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COGNITIVE ENHANCING AND ANTIOXIDANT EFFECTS OF TELMISARTAN IN SCOPOLAMINE-INDUCED AMNESIC RATS

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ABSTRACT

Brain Renin Angiotensin System (RAS) is involved in the pathogenesis of Alzheimer's disease (AD). Treatment with a RAS blocker thus may have beneficial effects in preventing the cognitive deficits of AD. The present study was designed to investigate the cognitiveenhancing activity ofa RAS blocker, telmisartan, in scopolamine-induced amnesic rats using the passive avoidance test. The study also analysed the antioxidant potential and acetylcholinesterase activity of telmisartan in mitigating the oxidative stress induced by scopolamine. Administration of scopolamine induced significant impairment of memory as indicated by a marked decrease in the step-through latency in the passive avoidance test which was reversed by pre-treatment with 3.60 mg/kg of telmisartan Moreover, treatment with 3.60 mg/kg telmisartanin scopolamine-induced amnesic rats significantly decreased malondialdehyde level which was accompanied by an increase in the activities or contents ofglutathione transferase, SOD and protein thiols.Further, the activity of acetylcholinesterase was significantly inhibited by telmisartan to a level similar to that observed in control rats.These data demonstrate that telmisartanhas potent cognitive-enhancing activity which may be attributed to its antioxidant properties or acetylcholinesterase inhibiting activity though other putative mechanisms need to be investigated.

KEYWORDS: Angiotensin, angiotensin receptor blocker, scopolamine, telmisartan

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1. INTRODUCTION

Alzheimer's disease (AD) is a progressive neurodegenerative disorder associated with cognitive and behavioural impairments. [1] It is characterized by the deposition of amyloid-β (Aβ) deposition, development of neurofibrillary tangles, inflammation, reduced acetylcholine (ACh) and abnormalities of glutaminergic and dopaminergic neurotransmission[2-5]. Oxidative stress induced by generation of free radicals also contributes significantly to the pathogenesis of AD [6]. RAS is one of the prime regulators in controlling blood pressure, and contributes significantly to development of cardiovascular and renal diseases. Pharmacological blockade of RAS thus is an effective strategy in controlling hypertension and preventing the progression of cardiovascular diseases [8]. Numerous studies have shown that brain RAS is involved in mediating cognitive functions including learning and memory consolidation, and processing of emotional responses, suggesting the existence of a localized RAS in the brain [9, 10]. Components of brain RAS like Angiotensin (Ang) II has been found to inhibit ACh release, mediate TNF-α and TGF-β signalling, and induce oxidative stress, all of which significantly contributes to the progression of AD [11, 12]. Antihypertensive medications such as RAS inhibitors act, either by inhibiting ACE to block the conversion of Ang I to Ang II, or by blocking the binding of Ang II to AT_1 or AT_2receptor subtypes. Treatment of hypertension with ACE inhibitors and ARBs lowers morbidity and mortality, and has been shown to improve cognitive function in such patients[13, 14]. The present study has thus been designed to investigate if treatment with telmisartan, an angiotensin receptor blocker, could mitigate the memory deficit induced by scopolamine in rats using passive avoidance test. In our study, telmisartan was chosen among other ARBs because telmisartan is highly lipophilic and it has been shown to have the strongest evidence for penetration into the brain compared to other ARBs [15]. The present study also evaluated the effects of telmisartan on the levels of malondialdehyde (MDA), and the activities of glutathione transferase (GST), protein thiol and superoxide dismutase within the brain were evaluated. Further, the effect of telmisartan on brain acetylcholinesterase (AChE) inhibitory activity was also analysed.

