Neuroprotective effect of renin angiotensin system blockers on experimentally induced Alzheimer’s disease in rats

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ABSTRACT

Background: Alzheimer’s disease (AD) is a major world-wide health problem. Much evidence points to a link between hypertension and AD. However, the exact effects of different antihypertensive drugs on AD need to be more assessed. The aim was to evaluate and compare the possible effects of perindopril, and candesartan on cognitive impairment, oxidative stress markers, and brain concentrations of amyloid beta-peptide (Aβ-P) in a rat model of induced dementia.

Methods: Thirty-two adult male Wistar rats were distributed among 4 groups; (1) normal controls; (2) rats with dementia induced by intracerebroventricular administration of streptozotocin (ICV-STZ) and received no treatment; (3) ICV-STZ rats treated orally with perindopril for 3 weeks; and (4) ICV-STZ rats treated orally with candesartan for 3 weeks. The assessed parameters were spatial memory by Morris Water Maze test, brain tissue level of total antioxidant capacity (TAC), reduced glutathione (GSH), lipid peroxidation product (malondialdehyde [MDA]), and Aβ-P.

Results: Both perindopril and candesartan attenuated STZ-induced memory impairment, caused a significant increase in TAC and GSH levels, reduced MDA levels, whereas only candesartan significantly reduced Aβ-P levels.

Conclusions: This study reports that candesartan and perindopril can reverse the free radical induced damages and resultant memory defects, and may suggest candesartan as worthy drugs for prevention of Aβ-P deposition in this animal model of AD.

Keywords: Streptozotocin, Amyloid beta-peptide, Hypertension, Perindopril, Candesartan, Oxidative stress

INTRODUCTION

Alzheimer’s disease (AD), the most common form of dementia, is an insidious, progressive disorder characterized by subtle behavioral and memory disturbances; progressing into global cognitive impairment, memory loss, behavioral and psychiatric disturbances, and functional impairment.¹ The greatest risk factor for the development of AD is advancing age. However, the exact mechanisms of AD are incompletely understood.²

One of the key pathological features of AD is deposition of a 40–42 amino acid peptide called amyloid-β (Aβ). Aβ is the building brick for Aβ peptide (Aβ-P) that is deposited in the brain as extracellular senile plaques, or within the walls of the cerebrovasculature.¹ Development of drugs that reduce Aβ deposition through inhibition of its production or increased promotion of its removal is now a major therapeutic strategy.

Much evidence points to a link between hypertension and AD.⁴ High systolic blood pressure is reported to be associated with low brain weight, whereas a high systolic and diastolic blood pressure are associated with increased prevalence of the neurofibrillary tangles (NFT) in hippocampal brain regions.⁵ Although the use of antihypertensive drugs has...
been suggested to reduce incidence of dementia, their exact effects need more assessment.\(^6\)

The renin angiotensin system (RAS) was initially described as a hormone system designed to mediate cardiovascular and body water regulation. Various tissues have the ability to synthesize angiotensin (Ang) II independently of the circulating RAS. Of concern, brain RAS has been implicated to modulate functions such as the regulation of cerebral blood flow, stress, exploratory behavior, anxiety, learning, and memory acquisition.\(^7\) Based on the available data, manipulation of brain RAS has been suggested as a potential therapeutic target for a variety of neurological diseases including AD.\(^6\)

The intracerebroventricular administration of streptozotocin (ICV-STZ) animal model of dementia is extensively used for researching the pathogenesis of AD and in the pharmacological testing of potential neuroprotective effects of various drugs.\(^8\) Therefore, this study was undertaken to evaluate and compare the possible effect of one of angiotensin-converting enzyme (ACE) inhibitors-perindopril and one of angiotensin receptor blockers (ARBs) candesartan on spatial cognitive deficit, brain concentrations of amyloid beta-peptide (Aβ-P), and some markers of oxidative stress in a rat model of ICV-STZ induced dementia.

**METHODS**

**Animals**

Thirty-two adult male *Wistar* (albino) rats with a body mass of 180-200 g were used in this study. They were kept in the animal house under the same standard environmental conditions of light and temperature. They were housed in metal cages with free access to rat chow and water. The animals were cared for in accordance with the Guide for the Care and Use of Laboratory Animals.\(^9\) The study protocol was approved by the Ethics Committee, Faculty of Medicine, Alexandria University, Egypt.

**Grouping of animals**

Rats were divided into 4 groups (n=8 rats per group) as follows:

- **Group I** (sham control): included 8 normal healthy rats that were subjected to ICV injection of 0.2 ml citrate buffer (0.05 M, pH 4.5) and considered as sham normal control group. Starting from the second day post injection, these rats received 1 ml gum acacia 2% orally/day for 3 weeks.

