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Antiamnesic and Neuroprotective Effects of Low Dose of Ramipril and Losartan in Scopolamine-Induced Amnesia Model of Dementia

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ABSTRACT

To investigate the potential role of ramipril and losartan in preventing scopolamine-induced memory impairment in rats. Memory impairment was induced in male Wistar rats by administration of scopolamine (1 mg/kg intraperitoneally). Rats received oral doses of ramipril (0.225 mg/kg) and losartan (2.25 mg/kg) for a period of four weeks. Passive avoidance paradigm was used to assess learning and memory. Biochemical estimations of malondialdehyde, glutathione transferase, protein thiols, and histological analysis of hippocampal morphology were performed to estimate the degree of oxidative stress and neuronal degeneration. Pre-treatment with ramipril and losartan reversed scopolamine-induced memory impairment, oxidative stress and hippocampal degeneration which were evident by the findings from behavioral, biochemical and histological studies.

Keywords: memory, hippocampus, oxidative stress, scopolamine, ramipril, losartan

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INTRODUCTION

Dementia is a neurodegenerative disorder associated with cognitive and behavioralimpairments[1]. Alzheimer's disease (AD) is the most common form of dementia in elderly, followed by vascular dementia and dementia with Lewy bodies[2]. The disease has a multifactorial pathology characterised by aggregation of amyloid proteins[3], cholinergic deficits, oxidative stress, neuroinflammation[4, 5] and abnormalities of hippocampal structure and function[6]. Drugs currently available for the treatment of AD such as anticholinesterases do not prevent or reverse the disease[1]. Therefore development of drugs for prevention of dementia is a critical need for the hour.

Over the recent years, the brain renin angiotensin system (RAS) has been shown to be involved in learning and memory consolidation[7]. Continuous activation of the brain RAS impairs cognitive functions through stimulation of AT_1receptor[8]. RAS is also believed to play a pathophysiological role in metabolic syndrome, contributing to enhanced levels of oxidative stress and inflammation[9].Therefore it is believed that drugs that act by inhibiting RAS such as angiotensin converting enzyme (ACE) inhibitors and angiotensin receptor blockers (ARBs) could have a potential role in the prevention of dementia.

The present study has been undertaken to investigate the effects of RAS inhibitors in memory-deficit of rats using scopolamine-induced amnesia model.

MATERIALS AND METHODS

Animals

In-house bred male Wistar rats weighing 200-250 grams were used in the study. All the animals were housed in polypropylene cage with four animals in each cage to prevent overcrowding. Animals were provided with standard laboratory feed (Gold Mohur; Lipton India Ltd) and water *ad libitum*. The experimental protocol was approved by the Institutional Animal Ethical Committee and experiments were conducted according to the CPCSEA guidelines on the use and care of experimental animals.

Drugs and reagents

An ACE inhibitor, ramipril and an ARB, losartan were used in the study. These drugs were received as gift samples from Zydus Health Care Ltd., Ahmadabad, India. Scopolamine was procured from Sigma Aldrich, Mumbai, India. The other chemical reagents used in the study were obtained from Merck Chemicals, Bangalore, India.

Experimental design

Four groups, each consisting of 6 animals, were used in the study.

Group I (Vehicle control): Rats received 2% gum acacia (1mg/kg) dissolved in distilled water, orally for a period of four weeks.

Group II (Scopolamine-alone): Rats were administered a single dose of scopolamine (1mg/kg i.p)[11] prior to the learning experiment.

Group III (Ramipril +scopolamine): Ramipril (0.225 mg/kg) was dissolved in 2% gum acacia and administered to rats orally for a period of four weeks followed by a single dose of scopolamine (1mg/kg i.p) prior to the learning experiment.

Group IV(Losartan +scopolamine): Losartan (2.25 mg/kg) was dissolved in 2% gum acacia and administered to rats orally for a period of four weeks followed by a single dose of scopolamine (1mg/kg i.p) prior to the learning experiment.

The doses of ramipril and losartan were derived by converting the human equivalent therapeutic dose based on the formula described by Paget and Barnes, 1964 [12].

