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ORIGINAL ARTICLE

The Effects of Systemic Coenzyme Q10 Treatment on Corneal Histology in Streptozocin-Induced Diabetic Rats

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ABSTRACT

Objective: This study investigate the histopathological changes and VEGF, IL-1β, and IL-6 immunoreactivities in cornea treated with Coenzyme Q10 (CoQ10) in a Streptozocin (STZ) induced diabetic rat model.

Methods: A total of 20 male Wistar Albino rats including a group of STZ diabetic rats, diabetic rats treated with CoQ10, rats were given CoQ10 without being diabetic and a Control group were included the study. The groups were followed up for 2 months. Eye tissues were stained with Hematoxylin-Eosin (HE), Periodic Acid-Schiff (PAS), and immunohistochemical staining (IHC).

Findings: The mean corneal thickness was found to be lower in the group with DM (126,62 ± 18,1) compared to the other groups. However, this decrease was found to be significant only in comparison with the control group (181,75 ± 13,87) ($p = 0.000$). In diabetic corneas, PAS positivity was observed in in Descemet's membrane ($p = 0.021$). Staining with VEGF, IL-1β, IL-6antibodies was found to be lower in the DM+CoQ10 group compared to the group with DM ($p < 0.001$, $p < 0.001$, $p < 0.001$).

Results: We observed that diabetes increases inflammation and tendency to angiogenesis in the corneal tissue, and CoQ10 treatment reduces the corneal thickness, inflammation, and tendency to angiogenesis caused by diabetes.

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Diabetes mellitus (DM) is an endocrine and metabolic disease common in all age groups on a worldwide scale and has high mortality rates in countries that have low socioeconomic status.[1–3] It reduces the quality of life with micro and macrovascular complications and annual mortality is approximately 5.4% in adult patients, which is twice that of non-diabetic adults.[4] Vasculogenesis (vascular system development) begins with the differentiation of the angioblasts, which are vascular endothelial cell precursors, into blood islets in the early stages of embryogenesis. The formation of new vessels from the vessels that develop with vasculogenesis is called angiogenesis. Angiogenesis provides vascularization of thin tissues such as kidney and retina during development and new vessel formation in adulthood, also playing active roles in conditions such as wound healing and inflammation.[5–7] Vascular Endothelial Growth Factor (VEGF) contributes to ocular neovascularization by stimulating pathological angiogenesis under the effect of long-term hypoxia in diabetic patients.[8,9]

Diabetes might cause a full-thickness epithelial defect in the cornea that can range from superficial erosion of the epithelium to subepithelial lesion.[10] In long-term diabetes, toxic glycosylation products accumulate in the basal membrane and this triggers cell death.[11] When the diabetic cornea is examined at the light microscopic level with PAS staining, it is seen that the epithelial basement membrane is thickened.[12] In a normal cornea, VEGF is expressed in keratocytes in the epithelium, endothelium, and stroma and its expression in the corneal epithelium is the same in the center and the periphery.[13] It is already known that VEGF and other growth factors and various cytokine levels increase in diabetic corneas.[14] With the effect of hyperglycemia, cytokines such as VEGF, IL-1, etc. increase not only in the serum but also locally in the damaged area.[15] Increased Interleukin-1 (IL-1) induced by high blood glucose plays roles in the pathogenesis of pancreatic beta-cell damage. The treatment for IL-1 inhibition reverses this situation and provides an improvement in blood glucose levels and beta-cell functions.[16–18] Another proinflammatory cytokine whose level increases in hyperglycemia is Interleukin-6 (IL-6). Although hyperglycemia improves, the levels of these two cytokines remain high for approximately the first 15 days.[19] Proinflammatory cytokine values increase further in the presence of diabetic complications. Diabetic patients who have renal complications also have a higher level of IL-6 in the blood compared to diabetics without such complications.[20]

Various histopathological changes such as corneal epithelial fragility and erosions, stromal edema, non-

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healing ulcers, decreased corneal sensitivity, and neurotrophic ulcerations are among the causes of decreased visual acuity in diabetic patients.[21,22] Various agents are employed to treat these effects; however, even initiating the treatment of diabetes in the early period and antidiabetic drugs can not prevent these effects from occurring in the long term. In the present study, the purpose was to investigate the histological effects of CoQ10, which is an anti-oxidant compound responsible for mitochondrial ATP production, on STZ diabetic rat corneas by using HE and PAS staining; and its inflammatory effects by using VEGF, IL-1β, and IL-6 antibodies.

Material and method

Experimental animals

In our study, a total of 20 male Wistar Albino rats with body weights ranging between 200 and 250 g were used. The power analysis showed that the minimum number of samples required to find a statistically significant difference of 0.7 between the two groups was 5 rats for each group (a = 0.05), Test Power = (1-b = 0.80). The animals were fed in standard plastic cages in a laboratory environment at a temperature of 21 ± 1°C and a humidity of 45–55% under standard conditions with free access to water and food with the maintenance of a natural day/night cycle (12/12 hours) in Afyon Kocatepe University Experimental Animals Unit. The experiment plan was made by dividing the rats, who were aged 2 months, into 4 groups as specified below.