- **AD** was induced in the remaining 24 rats through a single ICV injection of 0.2 ml freshly prepared STZ (Sigma-Aldrich) in a dose of 3 mg/kg body mass dissolved in citrate buffer (0.05M, pH 4.5).\(^10\) The site of injection was 2 mm from either side of the midline on a line drawn through the anterior base of the ears. From the second day after ICV-STZ injection, rats were randomly assigned, and grouping of animals continued as follows:

  - **Group II** (positive control): included 8 untreated ICV-STZ rats that received 1 ml of gum acacia 2% orally/day for 3 weeks.
  - **Group III** (perindopril-treatment group): included 8 ICV-STZ rats that received perindopril (Adwia-Pharmaceuticals) daily at a dose of 1 mg/kg body mass orally for 3 weeks.\(^11\)
  - **Group IV** (candesartan-treatment group): included 8 ICV-STZ rats that received candesartan (AstraZeneca) at a dose of 1 mg/kg body mass/day orally for 3 weeks.\(^12\)

The calculated dose of either perindopril or candesartan was dissolved in 1 ml gum acacia 2% (the vehicle).

**Evaluation of spatial memory by Morris water maze test**

During the 3rd week of therapy, rats were trained in a Morris water maze (MWM) for 3 consecutive days and then tested to assess spatial memory for 4 consecutive days. The MWM, as described previously, consisted of a black circular pool (with a diameter of 120 cm and a height of 60 cm), filled with water (26±2°C) to a depth of 30 cm. A rectangular platform (10 cm × 5 cm) was placed 2 cm below the water level at a fixed position in the water tank during the entire experiment. The water was colored with a non-toxic black dye to hide the location of the submerged platform.\(^13\) Each rat performed four trials per day for 4 consecutive days to find the hidden platform. Each trial began by placing a rat into one of the four quadrants of the pool, facing the wall of the tank. The trial was terminated when the rat climbed onto the hidden platform or when 120 sec had elapsed. After climbing onto the platform, the animal remained there for 30 sec before the commencement of the next trial. Rats that did not find the platform within the maximally allowed time of 120 sec were put on the platform by the experimenter and were also allowed to stay there for 30 sec.

After completion of the fourth trial, each rat was kept warm for one hour and was returned to its home cage. All tests were conducted between 09:00 and 18:00. Escape latencies (i.e., the time to reach the platform) were compared between groups.\(^14\)

**Estimation of biochemical parameters**

By the end of the behavioral tests (3 weeks after treatment period), rats of all groups were fasted overnight with free access to water. Rats were anesthetized with ether, sacrificed and their brains were taken out quickly. Dissected brains immediately washed with ice-cold saline, blotted on dry
filter papers weighed and then homogenized for assessment of the following parameters:

**Estimation of brain tissue level of total antioxidant capacity (TAC)**

Colorimetric determination of TAC is based on the reaction of antioxidants in the sample (supernatant of brain tissue homogenate in 10 mM phosphate buffer (pH 7.0) with a defined amount of exogenously provided hydrogen peroxide ($H_2O_2$). The residual is determined colorimetrically by an enzymatic reaction that evolves the conversion of 3,5 dichloro-2-hydroxy benzene sulfonate to a colored product that can be measured at 505 nm. The values are expressed in (mM/gm tissue).\(^{15}\)

**Estimation of brain tissue level of reduced glutathione (GSH)**

This method is based on reductive cleavage of 5,5'-dithiobis-2nitrobenzoic acid by SH group (contained in the supernatant of brain tissue homogenate prepared in 5% TCA/ethylenediaminetetraacetic acid) to yield a yellow color with a maximum absorbance at 412 nm using UV-visible spectrophotometer. GSH concentration was calculated by using standard curve prepared with reduced GSH, and the values are expressed in µg/g tissue.\(^{16}\)

**Estimation of brain tissue level of lipid peroxidation product; malondialdehyde (MDA)**

This method is based on the reaction of thiobarbituric acid (TBA) with MDA (contained in the brain tissue homogenate in 1.15% KCl) in acidic medium (20% acetic acid pH 3.5) at a temperature of 95°C for 1 hr to form TBA-reactive product that yield a pink color which can be measured spectrophotometrically at 532 nm. MDA concentration was calculated by using standard curve prepared with tetra methoxy propane. The values are expressed in nmol/g tissue.\(^{17}\)

**Estimation of brain tissue level of amyloid beta-peptide (Aβ-P)**

Rat Aβ-P enzyme-linked immunoassay kit was used (WKEA, MED SUPPLIES - USA). The values are expressed in pg/mg protein.\(^{18}\)

**Statistical analysis**

Values are expressed as the mean ± standard deviation. Statistical analysis was done using the Statistical Package of Social Sciences (SPSS) version 20. Comparisons among the different groups were done using analysis of variance (ANOVA; F test) followed by a post-hoc (Scheffe) test to compare variables between different groups. Values for p<0.05 were considered statistically significant.

