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Research Article

AMELIORATIVE EFFECTS OF ANGIOTENSIN RECEPTOR BLOCKERS AGAINST SCOPOLAMINE-INDUCED MEMORY IMPAIRMENT IN RATS

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ABSTRACT

Objective: The present study was designed to investigate the cognitive enhancing property of angiotensin receptor blockers (ARBs) in scopolamine-induced amnesic rats.

Methods: A total of 42 male Wistar rats were divided into seven groups. Group 1 received 2% gum acacia orally for 4 weeks, Group 2 received normal saline, and Group 3 received scopolamine (2 mg/kg/i.p.) as a single dose. Groups 4 and 5 received telmisartan (1.80 mg/kg and 3.60 mg/kg, respectively) while Groups 6 and 7 received losartan (2.25 mg/kg and 4.50 mg/kg, respectively), orally for 4 weeks, followed by scopolamine (2 mg/kg/i.p.) given 45 minutes prior to experimental procedure. Evaluation of learning and memory was assessed by using morris water maze test followed by estimation of hippocampal choline acetyltransferase (ChAT) activity. Alterations in hippocampal morphology and degree of neuronal survival were also analyzed following drug treatments.

Results: Scopolamine-induced marked impairment of memory in the behavioral test which correlated with reduced ChAT activity and morphological changes in the hippocampus. Treatment with higher doses of telmisartan and losartan improved memory deficits in scopolamine-induced amnesic rats while increasing the hippocampal ChAT activity. The treatments also attenuated hippocampal degeneration and increased the number of surviving neurons in hippocampus scopolamine-induced amnesic rats.

Conclusion: Pre-treatment with ARBs attenuated scopolamine-induced memory deficits which may be attributed to their angiotensin receptor blockade property or to improved cholinergic activity, and thus highlighting the potential of these drugs in dementia.

Keywords: Angiotensin scopolamine, Amnesia, Angiotensin receptor blockers, Losartan, telmisartan

INTRODUCTION

Learning and memory are complex higher functions in the brain, and they constitute an area of rapid advances in neurobiology. A decreased ability to learn and store information has important implications on the functioning of day-to-day and long-term practices, as seen in dementia. Dementia causes disruption of a number of higher cortical functions including memory, reasoning, learning, orientation, judgment and emotional stability [1]. The most common form of dementia is Alzheimer's disease (AD) followed in frequency by cerebrovascular dementia and Lewy body dementia [2]. AD has a tremendous impact on the health care system and poses an immense burden on the affected individuals, caregivers, and society at large. Cure of AD is still at a nascent stage in the field of medicine, and thus effective therapeutic interventions to prevent Alzheimer's dementia or halt its progression are an utmost priority. Many attempts have been made to reverse cognitive deficits by alternative treatments such as acetylcholinesterase inhibitors (AChEIs) and nootropics [3,4]. However, these drugs have been shown to provide only modest symptomatic benefits with no effect on the progression of the disease.

Over the recent years, drugs known to interfere with the renin-angiotensin system (RAS) have been shown to exert beneficial effects on memory and cognition [5]. Ang-II, the dominant effector molecule regulating RAS has been known to impair cognitive performance by inhibiting the release of acetylcholine (ACh), a primary neurotransmitter involved in learning and memory [6]. Further, angiotensin-II (Ang-II) can induce oxidative stress, neural injury and neuroinflammation, all of which are implicated in the pathogenesis of AD [7].

The angiotensins exert their function through AT_1, AT_2, and AT_4 receptor subtypes [8-10]. However, almost all the known physiological and clinical effects of Ang-II have been shown to be mediated largely by the AT_1 receptors. Studies have shown that the activity of Ang-II, AT_1 and AT_2 receptor binding are increased in brains of AD patients, suggesting an increased brain RAS activity during the disease process. The AT_4 receptors in the brain are known to mediate the actions of Ang-IV, which is an aminopeptide produced as a derivative of Ang-II [11,12]. However unlike Ang-II, Ang-IV has been postulated to exert a beneficial effect on memory acquisition and retrieval [13].

Angiotensin receptor blockers (ARBs) are a class of drugs affecting the RAS cascade and may have an influence on learning and memory [14]. The present study was thus designed to investigate and compare the effects of two clinically used ARBs, *viz*., losartan and telmisartan in scopolamine-induced amnesia model. Further, the effect of ARBs on brain cholinergic activity was done by estimating the level of choline acetyltransferase (ChAT). Hippocampal morphology and neuronal cell count at hippocampal subregions (CA1, CA3 and dentate gyrus [DG]) were also analyzed following treatment with losartan and telmisartan.

