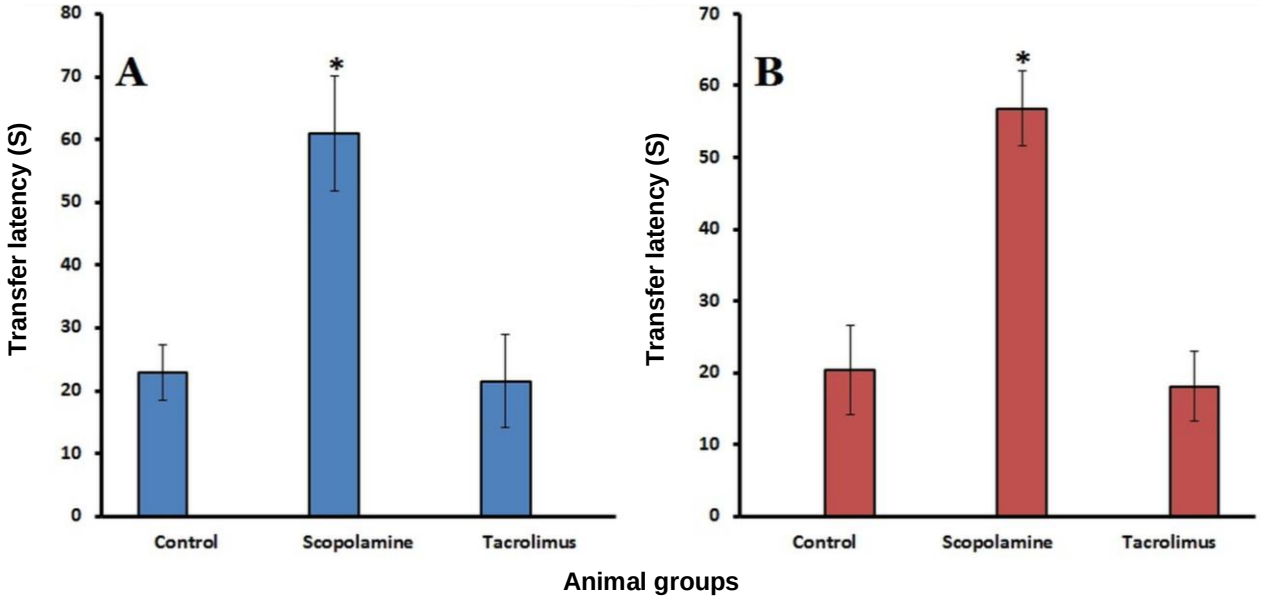

Figure 1. The impact of tacrolimus on transfer latency in scopolamine-induced memory loss. (a) Transfer latency on Day 7th; (B) Transfer latency on Day 8th (TL after 24 h (s)). Diseased versus all groups, *P<0.001.

sub-chronic calcineurin inhibitor treatment on scopolamine-induced cognitive dysfunction using the elevated plus maze model was tested.

Learning trails

During the learning (acquisition) phase, rats in all three groups were trained to find the closed arm under the same environment. Rats in the control, and tacrolimus/scopolamine groups learned the location of the covered arm at equivalent rates 45 min after the acquisition phase (22.87 ± 4.41; 21.50 ± 7.45). In contrast, the scopolamine group ability to find the closed arm was significantly impaired (61.00 ± 9.16) as indicated by more time needed as compared to the other two experimental groups (Figure 1A).

Spatial long-term memory

In the long-memory test administered 24 h after the end of the acquisition phase, the scopolamine group committed drastically more seconds (56.83 ± 5.22) in finding the closed arm in the elevated plus maze than the control group (20.34 ± 6.18) (Figure 1B) suggesting significant deficit of long-term memory. Sub-chronic tacrolimus treatment ameliorated long-term memory impairment in scopolamine-treated rats as found by the insignificant difference (18.10 ± 4.87) from control group (Figure 1B). These results suggest that pre-treatment of calcineurin inhibitor averts the deleterious effect of scopolamine on learning and memory.

