Investigating the Role and Mechanism of Octreotide in Long Standing Diabetes-induced Cognitive Impairment in Rats

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ABSTRACT

Background: Octreotide is a somatostatin analogue and it produces beneficial effects in diabetic-neuropathy. Studies have also shown its beneficial role in Alzheimer disease. However, the role of octreotide in diabetes-induced cognitive impairment is not explored yet. Aim: The present study was designed to explore the role and mechanism of octreotide in long standing diabetes-induced cognitive impairment in an experimental model. Materials and Methods: Streptozotocin (60 mg/kg)-injected rats were kept for 10 weeks to induce the development of cognitive impairment, which was assessed using Morris Water Maze test. The learning was assessed by comparing the escape latency time (ELT) of day 1 with ELT of day 4. The memory was assessed on the 5th day by measuring the time spent in target quadrant (TSTQ). Three different doses of octreotide (10, 20 and 40 µg/kg) were administered for the last two weeks. The levels of Nrf2, reduced glutathione, TBARS, IL-1 and TNF-α were measured in rat brain homogenates.

Results: Treatment with octreotide for two weeks led to significant increase in day 4 ELT and day 5 TSTQ in streptozotocin-injected rats suggesting the improvement in learning and memory. Moreover, octreotide attenuated streptozotocin-induced increase in neuroinflammation and oxidative stress. It also increased the nuclear: cytoplasmic ratio of Nrf2 suggesting the effect of octreotide in increasing the levels of endogenous antioxidants.

Conclusion: Octreotide has the potential to improve learning and memory in long standing diabetes-induced cognitive dysfunction and its beneficial effects may be possibly attributed to decrease in neuroinflammation and oxidative stress.

Key words: Oxidative stress, Diabetes, Memory, Octreotide, Neuroinflammation, Learning.

INTRODUCTION

Diabetes mellitus is a chronic metabolic disorder, which is characterized by persistent hyperglycemia and associated complications. Long standing diabetes mellitus is documented to induce a number of complications including the development of nephropathy, neuropathy, retinopathy, microvascular changes.$¹$ Along with it, persistent hyperglycemia may also produce cognitive impairment and there may be defective learning and memory in diabetic patients.$²$ Presently, there is no specific remedy to prevent or treat cognitive impairment in the diabetic condition. Accordingly, there is a need to identify new pharmacological agents to mitigate long standing diabetes-induced cognitive impairment.

Octreotide is an octapeptide and it acts as somatostatin analogue.$³$ Apart from acting as typical growth hormone inhibitor, somatostatin acts to produce diverse physiological functions. Indeed, somatostatin acts as a neuropeptide and produces analgesic, anti-inflammatory, and antidepressant effects without endocrine actions.$⁴$ Research studies have elaborated the wide spectrum of actions of somatostatin and its analogue, octreotide in a number of disease models. Their usefulness in cardiac injury, neuropathic
pain, cancer, pancreatic fibrosis, obesity and acute kidney injury has been demonstrated.\textsuperscript{5,7} Moreover, it is also found to alter the state of diabetic complications including neuropathic pain\textsuperscript{9} and endothelial dysfunction.\textsuperscript{9} Apart from it, octreotide is also found to be useful in improving the state of memory in Alzheimer disease.\textsuperscript{10} However, the role of octreotide in long standing diabetes-induced cognitive dysfunction is not explored yet. Therefore, the present study was designed to explore the role and mechanism of octreotide in long standing diabetes-induced cognitive impairment in an experimental model.

**MATERIALS AND METHODS**

**Animals and Drugs**

In this investigation, Male Wistar albino rats (220-270 g) of 4 months age were employed and maintained on the standard laboratory conditions. The experimental protocol was approved by Ethic Committee of Ninth Hospital of Xi’an, Ethic Number: 2020049. Octreotide, glucose oxidase-based colorimetric kit, lipid peroxidation assay kit and reduced glutathione colorimetric detection kit were procured from Sigma-Aldrich, USA. The ELISA kits for the quantification of IL-1, TNF-\(\alpha\) and Nrf2 were procured from abcam, USA. The extraction kit to separate nuclear and cytoplasmic fractions of Nrf2 was procured from BioVision, USA.