**RESULTS**

**Assessment of spatial memory (mean values of escape latency time) by Morris Water Maze test**

This study showed that normal control group (Group I) showed a significant decrease in escape latency time to reach the platform from the second day to the fourth day of the testing period in Morris Water Maze test. ICV-STZ injection caused a poor learning performance i.e., memory deficit; as shown by a non-significant decrease in escape latency time throughout all the sessions of Group (II) as compared with the normal control group (F=10.058, p=0.001). On the other hand, treatment with perindopril or candesartan attenuated STZ-induced memory impairment as shown by a significant decrease in escape latency time from session 2 onward (F=177.362 and 266.667, respectively, p<0.001) (Table 1).

**Comparison of mean brain tissue levels of TAC (mM/g tissue) and reduced GSH concentration (µg/g tissue) in the 4 studied groups**

The present study revealed that TAC and reduced GSH levels were significantly reduced in Group II (ICV-STZ untreated rats) as compared to normal controls of Group I. Oral administration of perindopril (Group III) or candesartan (Group IV) for ICV-STZ rats for 3 weeks resulted in a statistically significant increase in the brain tissue levels of TAC and GSH as compared to untreated rats of Group II, (F=12.565 and p=0.001), and (F=13.097 and p<0.001), respectively (Table 2).

**Comparison of mean brain tissue levels of MDA (nmol/g tissue) and Aβ-P (pg/mg protein) in the 4 studied groups**

The present study revealed that MDA and Aβ-P concentrations were statistically significantly elevated in Group II (ICV-STZ untreated rats) as compared to normal control group. Oral administration of perindopril (Group III) or candesartan (Group IV) to ICV-STZ rats for 3 weeks resulted in a statistically significant decrease in the brain tissue levels of MDA as compared to untreated rats of Group II, (F=28.601 and p<0.001) (Table 3).

On the other hand, oral administration of perindopril (Group III) resulted in a non-significant decrease in Aβ-P level, while oral administration of candesartan (Group IV) produced a statistically significant decrease in Aβ-P level as compared to untreated control group, (F=20.432 and p<0.001).

**DISCUSSION**

AD is a major world-wide health problem. Numerous observational studies have demonstrated that high blood pressure is strongly associated with an increased risk of this disease.\(^4\) However, conflicting results appeared regarding
the ability of different antihypertensive drugs to lower incidence of AD.  

The present study examined the potential protective effect of perindopril and candesartan on cognitive impairment, oxidative stress markers, and brain concentrations of Aβ-P in ICV-STZ-injected rats.

The ICV-STZ model is widely used to create an animal model of dementia and to evaluate neuroprotective properties of various compounds. The rationale for this model is that STZ, by the dose employed in this study, is shown to induce oxidative stress and inflammation leading to neuronal loss. STZ also alters enzymes involved in the glycolytic cerebral metabolism of glucose depleting ATP synthesis. Low ATP availability in the brain is followed by faulty Aβ-P metabolism and hyper-phosphorylation of the tau-protein that induce production of neuritic plaques and NFT, the prominent histopathological markers of AD.

In consistent with the previous reports, our results demonstrated that ICV-STZ caused impairment of learning and memory in rats as demonstrated by increased time (escape latency) to reach the submerged platform.

We found that both candesartan and perindopril treated rats learnt the location of the submerged platform in the water maze task significantly faster than ICV-STZ group

### Table 1: Comparison between different groups regarding escape latency time (seconds) according to Morris Water Maze test.