2.MATERIALS AND METHODS

a. Animals

Male Wistar rats weighing 200-250 grams were employed in the present 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 h 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 according to the CPCSEA guidelines on the use and care of experimental animals.

b. Drugs and reagents

Telmisartan in powdered form was obtained as generous gift samples from Zydus Health Care Ltd., Ahmedabad, India. Scopolamine hydrobromide was procured from Sigma Aldrich, Mumbai. The Superoxide Dismutase (SOD) assay kit was procured from Prolab Marketing Pvt. Limited, New Delhi, India. The other chemical reagents used in the study were obtained from Merck Chemicals, Bangalore, India. Rats equivalent doses in mg/kg body weight of clinical doses were calculated as mg/kg body weight as described by Paget and Barnes (1964) [16]. All the drugs except scopolamine were dissolved in 2% gum acacia while scopolamine was dissolved in normal saline.

c. Experimental design

6 animals were randomized into five groups and treated as below.

Group I: 2 ml/kg of 2 % gum acacia (normal control) orally for four weeks

Group II: 1 ml/kg of 0.9% normal saline i.p. (saline control) as single dose prior to behavioural test

Group III: 2% gum acacia orally for four weeks followed by scopolamine

Group IV: Telmisartan 1.80 mg/kg dissolved in 2% gum acacia orally for four weeks followed by scopolamine

Group V: Telmisartan 3.60 mg/kg dissolved in 2% gum acacia orally for four weeks followed by scopolamine

Scopolamine 2 mg/kg [17] was administered intraperitoneally as a single dose,45 minutes before the behavioural tests for induction of amnesia.

d. Behavioural test

Step-through passive avoidance test

Passive avoidance test is an exteroceptive behavioural model for testing learning and memory in experimental rodents. The apparatus has a box (27 cm x 27 cm x 27 cm) of three wooden walls and one Plexiglas wall, with a grid floor (made up of 3 mm stainless-steel rods set 8 mm apart), and a platform (10 cm x 7 cm x 1.7 cm) at the centre of the grid floor. The box was kept illuminated with a 15 W bulb during the experiment. Each rat was kept in the larger compartment facing away from the entrance to the dark compartment. Three exploratory trials were given to each rat in which the rat explored the apparatus for 3 minutes. The inter-trial interval was 5 minutes. The rat was removed from the cage during the inter-trial period. In each trial, the total time taken by the animal to enter the dark compartment was noted using a stop-watch. A decrease in the latency to enter the dark compartment was considered as an index of improved learning. After the third exploratory trial, the rat was kept in the light compartment and when it entered the dark compartment, the sliding door was closed and three foot shocks (50 Hz, 1.5 mA, and 1s duration) were delivered at 5-second intervals. The retention test was carried out after 24 hours of receiving the aversive stimuli. Rats received gum acacia or test compounds for 4 weeks; this was followed by scopolamine (2 mg/kg body weight, dissolved in normal saline) for induction of amnesia, 45 minutes before the acquisition trial. After 24 hours of acquisition trials, the rats were again placed in the light compartment. The latency time required for the animal to enter the dark compartment and the total time spent by the animal in the light compartment were recorded. The latency time was recorded as 3 minutes for those animals that did not enter the dark compartment within 3 minutes. Increase in the latency to enter the dark compartment and more time spent in the light compartment indicated positive memory retention[18].

e. Biochemical estimations

Collection of tissue samples

Following the behavioural tests, the rat was anesthetized, decapitated and the whole brain was rapidly dissected under standard conditions at 4 °C; the hippocampus was isolated and homogenized in0.02 M phosphate buffer (pH 7.4) at a concentration of 10% (w/v) using Teflon homogenizer. The tissue homogenate was centrifuged for 30 min at 3000 ×g at 4 °C and the supernatant was collected for assessing malondialdehyde (MDA), glutathione transferase (GST) and protein thiol activities. For SOD assay, the tissue was homogenized in HEPES buffer (pH 7.2) and centrifuged at 1500 x g for 5 min at 4 °C. The supernatant was then collected for assessing SOD enzyme activity.