The DM Group (n = 5): Rats that were made diabetic by administering intraperitoneal STZ at a dose of 50 mg/kg (DM)

DM+CoQ10 Group (n = 5): Rats that were made diabetic by administering intraperitoneal STZ at a dose of 50 mg/kg, after rats became diabetic, 0.35 mg CoQ10 dissolved in 50 µl pure olive oil was given daily for 2 months by gavage (DM +CoQ10)

CoQ10 Group (n = 5): Rats that were given 0.35 mg CoQ10 dissolved in 50 µl pure olive oil daily for 2 months by gavage without diabetics (CoQ10 alone).

Control (n = 5): Control group that was not diabetic and not given "CoQ10"

Induction of diabetes in rats by streptozocin (STZ)

In this study, STZ was dissolved in 0.1 M buffer citrate (pH:4.5) and given to the DM and DM+CoQ10 groups by intraperitoneal (ip) injection as a single dose (50 mg/kg) according to their body weight. Blood glucose values of the rats were measured with a glucometer 3 days after the STZ injection. Rats with fasting blood glucose of 200 mg/dL and above were considered diabetic.[23]

Histologic evaluation

Histologic monitoring

After the sacrification of rats, eyes were enucleated and eyes tissues obtained were placed in 10% neutral buffered formalin and fixed for 3 days. Later, tissue labeling was performed and the tissues were kept under running water for 1 night. The tissues removed from water were kept in 70%, 80%, and 90% pure alcohol consecutively for one hour each, in a xylene+alcohol mixture for 15 minutes, and in xylene twice for 15 minutes each. After the tissues were kept in liquid paraffin for 1 hour, they were embedded in paraffin blocks. 5 µm thickness sections were obtained from these blocks. Sections were taken serially from the optic nerve level and were placed on normal slides for HE and PAS staining and Poly-L-Lysine coated slides for IHC staining.

HE and PAS staining

After the 0.5 µm thickness sections placed on slides were deparaffinized, they were rehydrated and stained using the HE and PAS staining procedures. Then, the preparations were dehydrated and closed with entellan.

Immunohistochemical examination of the corneal tissues

For immunohistochemical evaluation, the sections placed on Poly-L-Lysine coated slides were deparaffinized and rehydrated. Then, microwave antigen retrieval was performed with 10% citrate buffer solution and the sections were kept in 3% H_2O_2 (Hydrogen Peroxide) for 20 minutes. Primary antibodies VEGF (Recombinant Anti-VEGFA antibody, ab52917, 1/100), IL-1β, (Recombinant Anti-IL-1 beta antibody, ab283818, 1/200), IL-6 (Anti-IL-6 antibody, ab9324, 1/250), antibodies were incubated at +4°C overnight, HRP secondary antibody kit (HRP Monoclonal Antibody) was applied on the next day and the tissues were colored with 3-Amino-9-ethylcarbazole (AEC) chromogen. Later, contrast staining of the preparations was performed with Mayers Hematoxylin, and they were dehydrated and closed with entellan.

Evaluation of the preparations and image analysis

The analysis program "Nikon NIS 4.2 Image Analysis Software" was used for image analysis. For corneal thickness measurement, the eye tissues were evaluated by staining the sections obtained sagitally from the level of the optic nerve, and in sagittal corneal sections, measurements were taken from 3 different regions of the cornea and Descemet's Membrane and their averages were recorded. Evaluations were made on 10 corneas from 5 rats in each group, two slides were used for cornea and Descemet's Membrane thicknesses. These assessments of corneal thickness were done using HE at 20X magnification and Descemet's Membrane thicknesses were done using HE at 40X magnification. (Nikon E-600 Light Microscope).

Evaluations were made on 10 corneas from 5 rats in each group, two slides (A total of 80 sections) were used for each antibody) and the corneas stained immunohistochemically were evaluated using the H score. At 20X magnification, the number of cells stained positively in the corneal epithelium in each preparation was specified. 500 cells were counted in each preparation by classifying the

cells as +1, +2, and +3 according to the severity of staining and as 0 for the cells that were not stained. The scoring was made using the formula (I + 1) X The Number of Cells Stained (I = severity of staining).[24] The data obtained were evaluated statistically.

Statistical evaluation

In our study, the "Anova" test was used to evaluate if there was a statistically significant difference between the mean values of the independent groups. The Post-Hoc Tukey Pairwise Test was used to evaluate the difference between the groups. For the numerical variables, descriptive statistics were expressed as mean and standard deviation.The version SPSS 22.0 was used for the analyses. A p-value of<0.05 was considered statistically significant.