METHODS

Animals

A total of 42 male Wistar rats weighing 200-250 g were used in the study. All animals were housed in polypropylene cage with only four animals in each cage to prevent overcrowding. The animals were kept at room temperature (25±3°C) with a 12 hrs dark/light cycle and were provided

with standard laboratory feed (VRK Nutritional Solutions, Pune, India Ltd) and water ad libitum. The experimental protocol was approved by the Institutional Animal Ethical Committee (No. IAEC/KMC/36/2011-2012, May 2011), and experiments were conducted in accordance with the CPSCEA guidelines on the use and care of experimental animals.

Drugs and reagents

Losartan and telmisartan in powdered form were obtained as generous gift samples from Zydus Health Care Ltd., Ahmedabad, India. Scopolamine hydrobromide was procured from Sigma Aldrich, Mumbai. The ChAT assay kit used in the study was obtained from Prolab Marketing Pvt. Limited, New Delhi, India.

Experimental design

The animals were randomly allocated into the following seven groups:

Group 1: Animals of this group received 2% gum acacia orally for 4 weeks (vehicle control)

Group 2: Animals of this group received 1 ml/kg of 0.9% normal saline (saline control) intraperitoneally (i.p.) as a single dose before the experimental procedure

Group 3: Animals in this group received 2 mg/kg of scopolamine i.p. as a single dose immediately before the behavioral test

Group 4: Animals in this group received telmisartan 1.80 mg/kg orally for 4 weeks followed by 2 mg/kg of scopolamine i.p. as a single dose immediately before the behavioral test

Group 5: Animals in this group received telmisartan 3.60 mg/kg orally for 4 weeks followed by 2 mg/kg of scopolamine i.p. as a single dose immediately before the behavioral test

Group 6: Animals in this group received losartan 2.25 mg/kg orally for 4 weeks followed by 2 mg/kg of scopolamine i.p. as a single dose immediately before the behavioral test

Group 7: Animals in this group received losartan 4.50 mg/kg orally for 4 weeks followed by 2 mg/kg of scopolamine i.p. as a single dose immediately before the behavioral test

Rats equivalent doses in mg/kg body weight of clinical doses were calculated as mg/kg body weight as described by Paget and Barnes [15]. All the drugs except scopolamine were dissolved in 2% gum acacia, whereas scopolamine was dissolved in normal saline. Scopolamine 2 mg/kg was administered intraperitoneally to the above groups of animals (except Groups 1 and 2) for induction of amnesia, 45 minutes before the behavioral test [16,17].

Morris water maze (MWM) test

MWM test is one of the gold standards for a test of spatial learning and memory. The apparatus consisted of a circular tank (diameter 150 cm and height 45 cm), which was filled with water and maintained at 25°C. The water was made opaque by adding milk. The tank was divided into four equal quadrants (Q1, Q2, Q3, and Q4). A white platform (10 cm_2) centered in one of the four quadrants (target quadrant) of the pool was submerged approximately 2 cm below the surface of water. In our study, the target quadrant was considered as Q4. To provide extra maze cues for allowing the rats to develop a spatial map strategy, a black and white symbol board was placed at a point near the maze. The positions of the platform and extra maze cues were kept consistent throughout the training session. The water maze test consisted of two phases [18].

Spatial task acquisition phase: The rats were trained in the water maze in 8 sessions on 4 consecutive days. Each animal was subjected to 4 consecutive acquisition trials on each day with an interval of 5 minutes, during which rats were allowed to locate the hidden platform and allowed to remain there for 20 seconds. The trials were always initiated from different positions in the tank. For each rat, the point of immersion into the pool varied between Q1, Q2, Q3 and Q4. If the animal was unable to locate the hidden platform within the specified time, it was gently guided to the platform and allowed to remain there for 20 seconds. During each trial, the latencies of rats to locate the hidden platform were recorded, and the latency was considered as an index of acquisition and learning [18].

Spatial probe trial: On the 5th day following the completion of learning sessions, each rat was subjected to a spatial probe trial, in which no platform was placed. Each rat was placed in the water as mentioned in the acquisition phase and was allowed to explore the pool for 60 seconds. As a measure of spatial working memory, the latency to enter the target quadrant Q4 and the total time spent in target quadrant Q4 were noted [18,19].

Estimation of brain cholinergic activity by ChAT assay

Following the behavioral test, animals were sacrificed under light ether anesthesia. The brains were immediately removed and washed in ice-cold physiological saline repeatedly. The hippocampus was carefully dissected out over ice-cold watch glass [20]. The hippocampus from each brain was blotted, weighed and placed in 0.01 mol/L phosphate buffer saline (pH 7.4) for homogenization.

