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Decrease of Serum Vascular Endothelial Growth Factor, along with its Ocular Level, after the Periocular Injection of Celecoxib and Propranolol in Streptozotocin-induced Diabetic Mouse Model

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Abstract

Background: There is a direct correlation between ocular vascular endothelial growth factor (VEGF) level and progression of pathological outcomes in diabetic retinopathy. In our previous study, the periocular administration of propranolol and celecoxib could significantly reduce ocular VEGF levels in a diabetic mouse model. Here, we investigated the changes of serum VEGF after periocular administration of propranolol and celecoxib in a diabetic mouse model.

Methods: Forty male BALB-C mice aged 4-6 weeks were divided into four groups as follows: non-diabetic, streptozotocin-induced diabetic, streptozotocin-induced diabetic + periocular injection of 200 µg celecoxib and streptozotocin-induced diabetic + periocular injection of 10 µg propranolol. Serum VEGF in all experimental groups was measured by using enzyme-linked immunosorbent assay (ELISA) method.

Results: In comparison to the non-diabetic group, serum VEGF levels were markedly elevated in diabetic groups and periocular injection of anti-VEGF agents could affect serum VEGF levels. Celecoxib was significantly more effective than propranolol in regulating serum VEGF levels.

Conclusion: The periocular injection of both celecoxib and propranolol is one of the most effective ways to prevent diabetic retinopathy and also has a beneficiary effect on down-regulation of serum VEGF levels in a diabetic mouse model. Therefore, periocular injection of anti-VEGF agents can play a significant role in preventing clinical side effects of diabetes.

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Introduction

Diabetes mellitus is a common endocrine disease which is characterized by hyperglycemia as a primary clinical factor. This increase is the result of defect in insulin secretion or insulin activity. Among the most common complications of diabetes mellitus, retinopathy is still known as the prominent cause of visual impairment and accounts for almost 25% of blindness in diabetic patients worldwide (1).

Various angiogenic factors are involved in the clinical development of diabetic retinopathy. Among them, vascular endothelial growth factor (VEGF) participates in pathological retinal vascular growth and its level elevates in an early stage of ocular neovascularization in diabetic retinopathy (2, 3). Prostaglandins, especially prostaglandin E2, which are biosynthesized through the cyclooxygenase pathway by cyclooxygenase 2 enzyme, are responsible for vascular leakage and intraocular inflammation with subsequent up-regulation of VEGF in patients with diabetic retinopathy (4). Therefore, cyclooxygenase-2 inhibitors are good targets among several different therapeutic strategies for preventing or delaying clinical manifestations of VEGF. Studies have shown that aspirin, as a non-selective cyclooxygenase inhibitor, blocks the progression of retinal vascular manifestation and hemorrhage in a diabetic dog model (5).

So, cyclooxygenase inhibitors can be considered as clinical biomarkers in the treatment of diabetic retinopathy. It is important to note that delivery of therapeutic amounts of a drug is as efficient as selection of an impressive drug. It is now well accepted that topical and systemic routes for retinal drug delivery are inefficient (6). Despite the high local concentration of intravitreal drug delivery in the retina, complications such as retinal detachment are among the side effect of this route of drug administration (7). The permeability of sclera, as well as sustained-release of the drug, makes periocular injection a more promising alternative route of administration among other retinal drug delivery systems (7, 8).

In authors' previous study, periocular injection of celecoxib as a selective cyclooxygenase inhibitor and propranolol as a beta-adrenergic blocking agent significantly down-regulated ocular VEGF levels in the diabetic mouse model (4). Therefore, we decided to investigate if this reduction of ocular VEGF levels also affects serum VEGF in diabetic mice.

Materials and Methods

Animals and drugs/compounds

Forty male BALB-C mice (4-6 weeks old) were housed for 7 days under the standard condition on a 12h light/dark cycle at 23º C and 45-55 % humidity with free access to food and water. All animal procedures were approved by the institutional animal ethics committee (IAEC) of Ahvaz Jundishapour University of Medical Sciences and were in accordance with the ethical guidelines of USA National Research Council Committee. Streptozotocin (STZ) was purchased from Sigma-Aldrich (Bangalore, India) and dissolved in 10mmol/L citrate buffer while celecoxib was dissolved in 0.5 % carboxymethylcellulose (4, 9).