<table>
<thead>
<tr>
<th>Escape latency time</th>
<th>Group I</th>
<th>Group II</th>
<th>Group III</th>
<th>Group IV</th>
<th>F</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>First trial</td>
<td>120.0±0.0</td>
<td>120.0±0.0</td>
<td>120.0±0.0</td>
<td>120.0±0.0</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Second trial</td>
<td>95.0±2.16</td>
<td>120.0±0.0</td>
<td>90.0±20.4</td>
<td>80.0±6.32</td>
<td>10.058*</td>
<td>0.001*</td>
</tr>
<tr>
<td>Third trial</td>
<td>34.0±2.45</td>
<td>96.0±1.83</td>
<td>64.0±6.32</td>
<td>50.0±6.35</td>
<td>177.362*</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>Fourth trial</td>
<td>24.0±4.08</td>
<td>86.0±2.94</td>
<td>28.0±3.65</td>
<td>30.0±3.65</td>
<td>266.667*</td>
<td>&lt;0.001*</td>
</tr>
</tbody>
</table>

ICV-STZ: Intracerebroventricular - Streptozocin injected rats; Group I, normal control group; Group II, untreated ICV-STZ rats; Group III, ICV-STZ rats treated with perindopril; Group IV, ICV-STZ rats treated with candesartan; F, ANOVA test, *Indicates statistical significance; values followed by the same letter are not significantly different, 1Significant with normal group, 2Significant with group II, 3Significant with Group III, 4Significant with the first trial

### Table 2: Comparison between different groups regarding TAC (mM/g tissue) and reduced GSH concentration (ug/g tissue) in brain tissue.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Group I</th>
<th>Group II</th>
<th>Group III</th>
<th>Group IV</th>
<th>F</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>TAC brain</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Range</td>
<td>21.4-24.3</td>
<td>-4.7-1.2</td>
<td>12.7-28.6</td>
<td>12.7-42.3</td>
<td>12.565*</td>
<td>0.001*</td>
</tr>
<tr>
<td>Mean±SD</td>
<td>22.98±1.26</td>
<td>-1.3±2.85</td>
<td>22.45±6.89</td>
<td>26.58±12.28</td>
<td></td>
<td></td>
</tr>
<tr>
<td>GSH</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Range</td>
<td>23.33-33.33</td>
<td>6.67-14.5</td>
<td>20-30</td>
<td>20-30</td>
<td>13.097*</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>Mean±SD</td>
<td>28.09±4.31</td>
<td>11.13±3.53</td>
<td>24.5±4.20</td>
<td>24.5±4.43</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

ICV-STZ: Intracerebroventricular - Streptozocin injected rats; Group I, normal control group; Group II, untreated ICV-STZ rats; Group III, ICV-STZ rats treated with perindopril; Group IV, ICV-STZ rats treated with candesartan; F, ANOVA test, *Indicates statistical significance; values followed by the same letter are not significantly different, 1Significant with normal group, 2Significant with Group II, SD: Standard deviation, TAC: Total antioxidant capacity, GSH: Glutathione

### Table 3: Comparison between different groups regarding MDA level (nmol/g tissue) and β-AP (pg/mg protein) in brain tissue.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Group I</th>
<th>Group II</th>
<th>Group III</th>
<th>Group IV</th>
<th>F</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>MDA</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Range</td>
<td>95.48-109.68</td>
<td>170.32-176.13</td>
<td>109.68-148.39</td>
<td>115.48-145.81</td>
<td>28.601*</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>Mean±SD</td>
<td>102.9±6.06</td>
<td>174.07±2.61</td>
<td>131.32±16.6</td>
<td>128.63±12.96</td>
<td></td>
<td></td>
</tr>
<tr>
<td>β-AP</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Range</td>
<td>156-205</td>
<td>363.2-434.64</td>
<td>299.52-324.39</td>
<td>195.23-327.14</td>
<td>20.432*</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>Mean±SD</td>
<td>177.5±23.10</td>
<td>388.0±31.76</td>
<td>312.6±11.05</td>
<td>269.59±66.19</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

β-AP: Beta-amyloid peptide, MDA: Malondialdehyde, SD: Standard deviation, ICV-STZ: intracerebroventricular - Streptozocin injected rats; Group I, normal control group; Group II, untreated ICV-STZ rats; Group III, ICV-STZ rats treated with perindopril; Group IV, ICV-STZ rats treated with candesartan; F, ANOVA test, *Indicates statistical significance; values followed by the same letter are not significantly different, 1Significant with normal group, 2Significant with Group II
reflecting some memory enhancing effects. These findings are in harmony with previous studies reported that chronic administration of ACE inhibitors improved memory function in various animal models. Similarly, ARBs were reported to prevent impairment of memory function in an animal model of scopolamine-induced amnesia. However, there is within-class difference in the prevention of cognitive impairment in some of these models.\textsuperscript{11}

There is a growing body of evidence that Ang II, via the AT1 receptor, affects spatial memory formation, cerebral blood flow, and acetylcholine release.\textsuperscript{6} It was found that prevention of the formation or action of Ang II helps to stop inhibition of potassium-mediated release of acetylcholine (a primary neurotransmitter in memory) in human and rat entorhinal cortex slices.\textsuperscript{26} Therefore, it is likely that the prevention of cognitive decline by perindopril or candesartan in this study is at least partially mediated by the reduction of brain angiotensin II, or blocking of AT1 receptor activation, respectively.