Ethics committee

Approval for our study was obtained from Afyon Kocatepe University Animal Experiments Local Ethics Committee with the number 49533702/89.

Findings

HE staining findings

HE staining showed large spaces and cells with cytoplasmic vacuoles in the DM group, while the numbers of stromal spaces and cells with cytoplasmic vacuoles were observed to be reduced in the DM+CoQ10 group (Figure 1). These structures were not observed in the control group.

Corneal thicknesses

The mean corneal thickness measured in the sections stained with HE was found to be lower in the diabetic group compared to the other groups. A considerably significant reduction was observed compared to the control group ($p < .001$). The corneal thickness was higher in the diabetic group that was treated with CoQ10 compared to the DM group, but this increase was not found to be statistically significant ($p = .410$). The corneal thickness was lower in the group that was given CoQ10 alone compared to the control group, but this reduction was not statistically significant ($p = .192$), and compared to the DM group it was found higher ($p = .010$) (Figure 1, Graphic 1).

PAS staining findings

Thickenings in the Bowman and Descemet's membranes in the corneas of the diabetic rats were observed considerably prominently with PAS positive staining. Sporadic positivity in the corneal stroma and epithelium was observed prominently. PAS positive staining was again observed in the DM+CoQ10 group, but to a considerably lower extent compared to the diabetic group. PAS positive staining was found considerably rarely in the group that was given CoQ10 alone and in the control group (Figure 2). In the measurement of Descemet's membranes, a significant thickening was observed in the DM group compared to the other groups ($p = .000$). While the membrane thickness decreased in the DM+CoQ10 group compared to the DM group ($p = .001$), it increased compared to the CoQ10 and Control groups ($p = .009$, $p = .001$). No difference was observed between the CoQ10 group and the Control group in terms of membrane thicknesses ($p = .781$).

Immunohistochemical findings

The severity of staining was at the highest level in the sections stained immunohistochemically with VEGF antibody in the diabetic groups. The severities of VEGF staining were found to be higher in the corneal epithelial cells in the diabetics compared to the control group and CoQ10 group ($p = .001$, $p = .001$). The severity of VEGF staining was found to be lower in the DM+CoQ10 group compared to the DM group ($p = .000$). The severity of VEGF staining was found to be higher in the DM+CoQ10 administered group compared to the control group ($p < .001$) (Figure 3, Graph 2).

In the sections stained with IL-1β and IL-6 antibodies, the increase in the severity of staining was more prominent in the diabetic groups compared to the other groups. The severities of

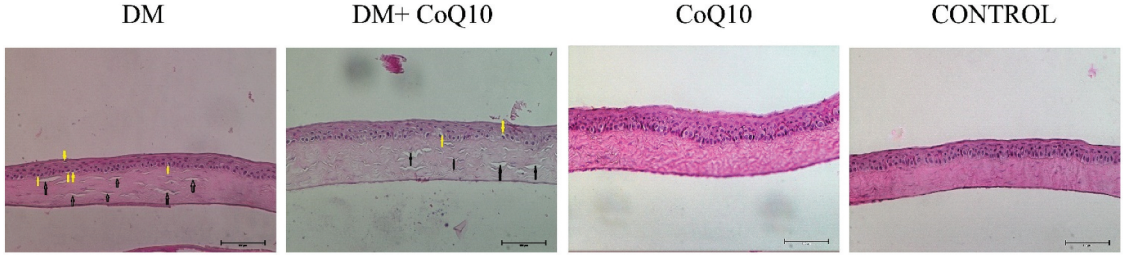
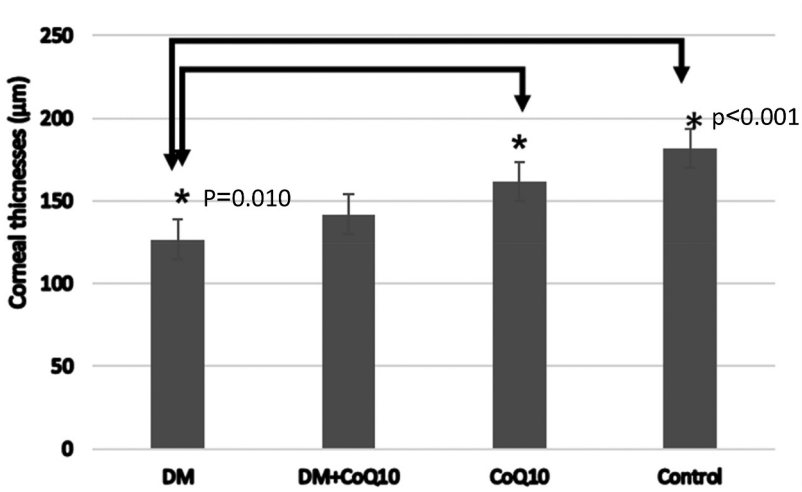


Figure 1. Corneal thicknesses, large spaces marked with black arrows and cells with cytoplasmic vacuoles marked with yellow arrows, HE staining, X100 magnification (scale bar = 100 μm).