2.5.1 Malondialdehyde assay

MDA, a measure of lipid peroxidation, was analyzed in the rat brain by the method of Okhawa *et al.* [19, 20]. Change in absorbance was read spectrophotometrically at 532 nm.

2.5.2 Glutathione-S-transferase and protein thiol estimations

GST activity was analyzed in the rat brain by the method of Beutler *et al.* [21] and change in absorbance was read spectrophotometrically at 340 nm. Protein thiol was measured using the method of Ellman *et al.* and the change in absorbance was read spectrophotometrically at 412 nm [22].

2.5.3 Superoxide dismutase assay

The SOD estimation was performed using Cayman's Superoxide Dismutase Assay Kit which utilizes a tetrazolium salt for determination of superoxide radicals generated by xanthine oxidase and hypoxanthine. One unit of SOD is defined as the amount of enzyme needed to exhibit 50% dismutation of the superoxide radical [23, 24].

2.5.4. Acetyl cholinesterase enzyme activity

The brain acetylcholinesterase activity was measured by the method of Ellman *et*

al.(1961).A volume of 3 ml of phosphate buffer (pH=8) was added to the test tubes labeled as sample blank and test to which 200 µl of supernatant was added and the mixture was vortexed. Subsequently DTNB was added to both the test tubes and incubated for 5 min at room temperature and absorbance was read at 412 nm using spectrophotometer and was set at zero absorbance when the enzyme activity had stopped increasing. Finally 20 µl of acetylthiocholine was added to the test sample, and 20 µl of phosphate buffer (pH=8) to sample blank. Mixture was vortexed and absorbance was read at 412 nm for 10 min at 37°C at 1 min interval. The enzyme activity was calculated based on the changes in absorbance/min [25].

f. Statistical analysis

Data obtained from the experiments were expressed as mean ± SE. Statistical differences between the treatment and the control groups were calculated by one-way analysis of variance (ANOVA) followed by Tukey's post hoc test. The data were considered to be statistically significant if the probability had a value of 0.05 or less.

3. RESULTS

a. Cognitive enhancing effect of telmisartan in scopolamine-induced amnesia using passive avoidance test

The efficacy of telmisartan in enhancing cognition after impairment of memory via scopolamine was evaluated through the passive avoidance test.The alterations of the step-through latencies (STL) as revealed in the passive avoidance test are shown in Table 1. During the exploratory trials, the latency to enter the dark compartment was decreased in all the groups from first to third trial. However, the scopolamine-treated animals took more time to enter the dark compartment during the three exploration trials. Pre-treatment with 1.80 mg and 3.60 mg/kg of telmisartan showed a significant decrease in step through latency during the exploratory trials compared to scopolamine group($^{**}p<0.05$ and $^{**}p<0.001$ for 1.80 and 3.60 mg/kg respectively). This is indicative of positive learning behaviour among telmisartan-treated rats. However when the effects of two doses were compared, only telmisartan 3.60 mg/kg showed a latency comparable to control group. During the memory retention test, the latency to enter the dark compartment was significantly reduced for scopolamine compared to normal control rats and rats treated with telmisartan alone ($^{**}p<0.001$). Rats which received scopolamine also spent lesser time in the light compartment compared to control group ($^{**}p<0.001$) as shown in Table 2. Pre-treatment with telmisartan at doses of 1.80 mg/kg and 3.60 mg/kg increased the entrance latency time of rats during the retention trial and the difference was statistically significant compared to scopolamine group ($^{**}p<0.05$ and $^{**}p<0.001$ respectively). Rats pre-treated with telmisartan also spent longer duration in the light compartment compared to scopolamine ($^{**}p<0.001$), indicating improved memory retention in the telmisartan treated groups.