Our results showed that ICV-STZ infusion caused an increase in the brain tissue levels of MDA, while it reduced TAC and GSH levels. These are in agreement with previous studies which demonstrated that ICV-STZ caused oxidative damage to brain tissues.\textsuperscript{21,27}

The imbalance between oxidative species and antioxidant defenses in the brain play a pivotal role in the pathogenesis of AD and other neurodegenerative diseases.\textsuperscript{26} The brain tissue, by virtue of its high contents of poly-unsaturated fatty acids and its high metabolic rate, is a vulnerable organ to oxidative stress. Oxygenate radicals cause damage to membrane lipids (lipid peroxidation), proteins, carbohydrates, and nucleic acids. These damages can lead to structural and functional disruption of the cell membrane, inactivation of enzymes, and finally cell death.\textsuperscript{28,29}

GSH reductase/GSH peroxidase (GR/GPx) system is one of the major defense systems in the brain. It utilizes reduced GSH to buffer free radicals in brain tissue. Moreover, Woltjer et al.\textsuperscript{30} found that selective inhibition of GSH synthase potentiated Aβ-P accumulation and increased cell death; corroborating that GSH is not simply a marker of oxidative damage but it is suggested to be a part of the cellular response to stressors associated with Aβ-P accumulation.

We found that perindopril, and candesartan treatment significantly attenuated all the oxidative alterations induced by ICV injection of STZ in rats. Previous studies found that modulation of RAS may lead to better circulation and prevent oxidative stresses caused by Ang II-mediated vasoconstrictions.\textsuperscript{31} Furthermore, attenuation of Ang II may diminish the activation of NADPH oxidase that plays a pivotal role in the production of free radicals.\textsuperscript{32}

The main finding of this study was that; candesartan but not perindopril resulted in a significant decrease in Aβ-P concentrations in brain tissues of candesartan treated rats compared to ICV-STZ control group. Aβ-P deposition is a key pathological feature of AD.\textsuperscript{28} It is known to be a potent mitochondrial poison that inhibits several mitochondrial enzymes (specifically cytochrome C oxidase) in the brain and in isolated mitochondria. Consequently, impairment of electron transport, ATP production, oxygen consumption, and mitochondrial membrane potential may follow.\textsuperscript{3,28}

On the other hand, it had been demonstrated that oxidative stress promotes the production of Aβ-P.\textsuperscript{33} Oxidative stress decreases the activity of alpha-secretase while promoting the expression and activation of beta-, and gamma-secretase enzymes critical for the generation of Aβ-P from its precursor and a vicious circle is developed.\textsuperscript{34} Thus, one of the mechanisms by which antioxidants can exert neuroprotective effect is by attenuating Aβ-P induced ROS generation and neuronal apoptosis.\textsuperscript{15}

The antioxidant effect of candesartan could explain, at least in part, the significant decrease in Aβ-P in candesartan treated group. Zhao and colleagues previously reported that; among several commercially available antihypertensive drugs, candesartan was one of only three drugs that prevented oligomerization of both Aβ-P (1-40) and Aβ-P (1-42) in primary neuron cultures.\textsuperscript{26}

There are conflicting findings with regard to the role of ACE in Aβ-P deposition. Some \textit{in vitro} studies have shown that ACE inhibitors increase, rather than decrease, Aβ deposition.\textsuperscript{37,38} This may be explained by the fact that brain ACE is not specific for angiotensin-I, but it can catabolize other amyloid peptides.\textsuperscript{39} Hu et al.\textsuperscript{37} reported that human ACE inhibited Aβ-P aggregation deposition and fibril formation \textit{in vitro}. This protective effect was nullified if the ACE inhibitor, lisinopril, was added to the cultures.

Taken together, perindopril may have short-term cognition-enhancing properties, as shown in our study. However, the long term effect of ACE inhibitors on Aβ-P brain burden need to be more assessed.

CONCLUSION

From the aforementioned results it could be concluded that both candesartan and perindopril can reverse the free radical induced damages and resultant learning and memory defects seen in this animal model of AD, as they share a common Ang II-blunting effect, antioxidant and free radical scavenging properties. However, the ARB; candesartan may have an additional protective effect over the ACEI; perindopril, as evidenced by decrease Aβ-P concentration in brain tissues.

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**Conflict of interest:** None declared

**Ethical approval:** The study was approved by the Institutional Animal Ethics Committee

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