The *in vitro* quantitative measurement of ChAT in rat tissue homogenates was performed using an ELISA kit. The microtiter plate provided in the kit is pre-coated with a monoclonal antibody specific to ChAT. Standards or samples were added to the appropriate microtiter plate wells with a biotin-conjugated polyclonal antibody preparation specific for ChAT. Next, avidin conjugated to horseradish peroxidase was added to each microplate well, incubated and a TMB substrate solution was added to each well. Only those wells that contain ChAT, biotin-conjugated antibody, and enzyme-conjugated avidin exhibited a change in color. The enzyme-substrate reaction was terminated by the addition of a sulfuric acid solution, and the color change was measured spectrophotometrically at a wavelength of 450 nm±10 nm. The concentration of ChAT in the samples was then determined by comparing the optical density of the samples to the standard curve [21].

Analysis of hippocampal morphology and neuronal cell count using hematoxylin and eosin (H & E) staining

H&E staining was done to analyze the cellular architecture of rat hippocampus and to quantify the healthy neuronal cells at different regions of the hippocampus *viz.,* CA1, CA3, and DG. The brain sections from each animal were evaluated qualitatively for analyzing the general morphology by a light microscope under ×40 (Magnus MLX, Microscope). A qualitative analysis was carried out in CA1, CA3 and DG regions of the hippocampus of left hemisphere. Images of these brain regions were captured using Olympus BX43 dual-headed microscope, with DP 21 microscope digital camera attached (Japan). These images were then observed for any morphological changes such as cell shrinkage, cell size, and cell number. Cell counting was done using an ocular micrometer. The number of surviving or viable neurons (neurons with a distinct nucleus) within a specific measured (using ocular micrometer) area under ×40 was counted. 10 sections were counted a rat and the mean was taken. Cells with darkly stained shrunken cell body and cells with fragmented nuclei were excluded from quantification.

Statistical analysis

All the data were analyzed using Statistical Package for Social Sciences (SPSS) vs. 16.0. The data were expressed as mean±standard error. For all the parameters studied, the gum acacia control group was considered as normal control group in our study and used for comparisons with drug treated groups. The significance of differences between the groups was analyzed using one-way Analysis of Variance (ANOVA) test followed by *post-hoc* Tukey's test for multiple comparisons. $p<0.05$ was considered as statistically significant.

RESULTS

The vehicle (gum acacia) control was considered as a normal control in our study and all the drug treated groups were compared with vehicle control. There was no statistically significant difference between vehicle control and saline control in any of the parameters studied.

MWM test

Spatial memory during the acquisition trials

Results of 4 consecutive training sessions in water maze revealed significant memory impairment in scopolamine group as represented in Fig. 1. Control rats rapidly learned the location of the hidden platform as reflected by a decrease in their latencies from day 1 (44.89±1.42 seconds) to day 4, indicating normal acquisition behavior (17.24±0.88 seconds). Rats which received scopolamine showed a significant increase in latency to locate the hidden platform during the acquisition trials ($$p<0.001, scopolamine vs. control), indicating impairment of acquisition in the scopolamine-treated rats.

Administration of telmisartan showed a marked decrease in escape latencies from Days 1 to 4. On day 4, the escape latencies for telmisartan 1.80 mg/kg and 3.60 mg/kg were 26.25±1.08 seconds and 24.10±2.38 seconds which revealed a significant difference compared to scopolamine (**p<0.001). Rats pre-treated with losartan 4.50 mg/kg showed an escape latency of 28.16±2.43 sec, which was significantly decreased compared to scopolamine group (*p<0.05). However, no significant decrease in Day 4 latency (31.29±1.63 seconds) was noted with losartan 2.25 mg/kg when compared with the scopolamine-treated group. When telmisartan and losartan treated groups were compared to control group, only 3.60 mg/kg of telmisartan showed an efficacy that was comparable to control group indicating that higher dose of telmisartan demonstrates better memory enhancing efficacy compared to a higher dose of losartan. Fig. 2 depicts the track plots of control rats and scopolamine-treated rats while Figs. 3 and 4 depict the video track plots of rats pre-treated with lower doses and higher doses of telmisartan and losartan, respectively.

Spatial probe trial

Following two parameters were studied in this trial: Latency to locate the target quadrant: Administration of scopolamine showed a significant increase in the latency (27.48±2.00 seconds) to locate the target quadrant (Q4) compared to vehicle control (14.59±1.54 seconds) and saline control (17.11±1.62 seconds) rats (p<0.001) during the probe trial indicating impaired memory in the scopolamine-treated group. A significant decrease in latency to locate the target quadrant was noted in rats that were pre-treated with lower (16.51±1.31 seconds) and higher doses (15.76±1.23 seconds) of telmisartan when compared to scopolamine group (**p<0.001). For rats that received lower (20.07±1.96 seconds) and higher doses (19.74±2.38 seconds) of losartan, the latency to locate the target quadrant was decreased compared to scopolamine group, the difference being significant at p<0.05 (Fig. 5).

