RESEARCH ARTICLE

A comparative evaluation of neuroprotective activity of perindopril and valsartan in rats

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ABSTRACT

Background: Angiotensin II receptor blockers and Angiotensin-converting enzyme inhibitors are widely used drugs for cardiovascular disorders, renal disease, and diabetes. Recently, they have been recognized for neuroprotective activity and are used in many brain disorders. Aim and Objective: The present study was done to explore effects of perindopril and valsartan on experimentally induced learning and memory impairment in Wistar rats. Materials and Methods: 40 Wistar rats were divided into 5 groups, eight rats in each group, namely normal control, disease control, positive control (Piracetam 600 mg/kg), test group I (Perindopril 4 mg/kg), and test group II (Valsartan 15 mg/kg). Except normal control group, all animals received intraperitoneal injection of Scopolamine 1 mg/kg for 21 days to induce memory impairment. Piracetam and Test drugs were administered once daily orally for 21 consecutive days. On day 0, 7th, 14th, and 21st of the experiment, muscle grip strength (Wire hanging grip test) and memory functions Elevated plus maze (EPM) of all the animals were assessed. On 8th, 15th, and 22nd day of the experiment, retention memory functions (EPM) were assessed. Results: Animals treated with Scopolamine showed significant reduction in grip strength and significant rise in transfer latency (TL) (EPM model). Rats treated with piracetam and test drugs showed significant increase in grip strength. The animals treated with piracetam and test drugs showed significant reduction in TL (EPM model) when compared with disease control group. Similar results were seen in retention memory test. Conclusion: Perindopril and valsartan demonstrated neuroprotective effect in scopolamine-induced memory impairment in rats. Memory improvement by these test drugs was comparable with positive control piracetam.

KEY WORDS: Scopolamine; Perindopril; Valsartan; Neuroprotection

INTRODUCTION

Alzheimer’s disease (AD) is a progressive neurodegenerative brain disorder characterized by extensive atrophy of neurons, accumulation of neurofibrillary tangles, deposition of β-amyloid neuro-inflammation, brain tissue oxidative damage.[1] Furthermore, the dysfunction of the cholinergic system has been reported as a contributing factor in memory loss and cognitive impairment in AD patients. Using its effect on the cholinergic system, scopolamine, an antagonist of muscarinic receptors of acetylcholine, can induce learning and memory impairment in animal models.[2]

To date, the management of dementia is limited offering symptomatic relief rather than curative effect. In clinical practice, choline esterase inhibitors, and N-methyl-d-aspartate receptor antagonists (NMDA) are used. There is a requirement to find alternative approaches that confer prophylactic or therapeutic cure for such syndromes.[3] Reversible acetyl cholinesterase inhibitors such as tacrine
and donepezil are the approved drugs for the treatment of mild to moderate dementia coupled with AD. Drawbacks of tacrine include hepatotoxicity and high cost but advantage of donepezil is its longer half-life about 70 h. Nootropic agents like piracetam, aniracetam enhance cognitive skills.

Renin-Angiotensin System (RAS) regulates various functions of the body like blood pressure and fluid balance. Apart from peripheral functions, it has been also documented to be found in the central nervous system (CNS) and plays an important role in various neurodegenerative diseases. In the RAS, both centrally and locally, the angiotensinogen is converted by the renin enzyme to form angiotensin I (Ang I) which is then converted to angiotensin II (Ang II), a potent vasoconstrictor, by the action of the angiotensin-converting enzyme (ACE). It is observed that RAS activation impairs cognitive functions and this activity is mainly occurring through stimulation of the AT1 receptor. Drugs such as angiotensin-converting enzyme inhibitors (ACEI) and angiotensin receptor blockers (ARB) are associated with a slower rate of AD progression.

The present study was aimed to examine whether perindopril and valsartan have a protective role against scopolamine-induced memory impairment in rats by comparing the neuroprotective activity of perindopril and valsartan with positive control piracetam and negative control scopolamine.

MATERIALS AND METHODS

The study was started after getting approval from Institutional Animal Ethics Committee (Approval Letter No. IAEC/ BVDUMC/007/2020 dated 18/08/2020). 40 Albino Wistar Rats of either sex weighing 150–200 g were taken from Central Animal House from our institute. Animals were housed in polyacrylic cages with 12 h day and night cycle. Water and food were provided to all animals ad libitum.