Induction of type 1 diabetes and treatment groups

Mice were injected with a single intraperitoneal dose of 200 mg/kg STZ or vehicle. The blood glucose levels were quantitatively measured using glucometer (Glucometer Elite XL, Bayer, Laubach, Germany). Hyperglycemia was approved within the first 2-5 days after the injection. Mice with glycemia higher and lower than 250 mg/dl were respectively grouped as

STZ-treated diabetic (group 2; n=10) and vehicle-treated non-diabetic (group1; n=10) mice. Following STZ injection, 10 µg propranolol (group 3) and 200 µg celecoxib (group 4) were injected in the right eyes and in the periocular tissues of diabetic mice for 4 days.

Enzyme-linked immunosorbent assay

After anesthetizing mice with 10/1of ketamine/xylazine, blood collection and serum separation in all groups, VEGF levels were measured by VEGF enzyme-linked immunosorbent assay (ELISA) kits (Bender Medsystems, Vienna, Austria). The level of VEGF was calculated by using the standard curve of VEGF (Figure 1).

Statistical analysis

Based on Kolmogorov-Smirnov test, our data were normally distributed in each group. Homogeneity of variance was done with Levene´s test for all groups and all groups had the same variance. So, statistical analysis was performed using One-way ANOVA. Tukey´s test was used as a post hoc test in the analysis And $P<0.05$ was considered as statistical significant level. Data regarding the effect of drug administration on the serum VEGF level were presented as mean ± SD.

Results

The effects of celecoxib and propranolol treatment on vascular endothelial growth factor levels

To check whether serum VEGF is affected by periocular injection of anti-VEGF treatments, we treated the STZ-induced diabetic mice with either celecoxib or propranolol. Figure.1 shows the representative standard curve for mouse vascular endothelial growth factor.

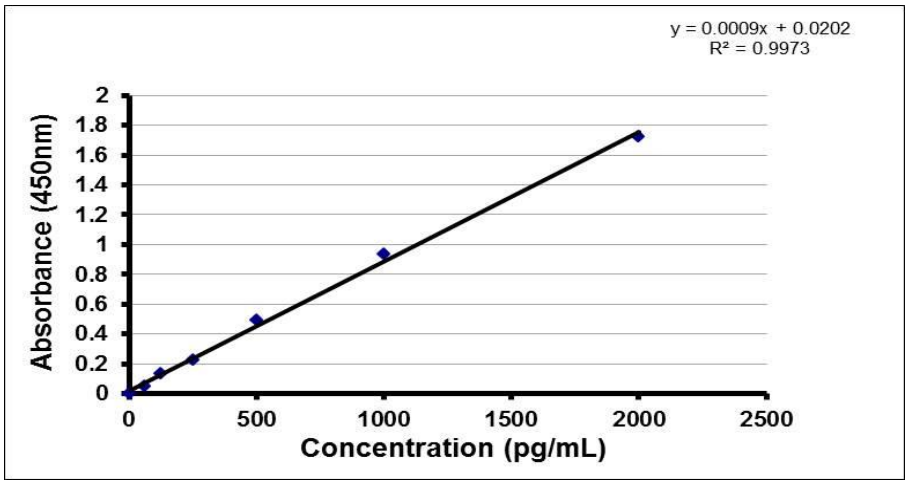

Figure1. The representative standard curve for mouse vascular endothelial growth factor (VEGF) Enzyme-linked immunosorbent assay (ELISA). 0 to 2 × 10 3 pg/ml standard curve of VEGF at 450 nm .

As shown in Figure 2, VEGF is slightly increased in the diabetic mice; however, it is significantly reduced in diabetic groups received periocular anti-VEGF therapy for diabetic retinopathy. Reduction of blood VEGF after periocular injection of celecoxib and propranolol suggests that these drugs can pass the blood-retinal barrier (BRB) and somehow cause an efficient reduction of serum VEGF.

Statistical analysis revealed that celecoxib (with decreasing blood VEGF by 44%) is more effective than propranolol (with decreasing blood VEGF by about 27%) compared to the diabetic group (Figure 2).