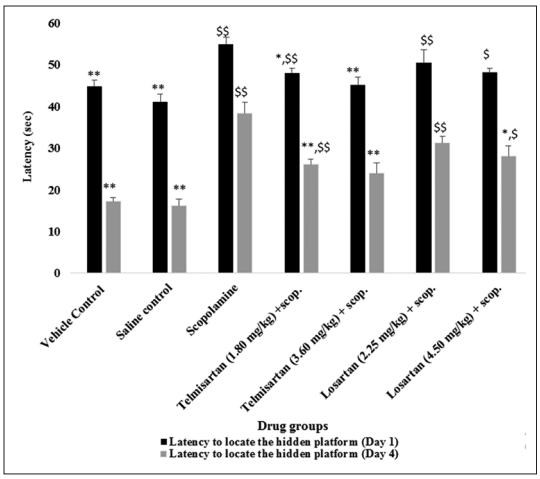

Fig. 1: Effect of angiotensin receptor blockers in scopolamine-induced amnesic rats during the acquisition trials of Morris water maze test. Values are expressed as mean±standard error; *vs. scopolamine (p<0.05); ** vs. scopolamine (p<0.001); $vs. control (p<0.05); $$vs. control (p<0.001)

Total time spent in target quadrant

Analysis of the swimming performance during the probe trial revealed that animals which received scopolamine spent significantly lesser time (12.58±0.42 seconds) in the target quadrant compared to vehicle control (26.11±0.54 seconds) and saline control (29.20±1.66 seconds) rats. Among the ARB-treated groups, the total time spent in Q4 for

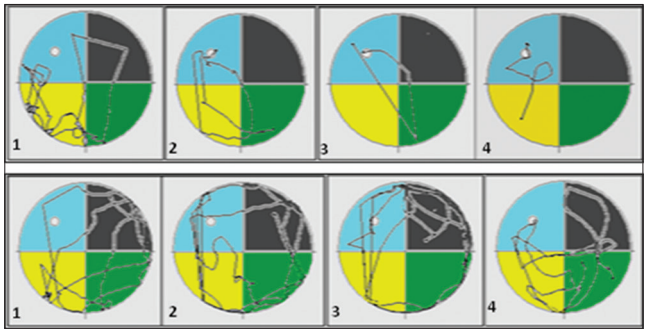

Fig. 2: Video tracking of morris water maze acquisitions trials in control and scopolamine group on 4 days; 1: Acquisition trials on day 1; 2: Acquisition trials on day 2; 3: Acquisition trials on day 3; 4: Acquisition trials on day 4

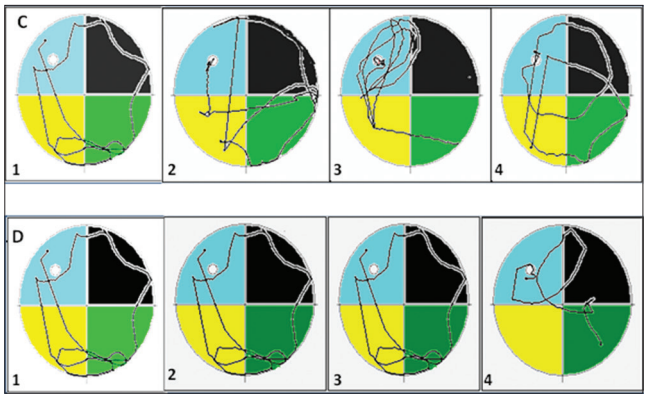

Fig. 3: Video tracking of morris water maze trials on 4 days in scopolamine-induced amnesic rats treated with: (c) Low dose of telmisartan; (d) high dose of telmisartan; 1: Acquisition trials on day 1; 2: Acquisition trials on day 2; 3: Acquisition trials on day 3; 4: Acquisition trials on day 4