Graphic 1. The corneal thicknesses were calculated as the average of three separate measurements by applying HE staining to the sections passing through the optic nerve. *p < .05 between two groups (Error bars show standard error).

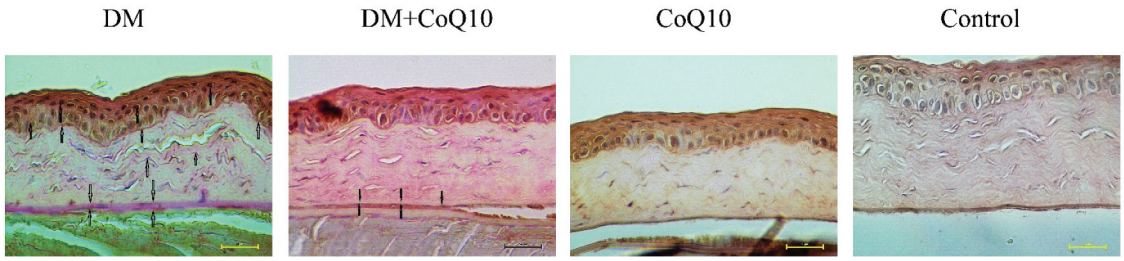


Figure 2. Figure displaying corneal glycogen stores, black arrows show PAS-positive staining. PAS staining, X200 magnification (scale bar = 50 µm).

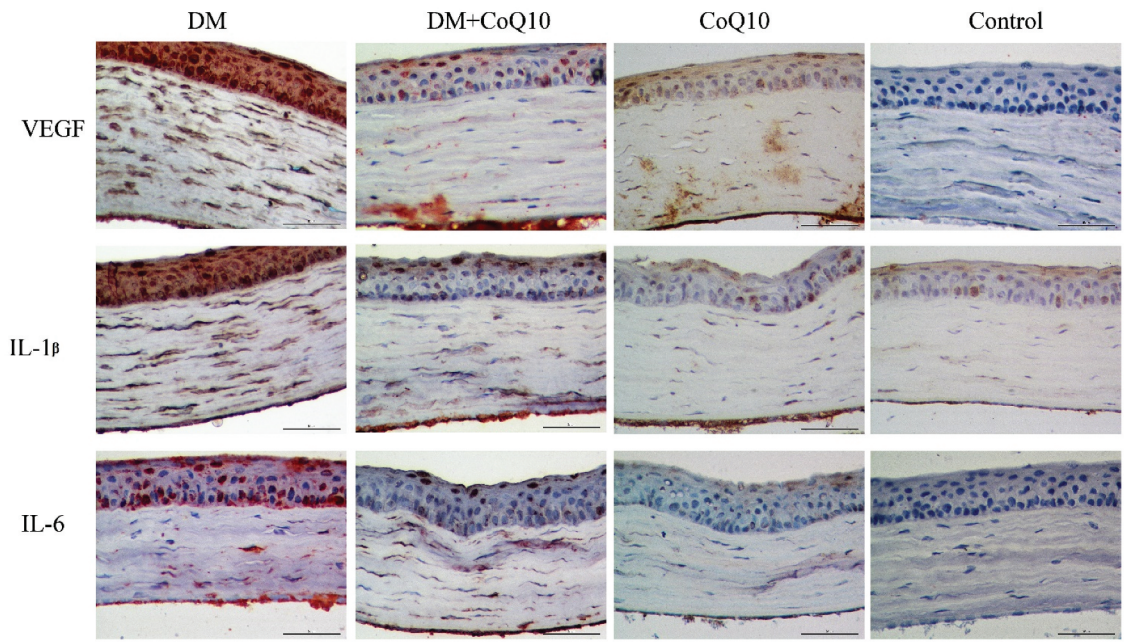
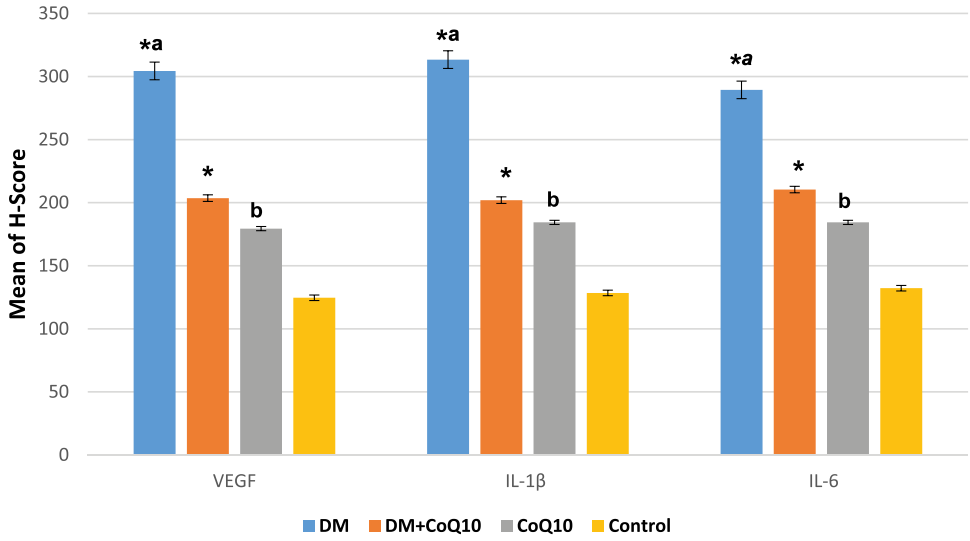