Molecular experiments

Levels of oxidative stress markers

This was carried out to validate the behavioral results and to elucidate the changes in the levels of reduced glutathione and thiobarbituric acid reactive substances that are indices for antioxidation and lipid peroxidation, respectively.

Effect of tacrolimus on reduced glutathione and thiobarbituric acid reactive substances

Considerable evidence suggests that calcineurin impairs memory through dephosphorylating the active form of CaMKII (Wang and Kelly, 1997). This study evaluated the effect of scopolamine on the basal levels of reduced glutathione in mixed tissue homogenate of hippocampus and cerebral cortex regions. There was significant reduction in the levels of reduced glutathione of scopolamine treated rats (6.93 ± 0.03) compared to control rats (10.11 ± 0.03) (Figure 3). However, tacrolimus pre-treated rats showed no difference in the levels of reduced glutathione (11.21 ± 0.06) compared to control rats. Therefore, tacrolimus pre-treatment prevented the decrease in the levels of reduced glutathione associated with scopolamine administration.

Thiobarbituric acid reactive substances play an important role in hippocampal and cortical dependent-memory. The present results show that scopolamine increased the activity of thiobarbituric acid reactive substances (100.12 ± 0.713) (Figure 2) compared to the

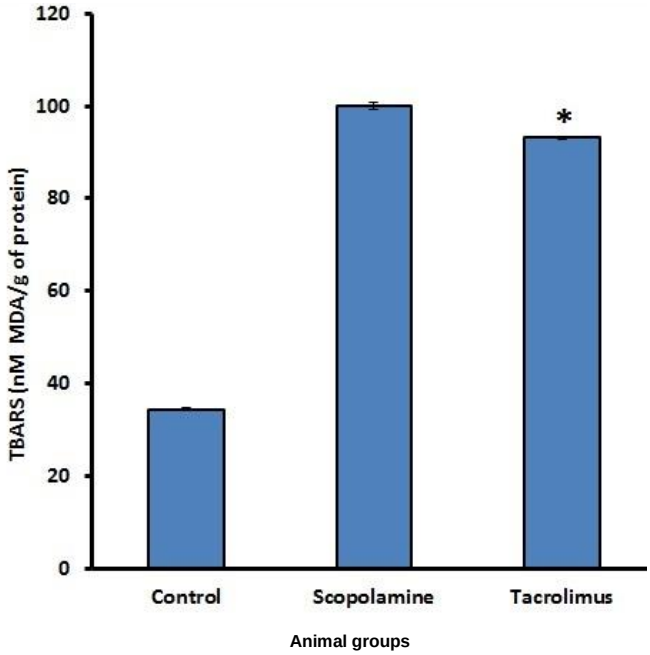

Figure 2. The effect of tacrolimus on lipid peroxidation levels. #Diseased versus all groups, *P<0.001.

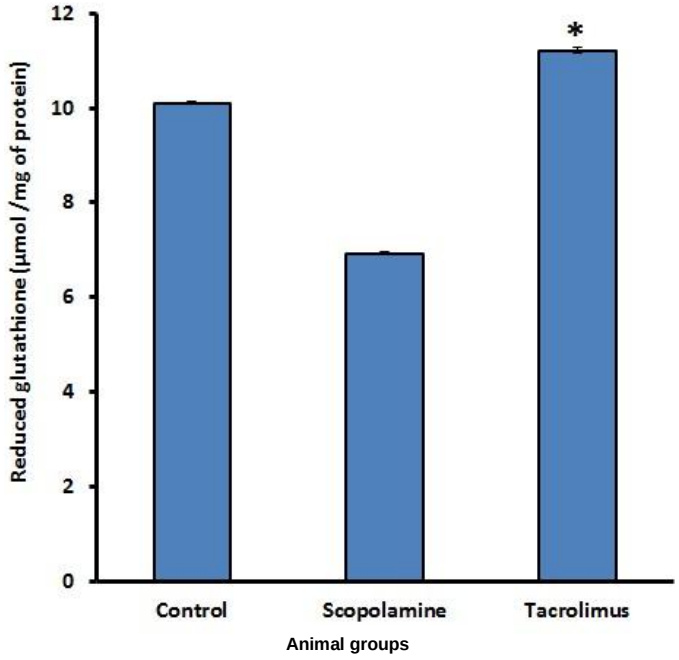

Figure 3. The effect of tacrolimus on reduced glutathione levels. Diseased versus all groups, *P<0.001.

control (34.41± 0.339). Even though sub-chronic tacrolimus treatment did not restore the activity of thiobarbituric acid reactive substances (93.20 ± 0.339) to that in control rats, it prevented the scopolamine-induced increase in the levels of thiobarbituric acid reactive substances in the scopolamine group (Figure 2).