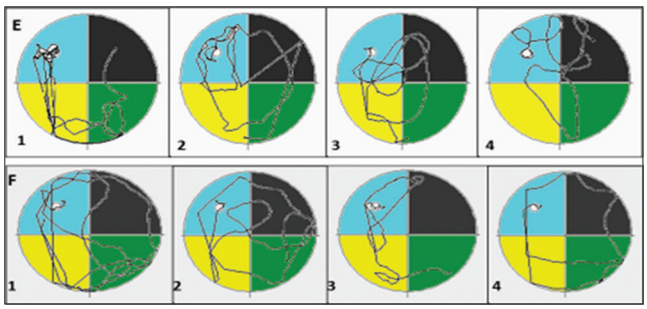

Fig. 4: Video tracking of morris water maze trials on 4 days in scopolamine-induced amnesic rats treated with: (e) low dose of losartan; (f) high dose of losartan; 1: Acquisition trials on day 1; 2: Acquisition trials on day 2; 3: Acquisition trials on day 3; 4: Acquisition trials on day 4

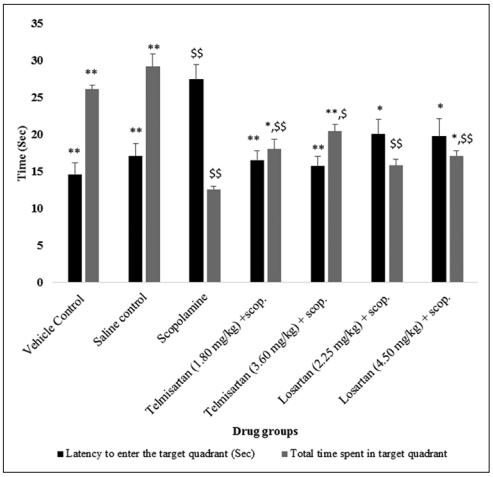

Fig. 5: Effect of angiotensin receptor blockers in scopolamine-induced amnesic rats during the probe trial of morris water maze test. Values are expressed as mean±standard error; *vs. scopolamine (p<0.05); **vs. scopolamine (p<0.001); $vs. control (p<0.05); $$vs. control (p<0.001)

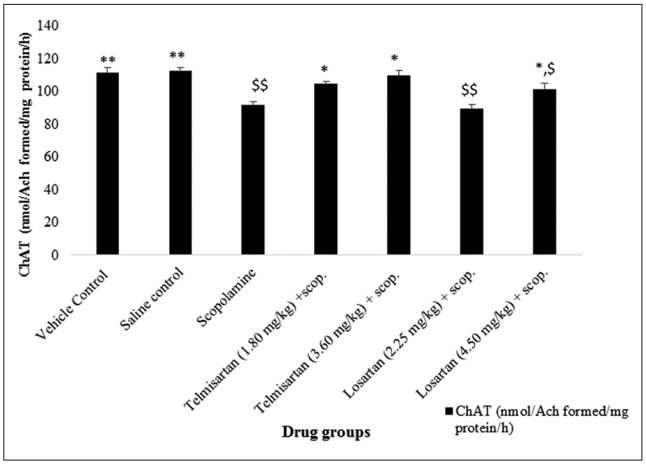

Fig. 6: Effect of angiotensin converting enzyme inhibitors and angiotensin receptor blockers on the choline acetyltransferase enzyme level within the hippocampus of scopolamine-induced amnesic rats. Values are expressed as mean±standard error; *vs. scopolamine (p<0.05); **vs. scopolamine (p<0.001); $vs. control (p<0.05); $$vs. control (p<0.001)

telmisartan 1.80 mg/kg and 3.60 mg/kg were 18.02±1.32 seconds and 20.43±0.91 seconds, respectively, while that for losartan 4.50 mg/kg was 17.13±0.63 seconds the difference significantly increased in comparison with scopolamine group (Fig. 5). However, only rats pre-treated with a higher dose of telmisartan demonstrated comparable efficacy with the control group.

Effect of ARBs on hippocampal ChAT activity

The cholinergic deficit in AD can be attributed to either neuronal death, increased AChE activity or decreased ChAT synthesis. To examine whether ARBs can alter ChAT activity, we studied their effects on scopolamine-induced amnesic rats. Among ARB-treated groups, telmisartan 1.80 mg/kg and 3.60 mg/kg showed ChAT activity of 104.66±1.32 and 109.61±3.63 nmol ACh formed/mg protein/h, respectively, which was significantly higher than scopolamine group (*p<0.05). However, only higher dose of telmisartan increased the ChAT activity to a level comparable with the control group. At lower dose, although losartan increased the ChAT level higher than scopolamine, the difference was not statistically significant. At higher doses, losartan (4.50 mg/kg) showed an increase (101.15±3.68 nmol ACh formed/mg protein/h) in ChAT activity that was significantly higher than scopolamine group (*p<0.05) (Fig. 6).