Figure 3. IHC staining with VEGF, IL-1β, and IL-6 antibody. X400 magnification, (scale bar = 50 µm).

staining with IL-1β and IL-6 were found to be higher in the diabetic corneal epithelial cells compared to the control group (p < .000, p < .000). The severities of staining with IL-1β and IL-6 were found to be lower in the DM+CoQ10 group compared to the DM group (p < .001, p < .001). The severities of staining with IL-1β and IL-6 were found to be higher in the



Graphic 2. The graph shows the H-score evaluation of IHC staining intensity with VEGF, IL1-beta, and IL-6 antibodies. Graphs illustrating the staining intensities in the corneal tissues of all groups. *: Comparison with Control group * p < .05. a: Comparison with DM+CoQ10 group, a = p < .05; b: Comparison with DM and CoQ10 groups, b = p < .05. (Error bars show standard error).

DM+ CoQ10 group compared to the control group (p < .001, p = .001), and also the staining intensities in these antibodies were found lower in the CoQ10 group compared to the DM group (p = .001, p = .001) (Figure 3, Graph 2).

IL-1β, IL-6, and VEGF staining intensities were higher in the CoQ10 group compared to the Control group, but these increases were not found to be statistically significant (p = .109, p = .287, p = .076)

Discussion

CoQ10 is an enzyme that increases endogenous antioxidant enzyme activity and has a therapeutic effect in DM and its complications.[25] Hussein J. et. al. showed that oxidative damage in the brains of rats diabetic with STZ and increased brain neurotransmitter level as a complication of DM decreased in the group treated with CoQ10.[26] In the study of Frederic M. et al., the damage caused by oxidative stress in many organs of DM such as brain, heart, kidneys and liver, CoQ 10 treatment, glutathione peroxidase, and superoxide dismutase, etc. reversed by increasing antioxidant enzyme activity.[27] CoQ10 has been tried for the treatment of various eye diseases in the literature. Postorino E. et al. used local hyaluronic acid and CoQ10 in the treatment of patients with mild and moderate dry eye and they reported that this could be a good preference in treatment.[28] Kernt M. et al. investigated if CoQ10 was an efficient agent in preventing the development of cataracts and showed that CoQ10 decreased apoptotic cell death in the epithelial cells in the lens by reducing oxidative stress.[29] In our study, we also observed that inflammatory response in the cornea caused by diabetes decreased with the use of CoQ10. We histopathologically examined the effects of diabetes on the corneal tissue by comparing the rats with STZ induced diabetes, diabetic rats that were given CoQ10 for 2 months, and rats that were given CoQ10 for 2 months without being diabetized and the control group that was not diabetized and not given CoQ10. We used HE and PAS stains for light microscopic evaluation and VEGF, IL-1β and IL-6 antibodies for immunohistochemical examination.

In the literature, different results have been reported in studies examining the corneal complications of diabetes. In a study conducted by Yin J. et al., the corneas of diabetic rats were injured and both wound healing and corneal thicknesses were examined. It was found that wound healing was delayed in diabetic corneas, while no significant difference was found in terms of corneal thicknesses compared to the control group.[30] In a study conducted by Kim et al., rats that were made diabetic with STZ were evaluated after 13 weeks, and stromal edema and increased stromal thickness were found in the cornea. The increase in corneal thickness was found to be prominent in the center.[31] Cai D. et al. evaluated total corneal thickness using in vivo confocal microscope at the end of the 12 week and 18 week periods in diabetic rat models. They found a 7.2% reduction in the corneal thickness at the end of 12 weeks, though this reduction was insignificant, and a 10.2% reduction in the diabetic rats for 18 weeks which was significant. They showed that the reduction in corneal thickness was mostly caused by the reduction in stromal thickness in the diabetic rats for 12 weeks and by both stromal and epithelial thinning in the diabetic group for 18 weeks.[32] In our study, the corneal thickness was found to be reduced in the rats that were made diabetic for 8 weeks compared to the control group. The mean corneal thicknesses in the DM+CoQ10 group were higher than the DM group but lower than the control group. These results (although there was no statistically significant difference in DM+CoQ10 group) made us think that diabetes causes thinning in corneal tissues in the long term (after diabetic complications begin) and CoQ10 prevents the reduction of corneal thickness. We detected signs of not only thinning but also glycogen storage in diabetic corneas. A prominent increase in PAS positive staining in the Bowman's and Descemet's membranes in the cornea in the DM group shows that the corneal complication of diabetes is

not limited in a single layer such as the epithelium or stroma. And the decrease in this storage in the DM+CoQ10 group showed that CoQ10 reduced this effect of diabetes.