Table 1

Effects of telmisartan on the exploratory behaviour of scopolamine-induced amnesic rats in passive avoidance test

Treatment	Exploration trial (Day 1)	Exploration trial (Day 2)	Exploration trial (Day 3)
Control	19.3 ± 0.95 **	17.16 ± 1.54 **	12.67 ± 1.31 **
Saline control	20.6 ± 1.28 **	16.00 ± 2.71 **	14.10 ± 1.92 **
Scopolamine	37.3 ± 2.55 $^{\$\$}$	33.5 ± 1.88 $^{\$\$}$	30.67 ± 1.85 $^{\$\$}$
Telmisartan 1.80 mg/kg + scop.	28.18 ± 2.66 $^{*,\$}$	26.41 ± 2.20 $^{*,\$}$	23.65 ± 1.92 $^{**,\$}$
Telmisartan 3.60 mg/kg + scop.	23.17 ± 2.81 **	19.86 ± 2.62 **	15.19 ± 2.10 **

Comparisons between control, scopolamine, and telmisartan during the exploration trials in passive avoidance test. Values are mean± SE; *vs. scopolamine (p<0.05); ** vs. scopolamine (p<0.001); $ vs. control (p<0.05); $$vs. control (p<0.001).

Table 2
Effects of telmisartan on memory retention behaviour of scopolamine-induced amnesic rats in passive avoidance test

Treatment	Latency to enter the dark compartment(sec) 24h after receiving foot shock	Total time spent in light compartment (sec) 24h after receiving foot shock
Control	51.83 ± 2.71 **	114.50 ± 3.87 **
Saline control	49.29 ± 2.21 **	109.78 ± 2.55 **
Scopolamine	14.83 ± 1.05 $$	57.33 ± 4.23 $$
Telmisartan 1.80 mg/kg + scop.	32.17 ± 3.92 *, $$	98.17 ± 3.53 **
Telmisartan 3.60 mg/kg + scop.	41.37±3.29 **	106.65 ± 2.83 **

Comparisons between control, scopolamine, and telmisartan during the retention trial in passive avoidance test. Values are mean± SE; *vs. scopolamine (p<0.05); ** vs. scopolamine (p<0.001); $ vs. control (p<0.05); $$vs. control (p<0.001).

b. Antioxidant effects of telmisartan on scopolamine-induced oxidative stress

To further elucidate the biochemical mechanism of anti-amnesic activity of telmisartan in brain tissue, we measured their antioxidant effects on lipid peroxidation, and on the activities of antioxidant enzymes. We therefore investigated the effects of ramipril on lipid peroxidation and antioxidant enzymes activity in scopolamine induced amnesia model. Administration of scopolamine significantly increased the level of MDA (Table 3), while reducing the activity of antioxidants like protein thiols, GST and SOD (Table 4) and the difference was significant compared to control rats. Treatment of amnesic rats with 1.80 mg/kg and 3.60 mg/kg telmisartan significantly reduced the brain MDA levels. The reduction in GST and protein thiol activities in hippocampus induced by scopolamine was also reversed by pre-treatment with telmisartan. Treatment with telmisartan could also significantly restore the activity of SOD in amnesic hippocampus and the activity in response to treatment with telmisartan was comparable to normal control rats. However the decrease in the level of MDA and increase in the activity of antioxidants were more marked and comparable to control group in rats that were pre-treated with 3.60 mg/kg of telmisartan

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Table 3
Effect of telmisartan on hippocampal MDA level in scopolamine-induced amnesic rats

Treatment	MDA (nmol/g tissue)
Control	13.76 ± 0.33**
Saline control	15.72 ±1.21**
Scopolamine	23.92 ±0.66 $$
Telmisartan 1.80 mg/kg + scop.	19.05 ± 0.34**, $$
Telmisartan 3.60 mg/kg + scop.	14.28 ±0.35**

Comparisons between control, scopolamine, and telmisartan on the brain MDA level. Values are mean± SE; *vs. scopolamine (p<0.05); ** vs. scopolamine (p<0.001); $ vs. control (p<0.05); $$vs. control (p<0.001).