Instruments and other material-Retort stands, Wire, Elevated plus maze (EPM), Feeding needles, weighing scale, butter paper, syringes, distilled water, stopwatch, disinfectant, cotton, etc.

Drugs and chemicals-Scopolamine, piracetam, perindopril, valsartan.

Animals (n = 40) were divided into five groups with eight animals in each group as follows:
- Group I - Distilled Water
- Group II - Scopolamine (1 mg/kg, i.p.)[7]
- Group III - Scopolamine (1 mg/kg, i.p.) + Piracetam 600 mg/kg, po[8]
- Group IV - Scopolamine (1 mg/kg, i.p.) + Perindopril 4 mg/kg, po[9]
- Group V - Scopolamine (1 mg/kg, i.p.) + Valsartan 15 mg/kg, po.[10]

Memory impairment was induced by i.p injection of freshly prepared scopolamine (1 mg/kg body weight) once daily for 21 days. Piracetam, perindopril, and valsartan were administered to the respective group once daily orally for 21 consecutive days.

Behavior and muscular activities: On the prestudy (Day 0), 7th, 14th, and 21st day of the study, muscular coordination (wire grip test), and memory function (EPM) were evaluated. Retention memory was assessed 24 h after drugs were administered i.e. on the 1st, 8th, 15th, and 22nd day.

Hanging Wire Grip Test[7]

The wire hanging grip test was conducted to assess the muscular strength and coordination of movement of an animal. Two heavy-based retort stands was kept at a distance of 55 cm, and 1-mm thick thread is tied between the poles at a height of 35 cm. A layer of bedding material was placed to prevent injury to the animal when it falls down. During the test, the thread was kept tightly attached to the frame to avoid vibration or unwanted displacement of the thread. Animal was placed at the center of the wire, and latency time of fall was recorded in seconds. Reduction in latency of falling time was considered as CNS depression.

EPM[7]

All the animals were screened before inclusion into the study by using EPM. Each rat is placed at the end of an open arm, facing away from the central platform. Rat was allowed to move in one of the closed arms with all its four limbs and this is called TL. Animals with TL less than 60 s on EPM were included for the EPM test. Screening was necessary to create all groups uniform.

All rats in each group were treated with the respective drug doses orally for 21 days. TL was recorded as the time (in seconds) taken by the animal to move from the open arm into one of the covered arms with all its four legs in EPM. The rats were allowed to explore the maze for 2 min. and returned to home cage. Retention of this learning task (Retention memory) was examined 24 hrs after the 1st day trial (i.e.24 after last dose). TL was recorded to assess learning ability of animals on the day 0, 7, 14 and 21, after 45 min of last dose of drug administration. TL was noted to assess animals learning retrieval i.e. memory in EPM, after 24 h of last drug dose administration, i.e. on 1st, 8th, 15th and 22nd day again. Significant improvement in memory function was indicated by reduction in TL.

Statistical Analysis

All the data were expressed as the mean ± SEM, and the statistical significance between the groups was tested using one-way Analysis of variance followed by Tukey’s post hoc test using SPSS software. \( P < 0.05 \) was considered statistically significant.
RESULTS

As seen in Figure 1, the baseline muscle coordination was almost similar in all groups on day 0 as observed by time of fall on a wire hanging grip test. The normal control group treated with distilled water showed similar muscle coordination on day 0, 7, 14, and 21. The negative control group treated with scopolamine showed a significant reduction in time of fall from the wire on 14th ($P \leq 0.05$) and 21st ($P \leq 0.001$) day in comparison with the normal control group.

On the 7th, 14th, and 21st day, a significant rise in time of fall was noted with piracetam, perindopril and valsartan groups in comparison with scopolamine treated group ($P \leq 0.05$ to $P \leq 0.001$). Animals treated with perindopril produced a significantly different change in time of fall from the wire ($P \leq 0.05$ to $P \leq 0.01$) on days 7, 14, and 21 when compared with standard drug piracetam.