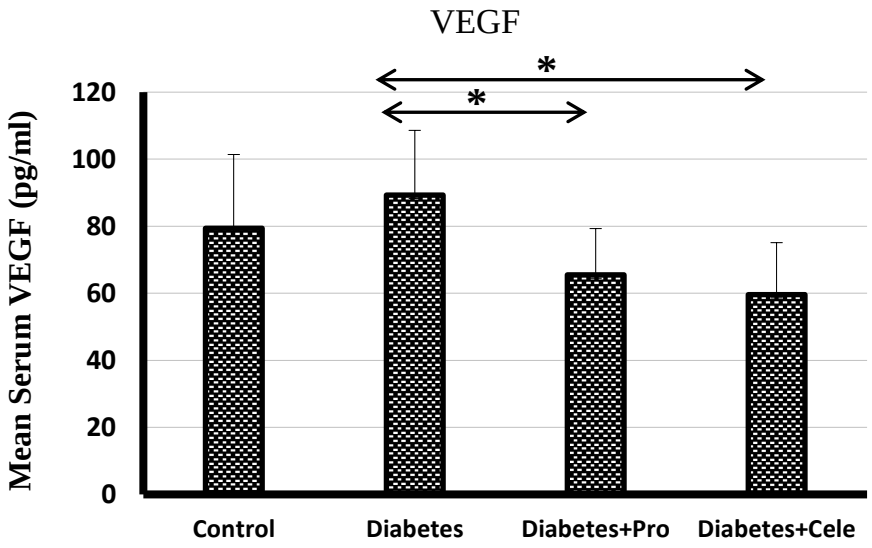

Figure2. Serum vascular endothelial growth factor (VEGF) level in Streptozotocin-induced diabetic mouse model treated with the periocular injection of either propranolol or celecoxib

Discussion

Diabetic retinopathy is one of the most prevalent diabetes-related complications (10) And its treatment in order to prevent visual loss is a persistent worldwide epidemic issue (5). VEGF which its expression is induced in an early stage of diabetic retinopathy is a desired candidate responsible for ocular neovascularization in diabetic retinopathy (11). As a result of the ongoing interest in anti-VEGF therapy for diabetic retinopathy, we expected that celecoxib as an optional cyclooxygenase-2 agonist and propranolol as a beta-adrenergic blocking agent can reduce retinal neovascularization and vessel leakage in diabetic retinopathy. Our previous study (4) and recent data together demonstrated that periocular injection of celecoxib and propranolol can reduce both ocular and serum VEGF levels.

The serum VEGF level which may speed up the risk of systemic diabetic microvascular and diabetes-related complications did not significantly increase in our STZ-induced diabetes model (Fig. 2); however, both anti- VEGF agents could down-regulate the VEGF levels even lower than that in normal control mice. There is a direct and positive correlation between hemoglobin A1c (HbA1c) and serum VEGF in type 2 diabetes, but in the present study, the reason of low increase of serum VEGF in the diabetic group might be due to using a short lifespan diabetic model. Although STZ-induced diabetes is a worldwide accepted model for study in the field of diabetes, use of diabetic mice with a longer lifespan is suggested for getting more reliable results.

Consistent with our findings, other studies reported inhibited expression of VEGF mRNA level in the retina of diabetic rat model (12). The difference of our study with others

was on the routes of drug administration, but according to the previous studies, among different routes of drug injection, topical and systemic ones are less impressive and periocular drug injection is a substantially alternative way in the transfer of a drug therapeutic dose in intraocular tissue (13, 14).

Of note, what makes celecoxib an interesting therapeutic drug is its ability to reduce cell proliferation and to initiate apoptosis in vitro. In this case, the protective effect of celecoxib has been also documented in the progression of malignancies like colorectal cancer (15). Observation based on evidence has also implied that adrenergic signaling promotes angiogenesis in which VEGF has pro-inflammatory effect in its neovascularization. So, it can be concluded that anti-adrenergic therapy or β-adrenergic receptor antagonist can be a potential target for inhibition of VEGF expression and subsequent angiogenesis (16, 17). This is what we observed in our recent and previous study; that is, propranolol down-regulated not only ocular VEGF level, but also its serum level.

In conclusion, the data presented here along with our previous results showed that periocular injection of both celecoxib and propranolol reduced ocular and serum levels of VEGF. The BRB becomes more permeable in diabetes (18) and therefore cause a leakage of celecoxib and propranolol to the bloodstream after periocular administration of anti-VEGF. The finding suggests that, as like as ocular, serum VEGF is affected by celecoxib and propranolol and these drugs have a systemic effect on VEGF level and considerably, VEGF reduction was more significant with celecoxib. Anti-proliferative and anti-VEGF expression of celecoxib and propranolol make them interesting drugs for controlled ocular edema through down-regulating ocular and serum VEGF level and also subsequent angiogenesis.

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Conflict of interest

All authors declare that there is no conflict of interest.

Authors´ contribution

Kheirollah A and Feghhi M designed the study, revised and approved the manuscript. Azizidoost Sh helped with the manuscript preparation and carried out the laboratory tests and animal care. Cheraghzadeh M and Nazeri Z were involved in data analysis and manuscript revision.

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