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Original Article

The Preventive Effect of Oxytocin on Retinopathy in Streptozotocin-Induced Diabetic Rats

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Abstract

Objectives: The aim of this study was to investigate the impact of intravitreal and intraperitoneal use of oxytocin (OT) on retinopathy in streptozotocin-induced diabetic rats.

Materials and Methods: Twenty-four 6–8-week-old adult male and female Sprague Dawley rats were used in the study. Diabetes was induced in the rats with a single injection of intraperitoneal streptozotocin. Diabetes was verified after 48 hours by measuring blood glucose levels of 260 mg/dl (14.4 mmol/L) or higher in diabetic rats. The rats were divided into 4 groups and treated as follows: intravitreal physiological saline group (0.01 mL saline weekly), intravitreal OT group (10 µU/µL OT weekly), intraperitoneal physiological saline group (1 mL daily), and intraperitoneal OT group (100 IU/kg OT daily). Hamilton syringes fitted with 27-gauge needles were used for intraperitoneal injections while 31-gauge needles were used for intravitreal injection. After 4 weeks of treatment the rats were euthanized to evaluate outer nuclear layer (ONL) thickness, vascular endothelial growth factor (VEGF) immunoexpression, and plasma VEGF levels from blood samples obtained by cardiac puncture.

Results: Morphometric analysis of retinal cross-sections showed that intravitreal and intraperitoneal OT significantly increased ONL thickness compared to physiological saline-treated groups. Also, OT treatment significantly decreased VEGF protein expression compared with the physiological saline groups. Plasma VEGF level was significantly higher in the physiological saline treatment group compared to the OT treatment group.

Conclusion: OT reduces diabetic retinopathy progression, particularly when administered intravitreally. To our knowledge, this is the first attempt to investigate the impact of OT on diabetic retinopathy and may provide a new area for further research.

Keywords: Immunohistochemistry, oxytocin, retinopathy, streptozotocin, VEGF

Introduction

Diabetes mellitus is a progressive disease that afflicts over 230 million people worldwide. Diabetes affects both microvascular and macrovascular structures throughout the body and consequently can cause retinopathy, neuropathy, and nephropathy. Diabetic retinopathy (DR) is the leading cause of preventable blindness. Sustained hyperglycemia causes the blood vessels to swell and leak fluid, resulting in damage to the microvascular structure of the retina. This can result in retinal ischemia that leads to vascular endothelial growth factor (VEGF) secretion and the growth of immature, fragile new vessels. These new vessels lead to neovascularization and proliferative DR and result in macular edema, vitreous hemorrhages, and tractional retinal detachment.[1,2,3,4,5]

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Oxytocin (OT) is nonapeptide synthesized in the supraoptic and paraventricular nuclei of the hypothalamus. OT establishes its effects through the OT receptor, a G protein-coupled receptor. It stimulates uterine contractions at parturition, myoepithelial cell contraction in mammalian glands for milk ejection, and also has vasoconstrictor or vasodilator effects on different vascular beds.[6,7,8] Recent studies have also reported the anti-inflammatory and anti-oxidant effects of OT.[9,10] OT receptors have been found in cone photoreceptors and retinal pigment epithelium. OT exerts its effects by increasing intracellular levels of Ca^{+2}, which facilitates smooth muscle contraction, nitric oxide synthesis, prostaglandin production, activation of the MAP-kinase cascade, and protein synthesis.[11,12]

In view of these previous studies and observations, we aimed to detect the effect of intravitreal and intraperitoneal administration of OT in the retina of streptozotocin (STZ)-induced diabetic rats.

Materials and Methods

Animals

In this study, 24 adult male and female Sprague Dawley rats weighing 200–250 g were used. Animals were fed ad libitum and housed in pairs in steel cages having a temperature-controlled environment (22±2 ºC) with 12-hour light/dark cycles. The experimental procedures were approved by the Committee for Animal Research of Ege University. All animal studies strictly conformed to the Committee on Animal Research and Ethics guidelines. All chemicals were obtained from Sigma-Aldrich Inc. unless otherwise noted.