Passive avoidance paradigm

Passive avoidance test is an exteroceptive behavioural model for testing learning and memory in experimental rodents. The apparatus consisted of a box (27 cm x 27 cm x 27 cm) with three walls of wood and one wall of Plexiglas, featuring a grid floor (made up of 3 mm stainless-steel rods set 8 mm apart) with a wooden platform (10 cm x 7 cm x 1.7 cm) in the centre of the grid floor. The box consists of a dark compartment and a light compartment separated by a sliding door. During the experimental procedure, the box was illuminated with a 15 W bulb. The rats were trained as follows: each rat was kept in the centre of the larger compartment facing away from the entrance to the dark compartment. The door between the two compartments was kept open. The rat was allowed to explore the apparatus (both larger and smaller compartments) for 3 minutes. In each trial, the total time taken by the animal to enter the dark compartment was noted using a stop-watch. At the end of the trial, the rat was replaced in the home cage, where it remained during an inter-trial interval of 5 minutes. After the last exploration trial, the rat was again kept in the light compartment. When the animal entered the dark compartment, the sliding door between the two compartments was closed and three strong foot shocks (50 Hz, 1.5 mA, and 1 s duration) were given at 5-second intervals. The ceiling was then opened and the rat returned to its home cage. The retention test was carried out after 24 hours of receiving the aversive stimuli. The latency time required for the animal to enter the dark compartment was recorded. The latency time was recorded as 3 minutes for those animals that did not enter the dark compartment within 3 minutes. The total time spent by the rats in each of the dark and light compartments during the memory retention trial was recorded. Absence of entry into the dark compartment and more time spent in the light compartment indicated positive memory retention[12, 13].

Biochemical estimations

Collection of tissue samples: The animals were sacrificed by cervical dislocation and brain tissues were carefully removed and chilled in ice-cold phosphate buffer. After washing in ice-cold buffer, the hippocampus was dissected out and homogenized in phosphate buffer (pH 7.4, 10% w/v) using Teflon homogenizer. The tissue homogenate was then utilized for

malondialdehyde (MDA) assay, glutathione-S- transferase (GST) assay and protein thiol estimation.

Malondialdehyde assay

Level of MDA was analyzed in the rat brain by the method of Okhawa *et al*with slight modifications[14].Change in absorbance was read spectrophotometrically at 532 nm.

Glutathione-S-transferase assay

GST assay was analyzed in the rat brain by the method of Beutler *et al*[15]. Change in absorbance was read spectrophotometrically at 340 nm.

Protein thiol assay

Protein thiol estimation was done using the method of Ellman *et al.*with slight modifications [16, 17]. Change in absorbance was read spectrophotometrically at 412 nm.

Histological examination

The animals were sacrificed with an overdose of anesthesia at the end of the experimental procedure. The animals were perfused with 250 ml 4% paraformaldehyde, followed by 0.01 mol/ L phosphate buffered saline (PBS). Brain part with hippocampus was cut and dehydrated in ascending grades of alcohol, defatted in xylene, and embedded in paraffin. Tissue is cleared in xylene for 1-2 hours. Five micron thick paraffin sections are obtained and mounted on clean glass slides and labeled. The sections were stained with hematoxylin and eosin (H&E) according to the standard procedure. The hippocampal CA_3 region was then observed for any morphological changes under a light microscope (Magnus, Olympus (India) Pvt. Ltd. New Delhi).

Statistical analysis

Values in the study are expressed as mean ± SEM. The data was analyzed using one-way ANOVA followed by post-hoc test using SPSS vs 16. The level of significance was set at $p<0.05$.

RESULTS

Effect of drug treatment on behavioural model

The effect of drug treatment was analyzed at the end of four weeks. Scopolamine (1mg/kg i.p.) significantly decreased step-through latency compared to control group ($p<0.001$). During the retention trial, scopolamine-treated rats spent an average of approximately 158 seconds in the dark compartment, while in control group, this decreased to 75 seconds, indicating induction of amnesia in the scopolamine group. Ramipril-treated rats and losartan-treated rats showed a significant increase in step-through latency compared to scopolamine group ($p<0.001$), indicating reversal of amnesia. The total time

spent by ramipril and losartan treated rats in the dark compartment were approximately 88 seconds and 110 seconds respectively, compared to 158 seconds in the scopolamine group [Table I].

Table I: Effect of drug treatment on passive avoidance test parameters

Groups	Latency to enter dark compartment (in seconds) on retention day	Total time spent in dark compartment (in seconds) on retention day
Group I (Normal control)	88.00 ± 2.85†	75 ± 6.45 †
Group II (Scopolamine)	7.16 ± 1.53**	158 ± 4.18**
Group III (Ramipril + scopolamine)	82.50 ± 3.88 †	88 ± 5.76†
Group IV (Losartan + scopolamine)	75.16 ± 3.73*†	110 ± 4. 86*†

† vs. scopolamine ($p<0.05$); * vs. control ($p<0.05$).
In the passive avoidance paradigm, scopolamine produced amnesia as evident by the decrease in latency to enter the dark compartment, and the values were statistically significant compared to control, ramipril and losartan-treated groups ($p<0.001$). Total time spent in dark compartment during the behavioral test also showed a significant decrease in control, ramipril and losartan-treated groups compared to scopolamine group ($p<0.001$), indicating reversal of amnesia.