In the literature, no agent can fulfill systemic treatment of diabetes alone by preventing chronic complications of diabetes. In studies conducted with CoQ10, it was shown that metabolic control was not adequate in diabetic patients.[33,34] In our study, the slight increase in corneal thickness with the administration of CoQ10 alone to diabetic rats as a therapeutic agent was found to be compatible with this information in the literature and suggests that CoQ10 will be a protective agent when used in addition to the treatment of diabetes

A well-known feature of diabetic corneas is delayed wound healing. Costa Pinto da FA and Malucelli BE induced injury by silver nitrate cauterization in the rats that were made diabetic with STZ, in diabetic rats treated with injections of insulin and in the control group, and they compared the development of corneal angiogenesis in the process of healing of this injury site by way of VEGF and FGF-2 immunoreactivity. Although these factors were observed more intensely in the control group and the group treated with insulin, a significant difference could not be found between the groups.[35] In Costa Pinto da FA and Malucelli's study, the response to inflammation between diabetes, treatment, and control groups was examined and it was thought that the finding of VEGF staining decreased in the diabetic group compared to the control group was due to insufficient angiogenesis in the diabetic groups during wound healing. There was an increase in VEGF at the wound site in the insulin-treated group, but no difference was found in the diabetic group. In this study, it was emphasized that although the treatment of diabetes with insulin delays the occurrence of chronic complications, it cannot completely prevent it. In our study, the corneas of diabetic rats were examined without causing any wounds, and the increase in VEGF immunoreactivity was found to be more prominent in the diabetic group than in the other groups. The increase in VEGF may cause a permanent increase in corneal vascularity in the following periods, resulting in a decrease in corneal transparency and visual acuity together with inflammation. The finding of decreased staining intensity in the corneas of diabetic rats treated with CoQ10 indicates that CoQ10 suppresses the tendency for diabetes-enhanced angiogenesis in the cornea.

Wang Y. et al. compared IL-1, IL-6, and VEGF levels in the serum samples of diabetic rats with ELISA, and in retinal tissues by qRT-PCR and Western blot methods, and found a significant increase in the levels of these proinflammatory cytokines in the diabetic group.[36] Alves M. et al. also evaluated the inflammation markers in the lacrimal gland, tears, and corneas of rats with diabetes and found increased IL-1β levels in their tears.[37] Liu X. et al. examined IL-1β expressions in the cornea and conjunctiva of rats, which were made diabetic with STZ, for possible curative effects of 8 weeks of NAC (N-acetylcysteine) treatment on ocular surface damage due to inflammation, a complication of diabetes. In this study, IL-1β expressions were increased in the diabetic cornea and conjunctiva; NAC treatment decreased IL-1β expressions, improved ocular surface damage, and increased tear secretion volume.[38] In our study, IL-1β and IL-6 staining intensities were observed to increase in the corneas of diabetic rats, and it was observed that the staining intensities decreased in the group treated with CoQ10. This result suggests that the inflammation increasing effect of diabetes can be reduced by CoQ10 treatment.

Conclusion

The most important step in the treatment of diabetic patients is keeping serum blood glucose levels under control. Although most of the pathogenic mechanisms of diabetes are known, there is no single agent that can control the disease in the long term. Since diabetes mellitus is a multisystemic disease, it influences many organs and tissues in the long term. In our study, we observed that diabetes increased the tendency to inflammation and angiogenesis also in the corneal tissue in the long term and it causes glycogen storage also in Bowman and Descemet's membranes, and we showed that CoQ10 decreased glycogen storage, inflammation, and angiogenesis in the corneas of diabetic rats. We think that CoQ10 is not effective in fully restoring corneal complication and visual morbidity by itself alone, but it could be used in addition to systemic treatment of diabetes (insulin, oral antidiabetic drugs … etc.), and additional studies are needed in this area.