Experimental Protocol

Diabetes was induced by a single intraperitoneal injection of streptozocin (STZ) (Sigma-Aldrich, Inc., Saint Louis, MO) (60 mg/kg in 0.9% NaCl, adjusted to a pH 4.0 with 0.2 M sodium citrate). Diabetes was verified after 48 h by evaluating blood glucose levels with the use of glucose oxidase reagent strips (Boehringer Mannheim, Indianapolis). Rats with blood glucose levels of 260 mg/dl (14.4 mmol/L) and higher were included in this study as diabetic rats.

The 24 rats were equally divided into 4 groups as follows: intravitreal physiological saline group, intravitreal OT group, intraperitoneal physiological saline group, and intraperitoneal OT group. The rats in the intraperitoneal groups were treated daily with either 1 mL physiological saline or 100 IU/kg OT intraperitoneally; the rats in the intravitreal groups received topical anesthesia with proparacaine hydrochloride followed by 0.01 mL physiological saline or 10 µU/µL OT in weekly intravitreal injections. Hamilton syringes fitted with 27-gauge needles were used for intraperitoneal injections while 31-gauge needles were used for intravitreal injections. After 4 weeks of treatment the rats were euthanized and blood samples were collected by cardiac puncture for enzyme-linked immunosorbent assay (ELISA) of plasma VEGF levels in the intraperitoneal treatment groups, then enucleation was performed in all groups.

Immunohistochemistry

Cross-sections 2 µm in thickness were taken with a microtome (Leica MR 2145) from paraformaldehyde-fixed paraffin-embedded eye tissues, floated in a sterile bath, placed onto poly-L-Lysine-coated glass slides, and dried at room temperature. After overnight incubation at 60 °C, the slides were dewaxed in xylene for 30 min, rehydrated through a graded ethanol series (100%, 95%, 80%, and 70%, sequentially), washed in distilled H_2O and PBS for 10 min, treated with 2% trypsin containing 50 mM Tris buffer (pH 7.5) at 37 °C for 15 min, and then washed again with PBS. Sections were delineated with a Dako pen (Dako, Glostrup, Denmark), incubated in 3% H_2O_2 solution for 15 min to inhibit endogenous peroxidase activity, and washed with PBS. The slides were incubated with VEGF primary antibody at 57 °C followed by washing with PBS. Afterwards, a biotinylated secondary IgG antibody was applied and washed with PBS before incubating with the streptavidin-peroxidase conjugate (Histostain Plus, Invitrogen, Camarillo, CA, USA) for 30 min to visualize the immunostaining. The whole procedure was finished after counterstaining the sections with Mayer's hematoxylin (Sigma Chemical Co., St. Louis, MO, USA). All sections were examined and photographed with an Olympus C-5050 digital camera mounted on an Olympus BX51 microscope (Olympus Corp., Tokyo, Japan).

Outer Nuclear Layer (ONL) Measurements

All sections were photographed and measured with the same Olympus C-5050 digital camera mounted on an Olympus BX51 microscope. The mean ONL thickness in the physiological saline groups was accepted as 100%.

Measurement of Plasma VEGF Levels

Plasma VEGF levels were measured using a commercially available ELISA kit according to the manufacturer's instructions (RayBiotech, Inc., GA, USA). VEGF levels were expressed in pg/mL. The detection limit was less than 2 pg/mL, and intra-assay and interassay coefficients of variation were less than 10%.

Statistical Analysis

Data analyses were performed using SPSS version 15.0 for Windows (SPSS Inc., Chicago, IL). The groups of parametric variables were compared using Student's t-test. The groups of non-parametric variables were compared with the Mann-Whitney U test. The results were reported as mean ± standard error of mean. A value of $p<0.05$ was accepted as statistically significant.