Analysis of hippocampal morphology and neuronal cell count

Analysis of the H&E stained sections of the hippocampus revealed notable changes in the hippocampus of scopolamine treated rats compared to control group. Cells with lightly stained nucleus, healthy cell membrane, and clear cytoplasm were considered as normal neurons while flame-shaped cells with pyknosed cell bodies (karyopyknosis), homogenous cytoplasm and intense basophilic appearance were considered as damaged cells.

In the control group, hippocampal neurons in all the three sub-regions *viz*., CA1, CA3 and DG of the hippocampus exhibited a regular arrangement with distinct edges and clear nucleus and cytoplasm. In contrast, the scopolamine treated rats demonstrated an unhealthy cellular architecture with irregularly arranged cells and evidence of karyopyknosis as shown in Figs. 7-9.

When the effect of ARBs on hippocampal morphology was analyzed, telmisartan-treated groups were found to prevent the hippocampal damage that was seen in scopolamine treated amnesic rats as evident in Figs. 7-9. While losartan in higher doses prevented the scopolamine-induced hippocampal damage, the effect was less marked compared to telmisartan (Figs. 7-9).

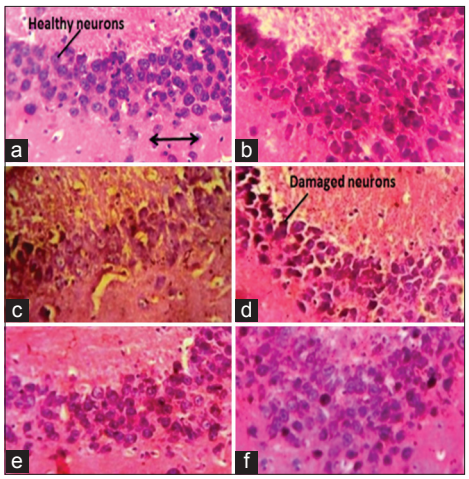

Fig. 7: Representative photomicrographs of CA3 region of hippocampus of rats treated with angiotensin receptor blockers. (a) control (b) scopolamine (c) telmisartan (1.80 mg/kg) + scopolamine (d) losartan (2.25 mg/kg) + scopolamine (e) telmisartan (3.60 mg/kg) + scopolamine (f) losartan (4.50 mg/kg) + scopolamine scale bar = 1 µ

Quantification of healthy neurons in the hippocampal CA3, CA1 and DG regions revealed a significant decrease in the mean number of healthy neurons in the scopolamine group compared to that of the control group (Table 1). The mean number of healthy surviving neurons in the hippocampal CA3, CA1, and DG of control group was found to be 35.00±1.29, 29.50±1.55 and 35.75±1.10, respectively. This was reduced to 13.30±1.37, 11.0±1.68 and 17.5±3.12 for CA3, CA1 and DG regions, respectively. A statistical analysis by ANOVA test revealed a significant difference in the mean values of control group compared to scopolamine group (**p<0.001) as shown in Table 1.

Among ARBs, telmisartan 1.80 mg/kg showed a significantly higher number of neurons in the CA3 (**p<0.001), CA1 (**p<0.001) and DG (*p<0.05) compared to scopolamine. However, the effects were not comparable to control group. At higher dose of 3.60 mg/kg, telmisartan produced marked increase in the form of healthier neurons in all the three subregions compared to scopolamine group (**p<0.001) and the numbers were comparable to control rats. While both lower and higher

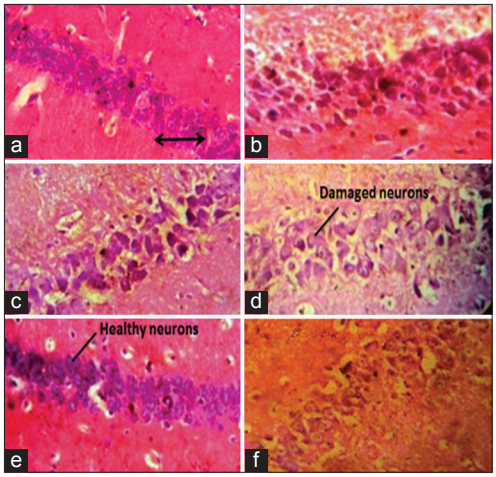

Fig. 8: Representative photomicrographs of CA1 region of hippocampus of rats treated with angiotensin receptor blockers. (a) control (b) scopolamine (c) telmisartan (1.80 mg/kg) + scopolamine (d) losartan (2.25 mg/kg) + scopolamine (e) telmisartan (3.60 mg/kg) + scopolamine (f) losartan (4.50 mg/kg) + scopolamine scale bar = 1 µ