Table 4
Effect of telmisartan on the hippocampal antioxidant levels in scopolamine- induced amnesic rats

Treatment	GST (nmol/mg protein)	Protein thiol (micromol/g tissue)	SOD (U/mg protein)
Control	177.13 ±8.50**	55.22 ± 0.56**	7.21±0.67 **
Saline control	171.09 ± 6.15**	51.79± 1.66**	8.17±1.34**
Scopolamine	119.92 ±5.13 $$	42.05±0.87 $$	3.91±0.36 $$
Telmisartan 1.80 mg/kg + scop.	159.43± 6.90 *	49.06±0.74**, $$	5.11±0.42 *, $
Telmisartan 3.60 mg/kg + scop.	173.82 ± 2.11**	52.79 ±0.59**	6.75±0.46 **

Comparisons between control, scopolamine, and telmisartan on the activity of GST, protein thiols and SOD in the hippocampus. Values are mean± SE; *vs. scopolamine (p<0.05); ** vs. scopolamine (p<0.001); $ vs. control (p<0.05); $$vs. control (p<0.001).

c. Acetylcholinesterase (AChE) inhibitory effects of telmisartan in scopolamine-induced amnesic rats

Drugs that inhibit AChE are known to antagonize scopolamine-induced amnesia. Thus the effects of telmisartan on AChE activities within the hippocampus of rats were evaluated. There was a significant inhibition of AChE activity in control rats compared to scopolamine group ($^{*}p<0.05$). Pre-treatment with telmisartan at doses of 1.80 mg/kg and 3.60 mg/kg, significantly inhibited the AChE activity in the hippocampus of amnesic rats compared to scopolamine ($^{*}p<0.05$) as shown in Table 5.

Table 5

Effect of telmisartan on the acetylcholinesterase activity within the rat hippocampus

Treatment	AChE ((micromol/L/g tissue)
Control	2.02 ± 0.34*
Saline control	2.18 ±1.61*
Scopolamine	4.41±0.37$^{\$}$
Telmisartan 1.80 mg/kg + scop.	2.51±0.33*
Telmisartan 3.60 mg/kg + scop.	2.65±0.21*

Comparisons between control, scopolamine, and telmisartan on AChE activity within the rat hippocampus. Values are mean± SE; *vs. scopolamine (p<0.05); ** vs. scopolamine (p<0.001); $ vs. control (p<0.05); $$vs. control (p<0.001).

4. DISCUSSION

The present study was designed to assess the memory enhancing effect of telmisartan on scopolamine- induced amnesic rats using the passive avoidance test. Scopolamine is one of the most frequently employed drugs to mimic the cholinergic deficits seen in AD. It blocks the muscarinic receptors and thus interferes with memory and cognitive function in humans and experimental animals [26, 27]. The passive avoidance paradigm is a fear-aggravated test used to examine the avoidance learning and memory in experimental rodents. Rats avoid bright illumination and prefer dim illumination as a part of their normal behavior. When placed in a brightly illuminated compartment connected with a dark enclosure, they rapidly enter the dark compartment and remain there. Once they receive an aversive consequence (foot shock) in the dark compartment, the animals modify their behaviour to avoid a noxious event by suppressing the learned habits of staying in the dark compartment and remain in the bright compartment. In our present study, administration of scopolamine clearly produced memory deficits (amnesia) in rat performance in passive avoidance test as indicated by their shorter latency to enter into the dark compartment in the memory retention test compared to the control group. The mean latency of rats treated with 3.60 mg/kg telmisartan was significantly higher compared to scopolamine group, indicating reversal of amnesia. This indicates that scopolamine-treated rats after being exposed to aversive stimulation in the passive avoidance task, failed to remember the task on the following day, but this effect could be attenuated following treatment with 3.60 mg/kg of telmisartan, indicating that the drug has a positive effect on memory retention. Several clinical studies have reported that oxidative stress is strongly involved in the pathogenesis of Alzheimer's disease. These reports suggested that activities of glutathione peroxidase and glutathione were found to be elevated the brains of patients with AD; this reflects a protective response to increased lipid peroxidation within the brain[28, 29]. The progression of neurodegenerative diseases is thus found to be inhibited by free radical scavengers and antioxidant agents. In our experimental conditions, scopolamine administration resulted in a significant increase in MDA, an important marker of lipid peroxidation, and caused a reduction in the activities of GST, thiols and SOD within the hippocampus of amnesic rats. The findings of the present study are in accordance with earlier reports which suggested that acute scopolamine administration does produces alterations in the activity of antioxidant enzymes in the brain [30]. The administration of telmisartan produced a significant decline in MDA level and restored the activities of antioxidants in the brain of scopolamine-induced amnesic rats but the effects were