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References

1. Patterson CC, Karuranga, S, Salpea, P, et al. Worldwide estimates of incidence, prevalence and mortality of type 1 diabetes in children and adolescents: results from the international diabetes federation diabetes atlas. *Diabetes Res Clin Pract*. 2019;157:107842. doi:10.1016/j.diabres.2019.107842.
2. Xia Y, Xie Z, Huang G, et al. Incidence and trend of type 1 diabetes and the underlying environmental determinants. *Diabetes Metab Res Rev*. 2019;35(1):e3075. doi:10.1002/dmrr.3075.
3. Morgan E, Cardwell C.R., Black C.J, et al. Excess mortality in Type 1 diabetes diagnosed in childhood and adolescence: a systematic review of population-based cohorts. *Acta Diabetol*. 2015;52 (4):801–807. doi:10.1007/s00592-014-0702-z.
4. Donnelly R, Emslie-Smith A.M, Gardner, I.D, et al. Vascular complications of diabetes. *Bmj*. 2000;320(7241):1062–1066. doi:10.1136/bmj.320.7241.1062.
5. Risau W, Flamme I. Vasculogenesis. *Annu Rev Cell Dev Biol*. 1995;11(1):73–91. doi:10.1146/annurev.cb.11.110195.000445.
6. Yancopoulos GD, Davis S, Gale NW, et al. Vascular-specific growth factors and blood vessel formation. *Nature*. 2000;407.6801 (6801):242–248. doi:10.1038/35025215.