**Streptozotocin-induced diabetes mellitus and measurement of plasma glucose levels**

A single intravenous dose of streptozotocin (STZ) (60 mg/kg) was injected to Wistar albino rats to induce the development of diabetes mellitus.\textsuperscript{11} STZ-injected animals were kept for ten weeks to develop cognitive impairment as a diabetic complication. The fasting plasma glucose levels were assessed before STZ injection and at the end of 8th week using commercially available kit based on glucose oxidase method.

**Learning and memory in the Morris Water Maze test**

In the last (10th) week, learning and memory was assessed on five consecutive days starting on 3rd day and ending on 7th day of week using the Morris Water Maze test.\textsuperscript{12,13} In first four days of trials (3rd day to 6th day) escape latency time (ELT) was assessed and day 1 ELT was compared to day 4 ELT. The decrease in ELT on day 4 describes the learning ability of rats. On the 5th day of trial, the time spent in the target quadrant was assessed. The increase in time spent in the target quadrant signifies the increase in memory.

**Assessment of oxidative and neuroinflammatory markers in the brain**

After the last episode of trial on the Morris Water Maze, the rats were sacrificed and brain was isolated. One portion of the brain was homogenized in the phosphate buffer saline (PBS, pH: 7.4) and centrifugation at 5500 g for 20 min to get the clear supernatant solution. The neuroinflammatory markers including IL-1 and TNF-\(\alpha\) were measured in the brain supernatant using commercially available ELISA kits. The levels of oxidative stress including thiobarbituric acid reactive substances (TBARS) and reduced glutathione were assessed quantitatively using commercially available colorimetric kits. The protein levels were estimated in the brain homogenate using Polin Lowry method.\textsuperscript{14} The other half portion of the brain was used to quantify nuclear: cytoplasmic ratio of Nrf2. The nuclear and cytoplasmic fractions were separated using an extraction kit (BioVision, USA) and the levels of Nrf2 were assessed in the nuclear and cytoplasmic fractions using commercially available ELISA kits.

**Experimental Design**

In this investigation, five experimental groups were employed. Each experimental group consisted of 8 rats. Non-diabetic animals in group I were kept for eight weeks. In the last five days of 8th week, rats were subjected to five consecutive day trials on the Morris Water Maze test to assess learning and memory. Afterwards, animals were sacrificed to remove brain, which was homogenized to measure biochemical parameters. Diabetic animals in group II were kept for eight weeks. The rest of the protocol was same as described in group I. In groups III, IV and V, octreotide 10, 20 and 40 \(\mu\)g/kg (intraperitoneal route) was administered in diabetic animals for the last two weeks of experimental protocol. The rest of the protocol was same as described in group I. There is a large variation in the previously published studies regarding the doses of octreotide in rats. The doses ranging from 1 to 10 \(\mu\)g/kg in cholera toxin-stimulated intestinal secretions;\textsuperscript{15} 20 \(\mu\)g/kg in doxorubicin-induced cardiac injury,\textsuperscript{16} 40 \(\mu\)g/kg in stress-induced liver injury,\textsuperscript{17} 30 \(\mu\)g/kg in spinal cord injury model;\textsuperscript{18} 50, 100 or 200 \(\mu\)g/kg in ischemia-reperfusion injury.\textsuperscript{19,20} Since there have been studies showing the beneficial effects of octreotide with low doses like 10, 20, 30 and 40 \(\mu\)g/kg, therefore, in this study the doses range of 10, 20 and 40 \(\mu\)g/kg was selected for the present study.
Statistical Analysis

In this study, data were represented using Mean±S.D. The data of ELT and plasma glucose levels were compared using Two Way ANOVA, while the data of other parameters were statistically compared using One-Way ANOVA. Tukey’s post hoc test was employed for multiple comparisons. p<0.05 was considered to be statistically significant.

RESULTS

Increase in the plasma glucose levels in STZ-injected rats

A single dose of STZ led to significant elevation in the plasma glucose levels as assessed at the end of the 8th week in comparison to basal (before STZ injection). Treatment with different doses of octreotide (10, 20 and 40 µg/kg) in the last two weeks of STZ-injected rats did not modulate the plasma glucose levels in a significant manner (Figure 1).