most marked in rats that received higher dose (3.60 mg/kg) of telmisartan. The antioxidant activity observed with telmisartan can be explained by that reactive oxygen species (ROS) are involved in many of the Ang II signaling pathways [11] and blockade of this pathway by a RAS blocker may be involved in inhibiting the generation of reactive oxygen species. Peripheral administration of telmisartan can penetrate the blood brain barrier in a dose-dependent manner and inhibit the centrally mediated effects of angiotensin II [31]. Telmisartan has been shown to be effective in reducing the generation of reactive oxygen species and pro-inflammatory mediators by its ability to prevent the activation of nuclear factor-κB signaling pathway that promotes transcription of NADPH oxidase, tumor necrosis factor-α and inducible nitric oxide synthase genes [32-34]. Since oxidative stress is mediated by an increased level of Ang-II, the beneficial effect of telmisartan in mitigating the oxidative stress could be due to its ability to inhibit the activity of Ang-II. Impairments in learning and memory observed in patients with AD are partly caused by changes within the cholinergic system [35]. Cholinergic transmission involves the activity of choline acetyltransferase enzyme which is involved in ACh synthesis and is terminated mainly by acetylcholine hydrolysis via the acetylcholinesterase enzyme [36]. It is believed that the activity of AChE could affect the underlying processes in Alzheimer's disease. Thus in our study, we evaluated the effects of telmisartan on AChE activity and correlated these activities with their anti-amnesic activities. Telmisartan at doses of 1.80 mg/kg and 3.60 mg/kg significantly inhibited the AChE activity within the hippocampus of rats (*$p<0.05$ compared to scopolamine) and showed a similar level of inhibition compared to control group. This suggest that the ameliorating effects on memory of shown by telmisartan could be explained, in part, by their inhibition on acetylcholinesterase activity within the hippocampus. Previous studies with ARBs have shown that ARBs protect cognition after stroke and during aging, and these compounds can also significantly reduce the incidence and progression of Alzheimer's disease [36]. Another study by Singh et al., (2012) demonstrated that telmisartan can attenuate streptozotocin induced experimental dementia of Alzheimer's disease type [37]. It has also been reported that telmisartan shows cerebroprotective effects and can improve cognitive decline in AD and render neuroprotection and these actions of telmisartan are attributed to its anti-inflammatory, anti-oxidative, anticholinesterase and anti-Aβ effects [37]. Our study is thus in line with earlier studies which have reported that telmisartan has the potential to improve cognitive function in AD.

5. CONCLUSION

To conclude, the findings from the present study demonstrated that telmisartan exhibits potent cognitive enhancing and antioxidant activities. It can scavenge reactive oxygen species and exert a protective effect against scopolamine-induced oxidative damage by ameliorating the reduction in the activities of GST and SOD. In conclusion, telmisartan might offer a useful therapeutic choice in the prevention or treatment of Alzheimer's disease. Nevertheless, further studies are needed to explore the full potential of telmisartan in memory deficits.

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CONFLICT OF INTEREST

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of this paper.

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