Effect of drug treatment on biochemical parameters

Effect on MDA levels

Treatment with scopolamine significantly increased the levels of MDA in the rat brain compared to control group ($p<0.001$) reflecting enhanced oxidative stress. Pre-treatment with ramipril and losartan significantly reduced the scopolamine-induced rise in MDA levels ($p<0.05$) [Table II].

Effect on protein thiol levels

Scopolamine significantly decreased the brain protein thiol levels ($p<0.001$) compared to control group. Pre-treatment with ramipril and losartan significantly attenuated the scopolamine-induced decrease in protein thiol levels ($p<0.05$) [Table II].

Effect on glutathione transferase levels

Scopolamine significantly decreased the brain protein thiol levels ($p<0.001$) compared to control group. Pre-treatment with ramipril and losartan significantly attenuated the scopolamine-induced decrease in protein thiol levels ($p<0.05$)[Table II].

Effect of drug treatment on hippocampal morphology

Control rats which showed a row of normal hippocampal nerve cells (Fig.1) while degeneration of hippocampal nerve cells in CA3 region was observed in scopolamine-induced amnesic rats as evident by the darkly-stained, shrunken nerve cells (Fig.2), in

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contrast to. Pre-treatment with ramipril and losartan attenuated the scopolamine-induced degeneration of hippocampal nerve cells, indicating reversal of amnesia (Fig. 3 and Fig. 4)

Table II: Effect of drug treatment on antioxidant parameters

Groups	MDA assay (nm /l / g of tissue)	GST assay (µmol /l / g of tissue)	Protein thiol assay (µmol /l / g of tissue)
Group I (Normal control)	0.126 ± 4.92 †	12.7 ± 0.61 †	144.57 ± 3.25†
Group II (Scopolamine)	0.315 ± 3.71 *	3.26 ± 0.35*	61.43 ± 2.57*
Group III (Ramipril + scopolamine)	0.225 ± 6.37 †*	5.85 ± 0.16 †*	78.73 ± 2.51†*
Group IV (Losartan + scopolamine)	0.212 ± 6.54 †*	4.71 ± 0.17 †*	82.37 ± 2.37†*

Table II: † vs. scopolamine ($p<0.05$); * vs. control ($p<0.05$).
Scopolamine treated rats showed an increase in MDA level and a decrease in GST and protein thiol levels indicating oxidative stress, and the values were significantly different compared to control, ramipril and losartan-treated groups ($p<0.05$).

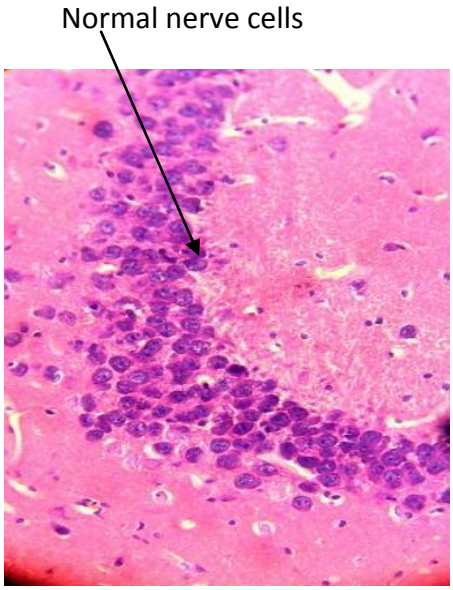

Fig. 1

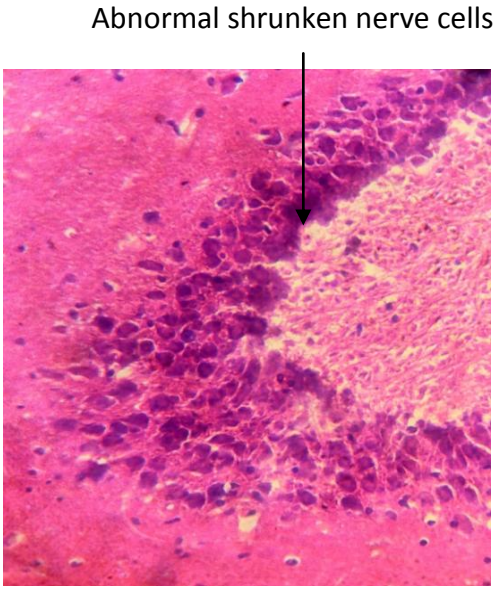

Fig. 2

Fig.1 and Fig. 2. Representative photomicrograph (40X) of sections of hippocampal CA3 region of the brain from control rats and scopolamine treated rats stained with hematoxylin and eosin. In control animals, a row of normal hippocampal nerve cells was seen in CA3 region while in scopolamine induced amnesic rats, dark, deeply stained, shrunken nerve cells were seen.