Modulation of impairment in learning and memory by octreotide in STZ-injected rats

There was a significant decline in the day 4 ELT in normal, non-diabetic rats in comparison to day 1 ELT indicating the normal learning ability (acquisition) (Table 1). Moreover, there was a significant increase in day 5 TSTQ in non-diabetic rats on the day 5 of the Morris Water Maze test, suggesting the normal retrieval of memory (Figure 2). However, there was no significant change in the day 4 ELT in comparison to day 1 ELT in STZ-injected rats, suggesting the impairment in learning ability. Moreover, the day 5 TSTQ was significantly decreased in STZ-treated rats in comparison to non-diabetic rats, suggesting the impairment in memory. Treatment of STZ-injected rats with the different doses of octreotide (10, 20 and 40 µg/kg) for two weeks led to a significant improvement in learning and memory in a dose-dependent manner. In octreotide-treated rats, there was a significant increase in the day 4 ELT (Table 1) and day 5 TSTQ (Figure 2) suggesting the improvement in the acquisition (learning) and retrieval (memory).

Influence of octreotide on STZ-induced neuroinflammation and oxidative stress in rat brains

In long standing diabetes rats, there was a significant increase in neuroinflammation in the brain homogenates as assessed by an increase in the levels of IL-1 (Figure 3) and TNF-α (Figure 4) in comparison to non-diabetic rats. Moreover, there was an increase in oxidative stress in the brains of STZ-treated rats as assessed by an increase in the TBARS (Figure 5) and decrease in the levels of reduced glutathione (Figure 6). Along with it, there was a significant decrease in the nuclear: cytoplasmic ratio of Nrf2 (Figure 7) suggesting the decrease in endogenous

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Table 1: Effect of different interventions on the escape latency time (ELT) measured in seconds (s) in Morris Water Maze test.

<table>
<thead>
<tr>
<th>S. No</th>
<th>Groups</th>
<th>Day 1 ELT (s)</th>
<th>Day 4 ELT (s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Non-diabetic</td>
<td>82.5 ± 3.2</td>
<td>33.2 ± 2.6</td>
</tr>
<tr>
<td>2.</td>
<td>Diabetic</td>
<td>95.3 ± 4.4</td>
<td>75.5 ± 3.7</td>
</tr>
<tr>
<td>3.</td>
<td>Octreotide (10 µg) in diabetic</td>
<td>81.3 ± 2.6</td>
<td>34.5 ± 2.5</td>
</tr>
<tr>
<td>4.</td>
<td>Octreotide (20 µg) in diabetic</td>
<td>85.6 ± 2.8</td>
<td>31.5 ± 1.7</td>
</tr>
<tr>
<td>5.</td>
<td>Octreotide (40 µg) in diabetic</td>
<td>84.2 ± 3.4</td>
<td>32.3 ± 2.4</td>
</tr>
</tbody>
</table>

Figure 1: Effect of different treatments on the plasma glucose levels, assessed at the start of experiment (basal) and at the end of experiment (8th week). Values are expressed as Mean ± SD. a = p< 0.05 vs. non-diabetic.

Figure 2: Effect of different treatments on the time spent in target quadrant (TSTQ), assessed on the fifth day of trial on the Morris Water Maze test. Values are expressed as Mean ± SD. a = p< 0.05 vs. non-diabetic, b = p< 0.05 vs. diabetic.
ability to protect from oxidative stress. Treatment of STZ-injected rats with octreotide (10, 20 and 40 µg/kg) led to significant attenuation of neuroinflammation and oxidative stress in a dose-dependent manner. There was a significant decline in the levels of IL-1, TNF-α, TBARS along with an increase in the reduced glutathione levels and Nrf2 ratio.

**DISCUSSION**

In the present study, a single injection of STZ led to the development of diabetic complications, which was manifested in the form of impairment in learning and memory. In STZ-injected rats, there was no significant difference in the day 4 ELT in comparison to day 1 ELT.
suggesting that STZ-injected rats failed to learn in the fours trials in the Morris Water Maze test. Moreover, there was no increase in day 5 TSTQ in the target quadrant in long standing diabetic rats in comparison to non-diabetic rats, which suggests that there was an impairment in retrieval process i.e. memory in diabetic rats. There have been previous studies showing that long standing diabetes leads to cognitive impairment in STZ-injected rats and the present study results are in consonance with previously published studies.22,23