Figure 2 shows baseline TL time in the EPM model was almost similar in all groups on day 0. The Control group treated with distilled water had shown comparable results on day 0, 7, 14, and 21. The negative control group treated with scopolamine had shown a significant increase in TL time on the 14th ($P \leq 0.05$) and 21st ($P \leq 0.01$) day in EPM in comparison with the normal control group. On the 14th and 21st day, a significant reduction in TL was noted in piracetam, perindopril and valsartan groups in comparison with the scopolamine treated group ($P \leq 0.05$ to $P \leq 0.001$). The test drugs perindopril and valsartan produced comparable results with the standard drug piracetam.

Table 1 shows the baseline retention TL time in the EPM model was almost similar in all groups on day 1. The control group treated with distilled water did not show any significant difference in retention TL in the elevated plus-maze on day 1, 8, 15 and 22. The scopolamine-treated group showed a significant rise in retention TL time on 15th ($P \leq 0.05$) and 22nd ($P \leq 0.01$) day in comparison with the normal control group. A significant reduction in retention transfer latency was noted in the piracetam group on day 8 ($P \leq 0.05$), 15 ($P \leq 0.05$), and 22 ($P \leq 0.01$) in comparison with the scopolamine-treated group. Also, a significant reduction of retention TL was noted on the 15th and 22nd day in perindopril and valsartan groups. The results of the perindopril and valsartan groups were comparable with standard drug piracetam.

As shown in Figure 3, the normal control group treated with distilled water did not show any significant difference in time of fall with the scopolamine-treated group ($P \leq 0.05$) and 21st ($P \leq 0.01$) day in comparison with the normal control group. A significant reduction in time of fall with piracetam, perindopril, and valsartan groups was noted on the 14th and 21st day in EPM in comparison with the normal control group. On the 14th and 21st day, a significant reduction in TL was noted in piracetam, perindopril and valsartan groups in comparison with the scopolamine treated group ($P \leq 0.05$ to $P \leq 0.001$). The test drugs perindopril and valsartan produced comparable results with the standard drug piracetam.

Table 1: Effect of piracetam, perindopril and valsartan on transfer latency time (EPM) in scopolamine treated rats for acquisition memory.

<table>
<thead>
<tr>
<th>Days→Drugs↓</th>
<th>Day 1</th>
<th>Day 8</th>
<th>Day 15</th>
<th>Day 22</th>
</tr>
</thead>
<tbody>
<tr>
<td>Distilled Water</td>
<td>14.65±2.64</td>
<td>15.38±2.05</td>
<td>16.23±1.46</td>
<td>21.82±3.18</td>
</tr>
<tr>
<td>Scopolamine</td>
<td>15.01±6.52</td>
<td>22.55±4.48</td>
<td>28.03±5.68$^a$</td>
<td>39.76±5.72$^{ab}$</td>
</tr>
<tr>
<td>Piracetam</td>
<td>15.38±2.14</td>
<td>9.5±1.71$^c$</td>
<td>6.15±1.47$^{bc}$</td>
<td>4.36±0.81$^{ab}$</td>
</tr>
<tr>
<td>Perindopril</td>
<td>15.01±2.28</td>
<td>10.18±1.52</td>
<td>7.63±0.53$^c$</td>
<td>5.76±0.55$^{ab}$</td>
</tr>
<tr>
<td>Valsartan</td>
<td>14.52±0.98</td>
<td>8.43±1.84</td>
<td>6.77±1.24$^a$</td>
<td>4.91±0.81$^{ab}$</td>
</tr>
</tbody>
</table>

All values as Mean±SEM ($n=8$), One-way ANOVA followed by Tukey’s post hoc test. $^aP \leq 0.05$, $^{ab}P \leq 0.01$ compared with normal control, $^bP \leq 0.05$, $^{bc}P \leq 0.01$ compared with disease control, $^cP \leq 0.05$, $^{ac}P \leq 0.01$ compared with disease control, EPM: Elevated plus maze.
spent on the close arm in the elevated plus-maze on day 0, 7, 14, and 21. Animals of all groups showed similar time, spent on the close arm in the elevated plus-maze on day 0. The scopolamine treated group showed a significant decrease in time spent on the close arm on the 21st (P ≤ 0.05) day in elevated plus-maze in comparison with the normal control group. On the 21st day, a significant increase in time spent on the close arm was seen in piracetam and perindopril (P ≤ 0.05), when compared to the scopolamine-treated group. But the results of perindopril and valsartan groups were comparable with standard drug piracetam.