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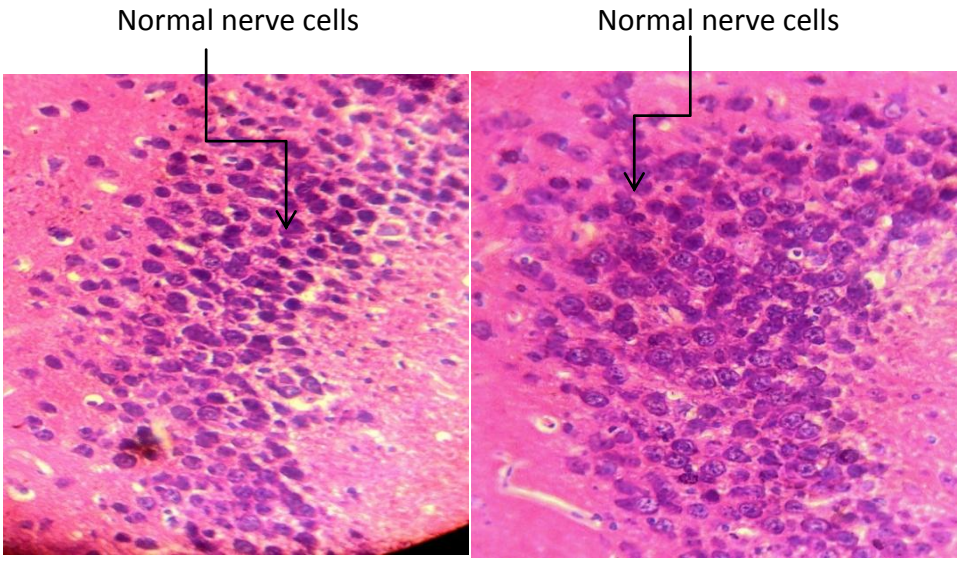

Fig. 3 **Fig. 4**

Fig. 3 and **Fig. 4**. Show representative photomicrograph (40X) of sections of hippocampal CA3 region from losartan-treated rats and ramipril-treated rats respectively, following administration of scopolamine. Treatment with ramipril and losartan could decrease the hippocampal atrophy that was seen following administration of scopolamine.

DISCUSSION

ACE inhibitors and ARBs are best known for their role in cardiovascular diseases [18]. However several components of RAS, namely angiotensinogen, ACE, angiotensin II and their receptors, are also found localized in the central nervous system[19]. RAS is involved in hippocampal and cortical atrophy, oxidative stress, neuronal inflammation,and in inhibiting acetylcholine release, all of which are involved in the pathophysiology of dementia[20-22].The significance of these properties is further emphasised by the likely links between hypertension and dementia which suggests that elevated midlife blood pressure is related to the development of dementia [18, 23].Hypertension-related pathological changes in neurofibrillary tangle numbers and atrophy in the regions of brain are also relevant to AD[23]. This raises a possibility that optimal antihypertensive therapy with RAS inhibitors may prove beneficial in the prevention and treatment of dementia.

In our study, the scopolamine-induced amnesia model has been used as an experimental model to study the memory deficits observed in AD. AD is associated with degeneration and dysfunction of cholinergic neurons[24]. Since scopolamine-induced amnesia is due to blockade of the cholinergic neurotransmission, it provides a rationale for the use of this drug to model the cognitive deficits that are observed in aging and dementia[25, 26].In the present study, administration of scopolamine produced impairment of acquisition and memory as reflected by the passive avoidance behaviour of rats, and this could be reversed with ramipril and losartan. Loss of neuronal cells in the CA3 region of the hippocampus which is seen in ADwas noted following scopolamine administration and it was improved in rats treated with ramipril and losartan.

Brain oxidative stress also plays a critical role in the pathophysiology of dementia [5]. MDA is a biomarker for oxidative stress and increased MDA level results in an increased generation of free radicals which can damage biomolecules. This study showed that MDA

level increased significantly in brains of scopolamine-treated rats and the rise in MDA level was controlled following pre-treatment with RAS inhibitors. Antioxidant enzymes show reduced activities in the affected brain region of patients of Alzheimer's disease[27]. Scopolamine treatment produced a significant fall in GST and protein thiol levels, which was attenuated following administration of ramipril and losartan. The effects of scopolamine on brain antioxidant levels is in line with previous studies, which demonstrated that administration of scopolamine was associated with an alteration in the brain antioxidant status [27, 28].

The change in behaviour of animals in scopolamine-induced amnesia model correlated with the histological changes in hippocampal morphology and decrease in brain antioxidant levels though other putative mechanisms may be involved. The findings of the present study suggest a possibility that RAS inhibitors could be beneficial in preventing cognitive deficits seen in AD and other forms of dementia. However, further studies with higher doses of the drug and analysis of other biochemical and enzymatic markers would be required before any conclusion can be drawn regarding the beneficial effects of ACE inhibitors and ARBs in dementia.

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