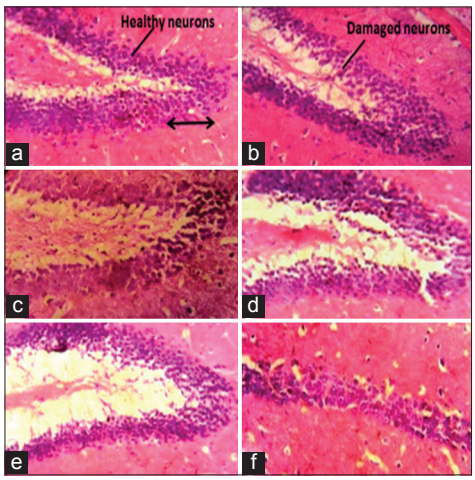

Fig. 9: Representative photomicrographs of dentate gyrus region of hippocampus of rats treated with angiotensin receptor blockers. (a) Control (b) scopolamine (c) telmisartan (1.80 mg/kg) + scopolamine (d) losartan (2.25 mg/kg) + scopolamine (e) telmisartan (3.60 mg/kg) + scopolamine (f) losartan (4.50 mg/kg) + scopolamine scale bar = 1 µ

doses of losartan increased the number of healthy neurons compared to scopolamine, the difference was more marked for rats, which were pre-treated with higher dose of losartan (**p<0.001, *p<0.05 and **p<0.001 for CA3, CA1 and DG, respectively) than rats which received lower dose (Table 1).

Table 1: Number of healthy neurons at hippocampal CA3, CA1 and dentate gyrus regions of scopolamine-treated amnesic rats following treatment with ARBs

Groups (n=6)	CA3	CA1	Dentate gyrus
Vehicle control	35.00±1.29**	29.50±1.55**	35.75±1.10**
Scopolamine	13.30±1.37$$	11.0±1.68$$	17.5±3.12$$
Telmisartan (1.80 mg/kg)+ scopolamine	28.25±1.25**,$	21.5±0.65$,**	25.5±0.64*
Telmisartan (3.60 mg/kg)+ scopolamine	29.25±0.75**	24.00±1.47**	33.00±2.16**
Losartan (2.25 mg/kg)+ scopolamine	22.00±1.08*,$$	19.00±1.41$$,*	18.75±1.65$
Losartan (4.50 mg/kg)+ scopolamine	28.25±1.20**,$	21.50±1.84$,*	30.50±0.95$,**

Comparison of the number of healthy neurons in CA3, CA1 and dentate gyrus regions of the hippocampus of scopolamine-induced amnesic rats. Values are expressed as mean±SE; *vs. scopolamine (p<0.05); **vs. scopolamine (p<0.001); $vs. control (p<0.05); $$vs. control (p<0.001), SE: Standard error, ARBs: Angiotensin receptor blockers

DISCUSSION

Critical analysis of the results of the present study showed that exposure to scopolamine impaired the learning and memory processing activities in experimental rodents, which could be reversed by administration of ARBs. In the present study, scopolamine was used as the amnesic agent. Scopolamine is a muscarinic receptor antagonist and is known to induce amnesia in rodents [22] and young healthy subjects [23]. Scopolamine induces amnesia by virtue of its muscarinic receptor antagonistic properties which disrupts the integrity of the cholinergic system essential to learning and memory [24].

The MWM test employed in the current study is one of the well-established models for evaluating hippocampal-dependent memory deficits in experimental animals and has often been used for the evaluation of anti-amnesic agents. A significant decrease in the escape latency in control rats during the acquisition trials denoted normal learning behavior, and an increase in total time spent in target quadrant during probe trial indicated retrieval of memory. These results are consistent with reports from other studies [25,26].

Administration of scopolamine produced a significant impairment of acquisition and retrieval of memory in the MWM test. Scopolamine interferes with the navigation test in MWM by probably blocking the cholinergic signals to hippocampus and neocortex [27,28] which are essential for mapping solution of the task. Further, scopolamine blocks NMDA receptors located at the hippocampus and prefrontal cortex [29], which is also believed to affect spatial memory.

In the current study, impaired learning and retention with scopolamine were associated with a decrease in the hippocampal ChAT activity and alterations in hippocampal morphology at CA1, CA3, and DG subregions. The findings of this study are in line with earlier reports suggesting that exposure to scopolamine causes severe deficits in cholinergic system reactivity indicated by reduced ACh levels and decreased ChAT activity [30,31]. The hippocampal damage could be due to the dysregulated hippocampal ACh neurotransmission caused by administration of scopolamine. Endogenous ACh released from cholinergic terminals has been known to modify long-term potentiation (LTP) and synaptic plasticity [32], the major cellular mechanisms that underlie learning and memory. In addition, scopolamine has been shown to disrupt the hippocampal activity during spatial learning and memory by causing dissociation between hippocampal-based and striatal-based memory systems [33]. The hippocampus plays a crucial role in learning and memory, and damage to the hippocampus and its related systems thus has been shown to produce amnesia [34], as also observed in the current study.