7. Bussolino F, Mantovani A, Persico G. Molecular mechanisms of blood vessel formation. *Trends Biochem Sci*. 1997;22(7):251–256. doi:10.1016/S0968-0004(97)01074-8.
8. Aiello LP, Wong J-S. Role of vascular endothelial growth factor in diabetic vascular complications. *Kidney Int*. 2000;58:S113–S119. doi:10.1046/j.1523-1755.2000.07718.x.
9. Klagsbrun M, D'Amore PA. Vascular endothelial growth factor and its receptors. *Cytokine Growth Factor Rev*. 1996;7(3):259–270. doi:10.1016/S1359-6101(96)00027-5.
10. Schultz R, Van Horn DL, Peters MA, et al. Diabetic keratopathy. *Trans Am Ophthalmol Soc*. 1981;79:180.
11. Lutty GA. Effects of diabetes on the eye. *Invest Ophthalmol Vis Sci*. 2013;54(14):ORSF81–ORSF87. doi:10.1167/iovs.13-12979.
12. Taylor HR, Kımsey RA. Corneal epithelial basement membrane changes in diabetes. *Invest Ophthalmol Vis Sci*. 1981;20:548–553.
13. Philipp W, Speicher L, Humpel C. Expression of vascular endothelial growth factor and its receptors in inflamed and vascularized human corneas. *Invest Ophthalmol Vis Sci*. 2000;41:2514–2522.
14. Saghizadeh M, Chwa M, Aoki A, et al. Altered expression of growth factors and cytokines in keratoconus, bullous keratopathy and diabetic human corneas. *Exp Eye Res*. 2001;73(2):179–189. doi:10.1006/exer.2001.1028.
15. Lutty GA. Effects of diabetes on the eye. *Invest Ophthalmol Vis Sci*. 2013;54(14):ORSF81–ORSF87. doi:10.1167/iovs.13-12979.
16. Dinarello CA. Biologic basis for interleukin-1 in disease. *Blood* 1996 15;87(6):2095–2147. doi:10.1182/blood.V87.6.2095.bloodjournal8762095.
17. Mandrup-Poulsen T. The role of interleukin-1 in the pathogenesis of IDDM. *Diabetologia*. 1996;39(9):1005–1029. doi:10.1007/BF00400649.
18. Larsen CM, Faulenbach M, Vaag A, et al. Interleukin-1–receptor antagonist in type 2 diabetes mellitus. *N Engl J Med*. 2007;356(15):1517–1526. doi:10.1056/NEJMoa065213.
19. Rosa JS, Flores RL, Oliver SR, et al. Sustained IL-1α, IL-4, and IL-6 elevations following correction of hyperglycemia in children with type 1 diabetes mellitus. *Pediatr Diabetes*. 2008;9(1):9–16. doi:10.1111/j.1399-5448.2007.00243.x.
20. Fathy SA, Mohamed MR, Ali MAM, et al. Influence of IL-6, IL-10, IFN-γ and TNF-α genetic variants on susceptibility to diabetic kidney disease in type 2 diabetes mellitus patients. *Biomarkers*. 2019;24(1):43–55. doi:10.1080/1354750X.2018.1501761.
21. Gül M, Emre S, Eşrefoğlu M, et al. Protective effects of melatonin and aminoguanidine on the cornea in streptozotocin-induced diabetic rats. *Cornea*. 2008;27(7):795–801. doi:10.1097/ICO.0b013e318169d67c.
22. Nahar N, Mohamed, S, Mustapha, N.M, et al. Metformin attenuated histopathological ocular deteriorations in a streptozotocin-induced hyperglycemic rat model. *Naunyn-Schmiedeberg's Arch. Pharmacol*. 2021;394(3):457–467. doi:10.1007/s00210-020-01989-w.
23. Brondum E, Nilsson H, Aalkjaer C. Functional abnormalities in isolated arteries from Goto-Kakizaki and streptozotocin-treated diabetic rat models. *Horm Metab Res*. 2005;37:56–60. doi:10.1055/s-2005-861370.
24. Sahin Z, Acar, N, Ozbey, O, et al. Distribution of Notch family proteins in intrauterine growth restriction and hypertension complicated human term placentas. *Acta Histochem*. 2011;113(3):270–276. doi:10.1016/j.acthis.2009.10.006.
25. Visnagri A, Kandhare AD, Kumar VS, et al. Elucidation of ameliorative effect of Co-enzyme Q10 in streptozotocin-induced diabetic neuropathic perturbation by modulation of electrophysiological, biochemical and behavioral markers. *Biomed. Aging Pathol*. 2012;2(4):157–172.doi:10.1016/j.biomag.2012.10.006.
26. Hussein J, El-Matty D, El-Khayat Z, ABDEL-LATIF Y. Brain neurotransmitters in diabetic rats treated with CO enzyme Q10. *Int J Pharm Pharm Sci*. 2012;4(4):554–556.
27. Rauscher FM, Sanders RA, Watkins III,JB. Effects of coenzyme Q10 treatment on antioxidant pathways in normal and streptozotocin-induced diabetic rats. *J Biochem Mol Toxicol*. 2001;15(1):41–46. doi:10.1002/1099-0461(2001)15:1<41::AID-JBT5>3.0.CO;2-Z.
28. Postorino EI, Rania L, Aragona E, et al. Efficacy of eyedrops containing cross-linked hyaluronic acid and coenzyme Q10 in treating patients with mild to moderate dry eye. *Eur J Ophthalmol*. 2018;28(1):25–31. doi:10.5301/ejo.5001011.
29. Kernt M, Hirneiss C, Neubauer AS, Ulbig MW, Kampik A. Coenzyme Q10 prevents human lens epithelial cells from light-induced apoptotic cell death by reducing oxidative stress and stabilizing BAX/Bcl-2 ratio. *Acta Ophthalmol*. 2010;88(3):e78–e86. doi:10.1111/j.1755-3768.2010.01875.x.
30. Yin J, Huang J, Chen C, Gao N, Wang F, Fu-Shin XY. Corneal complications in streptozocin-induced type I diabetic rats. *Invest Ophthalmol Vis Sci*. 2011;52(9):6589–6596. doi:10.1167/iovs.11-7709.
31. Kim J, Kim CS, Sohn E, Jeong IH, Kim H, Kim JS. Involvement of advanced glycation end products, oxidative stress and nuclear factor-kappaB in the development of diabetic keratopathy. *Graefes Arch Clin Exp Ophthalmol*. 2011;249(4):529–536. doi:10.1007/s00417-010-1573-9.
32. Cai D, Zhu M, Petroll WM, Koppaka V, Robertson DM. The impact of type 1 diabetes mellitus on corneal epithelial nerve morphology and the corneal epithelium. *Am J Pathol*. 2014;184(10):2662–2670. doi:10.1016/j.ajpath.2014.06.016.
33. Quan Y, Luo K, Cui S, et al. The therapeutic efficacy of water-soluble coenzyme Q10 in an experimental model of tacrolimus-induced diabetes mellitus. *Korean J Intern Med*. 2020;35(6):1443. doi:10.3904/kjim.2019.269.
34. Suksomboon N, Poolsup N, Juanak N. Effects of coenzyme Q10 supplementation on metabolic profile in diabetes: a systematic review and meta-analysis. *J Clin Pharm Ther*. 2015;40(4):413–418. doi:10.1111/jcpt.12280.
35. da Costa Pinto FA, Malucelli BE. Inflammatory infiltrate, VEGF and FGF-2 contents during corneal angiogenesis in STZ-diabetic rats. *Angiogenesis*. 2002;5(1):67–74. doi:10.1023/A:1021539315831.
36. Wang Y, Zhai W, Yang Y. Association between NDRG2/IL-6/STAT3 signaling pathway and diabetic retinopathy in rats. *Eur Rev Med Pharmacol Sci*. 2020;24(7):3476–3484. doi:10.26355/eurrev_202004_20806.
37. Alves M, Calegari V, Cunha D, Saad M, Velloso L, Rocha EM. Increased expression of advanced glycation end-products and their receptor, and activation of nuclear factor kappa-B in lacrimal glands of diabetic rats. *Diabetologia*. 2005;48(12):2675–2681. doi:10.1007/s00125-005-0010-9.
38. Liu X, Liu H, Lu X, Zhao S. N-acetylcysteine alleviates ocular surface damage in STZ-induced diabetic mice by inhibiting the ROS/NLRP3/Caspase-1/IL-1β signaling pathway. *Exp Eye Res*. 2021;209:108654. doi:10.1016/j.exer.2021.108654.