DISCUSSION

Animal experiments and clinical trials propose that scopolamine negatively affects performance of brain. It mainly impairs the ability to initiate leaning of new observation and interrupts memory retention as well (Klinkenberg and Blokland, 2010). Several research papers validate the harmful influence of scopolamine on cortex and hippocampus-dependent learning and memory (Fan et al., 2005; Oh et al., 2009; Chen et al., 2008). In agreement, the results of the current behavioral experiments demonstrate that scopolamine interferes with learning and memory. Use of scopolamine, non-selective muscarinic receptors blocker, as a model for amnesia has been validated through the ability of cholinomimetics including, rivastigmine and physostigmine, to avert the harmful effect of scopolamine on cognition (Gilles and Ertlé, 2000).

Accumulated evidence suggests that oxidative stress disrupts multiple functions in the central nervous system. In fact, oxidative stress harms the physiological perspective and lead to cell death. Additionally, oxidative imbalance has been considered as a common mechanism underlying neurodegenerative disorders. In the last decade, many reports have linked the alteration in the oxidative stress markers to the decline in memory consolidation using a variety of experimental tools (Wyss-Coray and Mucke, 2002; Zandi et al., 2004). Accordingly, in the current research paper, it was found out that the activity of reduced glutathione and thiobarbituric acid reactive substances was altered in scopolamine treated rats (Goverdhan et al., 2012).

Tacrolimus, a frequently used immunosuppressant, is implicated in the adjustment of the learning and memory performance (Benetoli et al., 2004). A large body of evidence has shown that tacrolimus enhances the cognitive function in clinical studies as well as experimental animal models (Benetoli et al., 2004). Additionally, tacrolimus administration alleviates learning and memory deficit associated with a diversity of animal paradigms of brain diseases, including ischemia, Parkinson's disease, Alzheimer's disease, and age-related cognitive impairment (Benetoli et al., 2004; Dineley et al., 2007). The beneficial mechanism of tacrolimus treatment on cognitive function is under investigation. However, existing researches suggest that tacrolimus facilitate memory through its action as a calcineurin inhibition (Cavallucci et al., 2013). The present results show that sub-chronic administration of tacrolimus prevents the harmful effects of scopolamine on memory. The elevated plus maze task was used as a behavioral test to assess the cognitive function. In consistent with the current findings, earlier reports demonstrated the ability of tacrolimus to prevent learning

and memory impairment in various models of behavioral tests, including Morris water maze, and eight-arm radial maze (Paganelli et al., 2004; Benetoli et al., 2004; Kumar and Kumar, 2009).

Substantial evidence shows that calcineurin, a phosphatase enzyme, is present in the cortical and hippocampal neurons. Calcineurin is involved in the process of dephosphorylation and deactivation of many phosphorylated signaling molecules. Recently, calcineurin inhibition has been reported to prevent the changes in long-term depression and post-synaptic density composition induced by Alzheimer's disease (Cavallucci et al., 2013). Interestingly, calcineurin has been found to activate calcium current in hippocampal neurons. In this study, tacrolimus-calcineurin inhibitor-prevented the oxidative stress induced by scopolamine as indicated by the increase and decrease in the activity of reduced glutathione and thiobarbituric acid reactive substances, respectively. The ability of tacrolimus to protect against oxidative stress is probably due to its ability to decrease the calcium release as reported by Norris et al. (2002). The calcium release is an essential factor to mediate apoptosis and cell death in hippocampus and cerebral cortex (Ermak and Davies, 2002).

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