In the present study, pre-treatment with a higher dose of telmisartan and to a lesser extent with losartan has been shown to significantly improve memory deficits in scopolamine-induced amnesic rats. The improvement in cognitive function observed with ARBs in MWM test could be due to blockade of AT_1 receptors which mediate the actions of Ang-II. Ang-II interferes with ACh release, and reducing

its synthesis removes an inhibitory influence upon Ang-II, thereby improving memory in scopolamine-treated rats [35,36]. The memory enhancement could also be due to conversion of Ang II to Ang IV, the latter being responsible for cognitive facilitation [37]. It has shown that Ang IV by activation of hippocampal AT_4 receptors can overcome the disruption of spatial memory accompanying treatment with the muscarinic receptor antagonist scopolamine [13,38], thus suggesting a role of angiotensin receptor subtypes in influencing cognition.

In the current study, pre-treatment with higher doses of ARBs produced a significant increase in ChAT activity compared to scopolamine treated rats. ChAT is an enzyme involved in the synthesis of ACh, and our results suggest that molecules that restore the synaptic ACh levels could have potential therapeutic value in dementia. The beneficial effect of ARBs on ChAT activity is not clearly understood, but it could be attributed to their AT_1 receptor blockade action, thus inhibiting the action of brain Ang-II. Micossi *et al*., (1992) has shown that Ang-II can cause a 25-35% reduction in activities of ChAT within the rat hippocampus, which is in line with our study [39].

Analysis of the gross morphology of CA1, CA3, and DG regions showed that administration of higher dose of ARBs could prevent the scopolamine-induced structural abnormalities and preserve the neuronal cells in the hippocampal subregions. This strongly suggests that treatment with RAS blockers maintain cognitive function in rats probably through protection of the vascular vessels and neuronal cells responsible for memory function. A possible mechanism could be due to removal of the inhibitory influence of Ang-II on ACh release thus preserving the cholinergic function. Further, the hippocampus receives intensive angiotensinergic synaptic inputs which can either promote or restrict the expression of long-term plasticity [40]. Ang-II suppresses LTP and treatment with telmisartan or losartan may facilitate synaptic plasticity by decreasing the Ang-II mediated effects [41,42].

In our study, comparison between the two ARBs showed that telmisartan at a higher dose exerts better cognitive enhancing efficacy compared to losartan. In a similar study in AD model, Mogi *et al*. compared the effects of telmisartan and losartan and found that telmisartan significantly increased avoidance scores in shuttle avoidance test compared to losartan, and it significantly decreased Aβ deposition that was not observed with losartan [43,44]. This difference could be attributed to variation in chemical structure and pharmacokinetic properties between the two drugs. Telmisartan differs from losartan in possessing the carboxyl group instead of imidazole group that plays a role in receptor binding [45]. Telmisartan also lacks a carboxylic acid near the imidazole nitrogen that further contributes to its receptor binding. Telmisartan binds with the strongest affinity to AT_1 receptor with a dissociation time of 213 min while that for losartan is only 67 minutes [46,47]. Further lipophilicity which governs the degree of passage across the blood brain barrier is highest for telmisartan and least with losartan [48,49]. Thus, despite belonging to the same drug class, ARBs differ in certain aspects of their chemical structure which account for their important differences in pharmacokinetic and pharmacodynamic characteristics.

In addition, telmisartan is highly selective for AT_1 receptor compared to losartan. Greater selectivity for AT_1 receptor implies that the AT_2 receptor may be exposed to a higher concentration of Ang II because of the renin–angiotensin feedback loop following ARB administration [50]. AT_2 receptor is suggested to be an important regulator of brain functions including cognition, behavior and locomotion, besides modulating neuronal excitability, and neuronal migration [51,52].

Overall, these factors taken together may help explain why telmisartan-treated rats demonstrated better memory enhancing and cholinergic activity in the present study compared to losartan-treated rats. Decreased efficacy with losartan could be due to its poor lipophilicity or decreased selectivity for the AT_1 receptor, but it also suggests that losartan probably may display better memory enhancement when given at higher doses.

CONCLUSION

It may be concluded that pre-treatment with telmisartan and losartan exerted beneficial effects on scopolamine-induced memory deficits by virtue of their angiotensin receptor blockade action and enhancement of cholinergic activity. It could also be due to increased formation of Ang-IV which however needs further evaluation to support the use of these drugs in dementia.

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