In this study, treatment with octreotide led to significant improvement in learning and memory of STZ-treated rats as there was a significant increase in day 4 ELT and day 5 TSTQ in octreotide-treated rats. Octreotide is a somatostatin analogue and a large number of studies have found its usefulness in the management of ischemia-reperfusion injury,24 pancreatic injury, liver injury,17 and heart injury.16 Moreover, it has also been shown to attenuate cerebral injury in experimental stroke model.25 The role of octreotide in preventing STZ-induced diabetic neuropathic pain has also been reported.8 Along with it, octreotide is also reported to improve memory in the patients of Alzheimer's disease.10 However, it is the first report documenting the protective role of octreotide in long standing diabetes-induced impairment in learning and memory.

In this study, long standing diabetes was also associated with significant alterations in the biochemical milieu in the rat brains. In STZ-injected rats, there was an increase in the levels of IL-1 and TNF-α in the brain suggesting the presence of neuroinflammation in response to long standing diabetes. Moreover, there was a decrease in the levels of reduced glutathione along with the decrease in nuclear: cytoplasmic ratio of Nrf2 suggesting the presence of oxidative stress in response to diabetes. The significant increase in the brain levels of TBARS also indicates the presence of oxidative stress. There have been studies showing that diabetes-induces neuroinflammatory changes that may contribute in inducing cognitive decline.22 Moreover, the significant increase in the levels of oxidative stress in the brain in response to long standing diabetes has also been reported.26

In this study, along with the improvement in cognitive functioning, octreotide also attenuated diabetes-induced neuroinflammatory changes and oxidative stress. Indeed, octreotide led to significant decline in IL-1, TNF-α, TBARS levels along with increase in reduced glutathione levels and Nrf2 ratio. There have been studies showing that octreotide attenuates inflammation27 and reduces oxidative stress28 to produce beneficial effects in a number of disease states. However, it is the first report documenting the beneficial effects of octreotide in attenuating neuroinflammatory changes and oxidative stress in the rat brain. Octreotide is also shown to produce biological effects through Nrf2 signalling.29 Therefore, it is possible to suggest that octreotide may inhibit neuroinflammation and oxidative stress to attenuate the deleterious effects of diabetes on learning and memory.

It is important to mention that in this study octreotide did not modulate STZ-induced increase in glucose levels, but attenuated long standing diabetes-induced neuroinflammation and oxidative stress. It suggests that octreotide-mediated beneficial effects are independent of glucose lowering effects. There have been studies showing the different pharmacological agents may attenuate diabetes-induced deleterious effects without reducing glucose levels.30,31 Therefore, it is possible that octreotide-mediated direct antioxidant effects and anti-inflammatory effects may contribute in attenuating diabetes-induced cognitive decline in rat model of diabetes. The major limitation of this study is that histopathological changes were not performed in the brain of diabetic rats because the brains were employed for biochemical testing. More studies may be done in future projects to explore the effects of octreotide on histopathological changes in the brains of diabetic rats.

CONCLUSION
Octreotide has the potential to improve learning and memory in long standing diabetes-induced cognitive dysfunction in a dose-dependent manner and its beneficial effects may be possibly attributed to decrease in neuroinflammation and oxidative stress.

CONFLICT OF INTEREST
The authors declare no conflict of interests.

ABBREVIATIONS
ELT: Escape latency time; PBS: Phosphate buffer saline; STZ: Streptozotocin; TBARS: Thiobarbituric acid reactive substances; TSTQ: Time spent in target quadrant.

REFERENCES
Octreotide in Diabetes-induced Cognitive Impairment

Yuan and Feng.


The present study investigated the role of octreotide in long standing diabetes-induced cognitive impairment in rats. Diabetes was induced in rats by injection of STZ and cognitive impairment was assessed by Morris Water Maze test after 10 weeks of diabetes induction. Treatment with octreotide decreased ELT and increased TSTQ in suggesting improvement in learning and memory. Octreotide increased the levels of Nrf2, reduced glutathione and attenuated the levels of TBARS, IL-1 and TNF-α suggesting the reduction in oxidative stress and neuroinflammation. It is concluded that octreotide may improve learning and memory in long standing diabetes-induced cognitive dysfunction, which may be due to decrease in neuroinflammation and oxidative stress.

About Authors

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