Results

VEGF protein expression was examined by immunohistochemistry and ONL thickness was measured. The expression was scored as follows: 0 represented no expression while 1, 2, and 3 represented expressions of 0-24%, 25-49%, 50-74%, and >75%. All comparisons of ONL measurements and staining intensities were carried out at X40 magnification from 10 different sections.

ONL Measurements

Figure 1 represents the alterations in retinal ONL thickness in the study groups. Results from the comparison of ONL thickness between groups is shown in Table 1. Morphometric analysis of the rat retinal cross-sections showed that intravitreal and intraperitoneal OT significantly increased ONL thickness compared to physiological saline-treated groups (23% and 25%, respectively, $p<0.001$).

VEGF Protein Expression

Figure 2 demonstrates VEGF protein expression in the rat retina after cessation of treatment. The mean score for VEGF expression in the intraperitoneal physiological saline group was 1.6±0.2, which decreased significantly to 0.3±0.2 in the intraperitoneal OT group. In the intravitreal treatment groups, the VEGF expression score was 1.3±0.3 in the physiological saline group, which decreased significantly to 0.3±0.2 in the OT group.

Plasma VEGF Levels

Plasma VEGF level was 161.29±47.36 pg/mL (range: 115.75-225.25) in the intraperitoneal saline group and 76.74±14.15 pg/mL (range: 25.50-142.75) in the intraperitoneal OT group. There was a statistically significant difference between the groups.

Discussion

To the best of our knowledge, this is the first report describing the effects of OT on the retina of diabetic rats. The study revealed that OT has protective effects on diabetic rat retina, as evidenced by reduced VEGF protein expression and plasma VEGF levels, as well as prevention of outer nuclear layer thinning.

As the pathogenesis of DR is better understood, novel treatment options are likely to become available. Postulated mechanisms of DR are hyperglycemia, accumulation of advanced glycation end-products (AGEs), activation of protein kinase C, oxidative stress, and inflammation. Chronic hyperglycemia results in the production of reactive oxygen species, and low-grade inflammation. This induces apoptosis of the retinal pigment epithelium and progression of DR. Hyperglycemia also causes accumulation of AGEs beneath the endothelial layer and changes the vascular structure, increases vascular stiffness, and initiates intracellular signaling pathways that lead to increased oxidative stress and inflammation. Oxidative stress is considered to be the most common mechanism in the etiology of DR. Damage or dysfunction due to oxidative stress can proceed even after glycemic control. Inflammation also plays an important role in the progression of DR and its complications. Therefore, new treatment strategies should be developed that target oxidative stress and inflammation.[1,13,14,15,16,17,18,19,20]

Table 1. Comparison of outer nuclear layer thickness (x40 magnification)

Groups	Intraperitoneal (%)	Intravitreal (%)
Physiologic saline	100±5.1	100±8.3
Oxytocin	115.6±6.1	128.8±7.5

OT is nonapeptide and its receptors have been identified in different tissues including kidney, heart, pancreas, adipocytes, and thymus. The role of OT in immune and inflammatory modulation is well defined and attributed to the activation of its receptors.[6,7,8,21] Based on these studies, we hypothesized that OT may act as an antioxidant and anti-inflammatory agent and therefore serve as a therapeutic agent in DR. Cone photoreceptors and the retinal pigment epithelium have OT receptors. Activation of those receptors causes an increase in intracellular Ca^{+2} level and downstream activation of phospholipase C and phosphatidylinositol 4,5-bisphosphate.[13,14]

In diabetic patients, sustained hyperglycemia can cause dysregulation in retinal blood flow, loss of pericytes, basal membrane thickening, microaneurysms, capillary occlusion, and ischemia. Ischemia eventually leads to the production of VEGF