Table 2 demonstrates that the normal control group treated with distilled water did not show any significant difference in time spent on the open arm in the elevated plus-maze on day 0, 7, 14, and 21. Animals of all groups showed similar time, spent on the open arm in the elevated plus-maze on day 0. The scopolamine-treated group showed a significant increase in time spent on the open arm on the 21st (P ≤ 0.05) day in elevated plus-maze in comparison with the normal control group. On the 14th and 21st day, a significant decrease in time spent on the open arm was seen in piracetam, perindopril, and valsartan groups (P ≤ 0.05 to P ≤ 0.001) when compared to the scopolamine treated group. But the results of perindopril and valsartan groups were comparable with standard drug piracetam.

**DISCUSSION**

Cognitive decline occurs in neurodegenerative disorders such as AD where cholinergic neuronal system plays a vital role. Clinical management of dementia includes drugs from different classes e.g. cholinergic activators like anticholinesterase, glutamate (NMDA) receptor antagonists, miscellaneous drugs like nootropic agents, and some herbal products. However, recently promising results are also shown by ACEIs and ARBs for neurodegenerative diseases. These RAS inhibitors are used commonly in the management of hypertension. In this study, we evaluated neuroprotective effects of perindopril (ACEI) and valsartan (ARB) in scopolamine induced memory impairment in animals. The drug scopolamine induced memory impairment significantly as indicated by increased time of TL in EPM model and reduced muscle coordination as shown by reduced time of fall on wire hanging grip test. The test drugs perindopril and valsartan reduced time of TL in EPM model and improved muscle co-ordination in wire hanging grip test which indicated improvement in memory impairment. The neuroprotective activity of test drugs perindopril and valsartan were comparable with positive control piracetam.

Various neurotoxins are usually used to induce neurological impairment in animals. It includes scopolamine, diazepam, glucocorticoids, streptozotocin, etc. As cholinergic system regulates the memory functioning, scopolamine is administered to animal it produces transient memory loss by blocking muscarinic receptor. Furthermore, in humans it interferes with memory and cognitive function by same mechanism of action as in animals. In the present study, scopolamine had induced transient but significant memory impairment on day 7, 14, and 21 as shown by the increased time of TL and time spent on the open arm of the EPM model [Figure 2 and Table 2]. The reduction in time on the close arm was seen with scopolamine [Figure 3]. It also shows reduction in time of fall in the wire hanging test [Figure 1]. It also affected retention TL significantly [Table 1]. Some animal studies also observed consistent findings and they concluded that scopolamine produced these effects by blocking muscarinic receptors/increasing acetylcholinesterase activity. Piracetam, one of the nootropic agent, is Gamma-amminobutyric acid derivative.

![Figure 3: Effect of piracetam, perindopril and valsartan on time spent on close arm (Elevated plus maze) in scopolamine treated rats for acquisition memory. All values as Mean ± SEM (n=8), One-way Analysis of variance followed by Tukey’s post hoc test. *P ≤ 0.05 compared with normal control, **P ≤ 0.05 compared with disease control](image)

### Table 2: Effect of piracetam, perindopril, and valsartan on time spend on open arm (EPM) in scopolamine treated rats for acquisition memory

<table>
<thead>
<tr>
<th>Days→Drugs</th>
<th>Day 0</th>
<th>Day 7</th>
<th>Day 14</th>
<th>Day 21</th>
</tr>
</thead>
<tbody>
<tr>
<td>Distilled Water</td>
<td>22.28±3.02</td>
<td>19.55±1.98</td>
<td>15.15±0.76</td>
<td>15.07±2.07</td>
</tr>
<tr>
<td>Scopolamine</td>
<td>23.13±2.84</td>
<td>24.55±4.70</td>
<td>26.48±0.97</td>
<td>30.05±3.22</td>
</tr>
<tr>
<td>Piracetam</td>
<td>20.51±2.30</td>
<td>16.83±4.92</td>
<td>5.025±1.46 **</td>
<td>7.17±1.60 ***</td>
</tr>
<tr>
<td>Perindopril</td>
<td>21.12±1.65</td>
<td>11.12±2.81</td>
<td>10.33±2.45</td>
<td>13.06±2.10</td>
</tr>
<tr>
<td>Valsartan</td>
<td>20.51±1.12</td>
<td>19.63±5.88</td>
<td>14.46±1.28 ***</td>
<td>11.4±2.44 ***</td>
</tr>
</tbody>
</table>