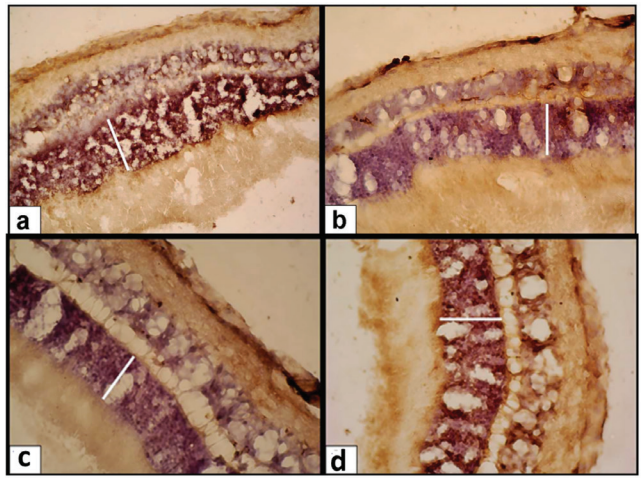

Figure 1. Outer nuclear layer of the a) intraperitoneal physiologic saline group, b) intravitreal physiologic saline group, c) intraperitoneal oxytocin group, and d) intravitreal oxytocin group

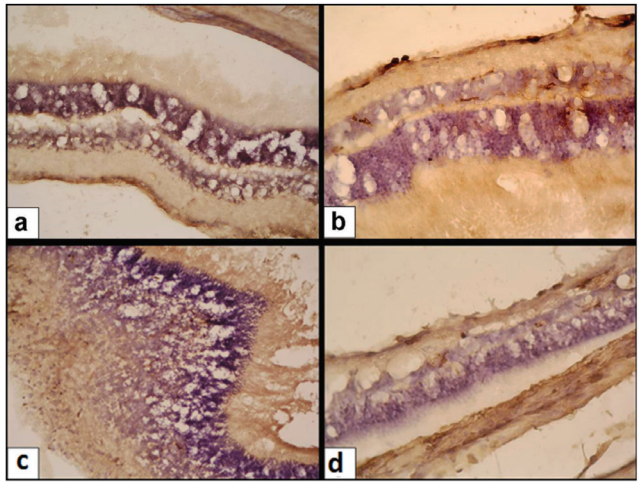

Figure 2. Vascular endothelial growth factor immunostaining in the a) intraperitoneal physiologic saline group, b) intravitreal physiologic saline group, c) intraperitoneal oxytocin group, and d) intravitreal oxytocin group

that activates tyrosine kinase receptors, VEGFR-1 and VEGFR-2.[22] VEGF is a growth factor and potent vasoactive cytokine that promotes angiogenesis, breakdown of the blood-retinal barrier, and induces endothelial cell growth and neovascularization.[23,24] Matsuoka et al.[25] reported that expression of VEGF in diabetic retinas was significantly increased while Funatsu et al.[26] suggested that VEGF levels in vitreous and plasma were elevated in diabetic patients with retinopathy when compared to normal subjects and diabetic patients without retinopathy. Consistent with this opinion, Aiello et al.[27] found that VEGF concentration was elevated in the ocular fluids of patients with retinal ischemia. The present study showed that VEGF expression in retina and plasma VEGF levels were elevated, but with OT treatment, the expression and plasma levels were significantly decreased.

Thinning of the outer nuclear layer has been reported in diabetic rats. A number of studies have implicated apoptosis for the thinning effect.[28,29,30] In the present study, in accordance with published reports, administration of OT was found to prevent the thinning of the ONL in STZ-induced diabetic rats when compared to the physiological saline group.

Conclusion

In conclusion, the treatment of STZ-induced diabetic rats with OT was effective in mitigating retinal degeneration. The significant reduction in VEGF expression and plasma VEGF levels and the protective effect against retinal thinning suggest that OT may be an alternative treatment in diabetic retinopathy. The beneficial effects of OT in diabetic retinal degeneration might be through its anti-oxidative and anti-inflammatory effects. To our knowledge this is the first report about the effect of OT on the retina of diabetic rats, and this subject needs to be explored with further studies.

Ethics

Ethics Committee Approval: Ege University Faculty of Medicine Ethic Committee, (2011-162).

Informed Consent: Experimental animal research.

Peer-review: Externally and internally peer-reviewed.

Authorship Contributions

Surgical and Medical Practices: Cumali Değirmenci, Concept: Cumali Değirmenci, Filiz Afrashi, Design: Oytun Erbaş, Cumali Değirmenci, Data Collection or Processing: Cumali Değirmenci, Filiz Afrashi, Analysis or Interpretation: Hüseyin Aktuğ, Dilek Taşkıran, Literature Search: Cumali Değirmenci, Filiz Afrashi, Hüseyin Aktuğ, Dilek Taşkıran, Oytun Erbaş, Writing: Cumali Değirmenci, Filiz Afrashi.

Conflict of Interest: No conflict of interest was declared by the authors.

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References

1. Kowluru RA, Chan PS. Oxidative stress and diabetic retinopathy. Exp Diabetes Res. 2007;2007:43603.
2. Semeraro F, Cancarini A, dell'Omo R, Rezzola S, Romano MR, Costagliola C. Diabetic Retinopathy: Vascular and Inflammatory Disease. J Diabetes Res. 2015;2015:582060.
3. Ding J, Wong TY. Current epidemiology of diabetic retinopathy and diabetic macular edema. Curr Diab Rep. 2012;12:346-354.
4. Stewart MW. The clinical utility of aflibercept for diabetic macular edema. Diabetes Metab Syndr Obes. 2015;8:473-482.
5. Behl T, Kaur I, Kotwani A. Implication of oxidative stress in progression of diabetic retinopathy. Surv Ophthalmol. 2016;61:187-196.
6. Iseri SO, Sener G, Sağlam B, Gedik N, Ercan F, Yeğen BC. Oxytocin ameliorates oxidative colonic inflammation by a neutrophil-dependent mechanism. Peptides. 2005;26:483-491.
7. Akman T, Akman L, Erbas O, Terek MC, Taskiran D, Ozsaran A. The preventive effect of oxytocin to Cisplatin-induced neurotoxicity: an experimental rat model. Biomed Res Int. 2015;2015:167235.
8. Gimpl G, Fahrenholz F. The oxytocin receptor system: structure, function, and regulation. Physiol Rev. 2001;81:629-683.
9. Rashed LA, Hashem RM, Soliman HM. Oxytocin inhibits NADPH oxidase and P38 MAPK in cisplatin-induced nephrotoxicity. Biomed Pharmacother. 2011;65:474-480.
10. Erbas O, Ergenoglu AM, Akdemir A, Yeniel AÖ, Taskiran D. Comparison of melatonin and oxytocin in the prevention of critical illness polyneuropathy in rats with experimentally induced sepsis. J Surg Res. 2013;183:313-320.
11. York N, Halbach P, Chiu MA, Bird IM, Pillers DM, Pattnaik BR. Oxytocin (OXT)-stimulated inhibition of Kir7.1 activity is through PIP(2)-dependent Ca(2+) response of the oxytocin receptor in the retinal pigment epithelium in vitro. Cell Signal. 2017;37:93-102.
12. Halbach P, Pillers DA, York N, Asuma MP, Chiu MA, Luo W, Tokarz S, Bird IM, Pattnaik BR. Oxytocin expression and function in the posterior retina: a novel signaling pathway. Invest Ophthalmol Vis Sci. 2015;15;56:751-760.
13. Wan TT, Li XF, Sun YM, Li YB, Su Y. Recent advances in understanding the biochemical and molecular mechanism of diabetic retinopathy. Biomed Pharmacother. 2015;74:145-147.
14. Wang XL, Yu T, Yan QC, Wang W, Meng N, Li XJ, Luo YH. AGEs Promote Oxidative Stress and Induce Apoptosis in Retinal Pigmented Epithelium Cells RAGE-dependently. J Mol Neurosci. 2015;56:449-460.
15. Brownlee M. The pathobiology of diabetic complications: a unifying mechanism. Diabetes. 2005;54:1615-1625.
16. Chen M, Curtis TM, Stitt AW. Advanced glycation end products and diabetic retinopathy. Curr Med Chem. 2013;20:3234-3240.
17. Yu Y, Chen H, Su SB. Neuroinflammatory responses in diabetic retinopathy. J Neuroinflammation. 2015;12:141.
18. Kowluru RA, Mishra M. Oxidative stress, mitochondrial damage and diabetic retinopathy. Biochim Biophys Acta. 2015;1852:2474-2483.
19. Zong H, Ward M, Stitt AW. AGEs, RAGE, and diabetic retinopathy. Curr Diab Rep. 2011;11:244-252.
20. Deliyanti D, Zhang Y, Khong F, Berka DR, Stapleton DI, Kelly DI, Wilkinson-Berka JL. FT011, a Novel Cardiorenal Protective Drug, Reduces Inflammation, Gliosis and Vascular Injury in Rats with Diabetic Retinopathy. PLoS One. 2015;10:e0134392.
21. Petersson M, Wiberg U, Lundeberg T, Uvnas-Moberg K. Oxytocin decreases carrageenan induced inflammation in rats. Peptides. 2001;22:1479-1484.
22. Osaadon P, Fagan XJ, Lifshitz T, Levy J. A review of anti-VEGF agents for proliferative diabetic retinopathy. Eye (Lond). 2014;28:510-520.
23. Tarr JM, Kaul K, Chopra M, Kohner EM, Chibber R. Pathophysiology of diabetic retinopathy. ISRN Ophthalmol. 2013;2013:343560.
24. Kastelan S, Tomic M, Gverovic Antunica A, Salopek Rabatic J, Ljubic S. Inflammation and pharmacological treatment in diabetic retinopathy. Mediators Inflamm. 2013;2013:213130.
25. Matsuoka M, Ogata N, Minamino K, Matsumura M. Expression of pigment epithelium-derived factor and vascular endothelial growth factor in