All values as Mean±SEM (n=8), One-way ANOVA followed by Tukey’s post hoc test, *P≤0.05 compared with normal control, **P≤0.05, ***P≤0.01, ****P≤0.001 compared with disease control.
Due to its nootropic action, it is used as standard positive control drug in many studies.[10] In our study, it had resulted in reduction in time of TL and time spent on the open arm of the EPM model which means rats were able to locate the closed arm/dark zone immediately after exposure to the open arm in the EPM paradigm, which is an indicator of cognition improvement [Figure 2 and Table 2]. The increase in time on the close arm was also seen with the piracetam [Figure 3]. Piracetam also improved time of fall in wire hanging test [Figure 1]. All these changes in different parameters indicated that piracetam had prevented scopolamine-induced behavioral and memory impairment in rats on days 7, 14, and 21. The retention of spatial memory is enhanced which is mainly due to its facilitatory effect [Table 1]. Piracetam, when given to experimental rats, produced improvement in spatial learning memory.[14]

In the present study, memory impairment in rats was assessed by the EPM model and muscular coordination in experimental rats was evaluated by wire hanging grip test. Acquisition memory was evaluated on day 0, 7, 14, and 21 of the study. On day 1, 8, 15, and 22 of the study, retention memory was evaluated after 24 h of administration of the test dose. The present work was undertaken to study the neuroprotective effect of ACE inhibitor perindopril and ARB valsartan on memory impairment in animals. RAS in the brain regulates systemic blood pressure and cerebral blood flow as well.[13] Angiotensin II is the main culprit of RAS which has a role in the pathophysiology of tissue dysfunction.[16,17] The inhibitors of RAS like ACE inhibitors and ARBs are widely used as antihypertensive drugs. They also have beneficial effects on cognitive functions.[18,19] As compared to ACEIs (perindopril), ARBs (Valsartan) are strongly inversely associated with AD.[8] Reduced cerebral blood flow, increased in oxidative stress[20] and anxiety,[21] inflammation could induce cognitive impairment by AT1 receptor activation.[22] The test drugs perindopril and valsartan improved acquisition and retention memory in rats as seen by the reduction in time of TL was found to be comparable with standard control piracetam on day 14 and 21 of the study [Figure 2 and Table 1]. This decrease in the TL, i.e., rats were able to locate the dark zone immediately after exposure to the open arm in the EPM paradigm, which is an indicator of cognition improvement.[11]

In the present study, the muscle strength of animals treated with valsartan was comparable with piracetam. Though the time of fall of animals treated with perindopril was improved, it was significantly less when compared with piracetam [Figure 1]. These findings suggest that perindopril and valsartan possess memory enhancing activity in view of its facilitatory effect on the retention of spatial memory in scopolamine-induced memory impairment.

**Strengths and Limitations of Study**

In the present study, comparison of two drugs of different classes were evaluated for their neuroprotective activity. Both the drugs were RAS inhibitors but one was ACEI and other was ARB. But the limitations of the study were that different models were not used to assess neuroprotection. Furthermore, the mechanism of this neuroprotection was not evaluated. The parameters of oxidative stress and cholinesterase levels of brain homogenate were not assessed.

**CONCLUSION**

The effect of ACEI-perindopril, and ARB-valsartan on scopolamine-induced memory impairment in rats was studied. Scopolamine, in the dose of 1 mg/kg intraperitoneally for 21 consecutive days, produced significant impairment in learning and memory, and muscle coordination. The ACEI perindopril, and ARB valsartan, improved memory deficits induced by scopolamine. The neuroprotective action of these drugs was comparable with the standard positive control drug piracetam.

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