fibrovascular membranes from patients with proliferative diabetic retinopathy. Jpn J Ophthalmol. 2006;50:116-120.
26. Funatsu H, Yamashita H, Ikeda T, Nakanishi Y, Kitano S, Hori S. Angiotensin II and vascular endothelial growth factor in the vitreous fluid of patients with diabetic macular edema and other retinal disorders. Am J Ophthalmol. 2002;133:537-543.
27. Aiello LP, Avery RL, Arrigg PG, Keyt BA, Jampel HD, Shah ST, Pasquale LR, Thieme H, Iwamoto MA, Park JE, et al. Vascular endothelial growth factor in ocular fluid of patients with diabetic retinopathy and other retinal disorders. N Engl J Med. 1994;331:1480-1487.
28. Boretsky A, Gupta P, Tirgan N, Liu R, Godley BF, Zhang W, Tilton RG, Motamedi M. Nicotine accelerates diabetes-induced retinal changes. Curr Eye Res. 2015;40:368-377.
29. Zhang J, Wu Y, Jin Y, Ji F, Sinclair SH, Luo Y, Xu G, Lu L, Dai W, Yanoff M, Li W, Xu GT. Intravitreal injection of erythropoietin protects both retinal vascular and neuronal cells in early diabetes. Invest Ophthalmol Vis Sci. 2008;49:732-742.
30. Park SH, Park JW, Park SJ, Kim KY, Chung JW, Chun MH, Oh SJ. Apoptotic death of photoreceptors in the streptozotocin-induced diabetic rat retina. Diabetologia. 2003; 46:1260-1268.