·Basic Research·

Effect of periocular injection of celecoxib and propranolol on ocular level of vascular endothelial growth factor in a diabetic mouse model

Saman Nassiri[1], Gholamreza Houshmand[2], Mostafa Feghhi[1], Alireza Kheirollah[3], Mohammad Bahadoram[4], Nariman Nassiri[5]

[1]Department of Ophthalmology, Infectious Ophthalmic Research Center, Schoolof Medicine, Ahvaz Jundishapur University of Medical Sciences, Ahvaz 61357-15794, Khuzestan, Iran
[2]Department of Pharmacology, School of Pharmacy, Ahvaz Jundishapour University of Medical Sciences, Ahvaz 61357-15794, Khuzestan, Iran
[3]Department of Biochemistry, Cellular&Molecular Research Center, Ahvaz Jundishapur University of Medical Sciences, Ahvaz 61357-15794, Khuzestan, Iran
[4]Medical Student Research Committee and Social Determinant of Health Research Center, Ahvaz Jundishapur University of Medical Sciences, Ahvaz 61357-15794, Khuzestan, Iran
[5]Jules Stein Eye Institute, David Geffen School of Medicine, University of California at Los Angeles, Los Angeles 90095, California, USA
Correspondence to: Gholamreza Houshmand. Departmentof Pharmacology, School of Pharmacy, Ahvaz Jundishapur University of Medical Sciences, Mailbox: 159, Ahvaz 61357-15794, Khuzestan, Iran. dr.houshmand_pharmaco@yahoo.com
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Abstract

• AIM: To investigate the effects of periocular injection of propranolol and celecoxib on ocular levels of vascular endothelial growth factor (VEGF) in a diabetic mouse model.

• METHODS: Forty 4-6wk BALB-C male mice weighing 20-25 g were used. The study groups included: non-diabetic control (group 1), diabetic control (group 2), diabetic propranolol (group 3), and diabetic celecoxib (group 4). After induction of type 1 diabetes by streptozotocin, propranolol (10 μg) and celecoxib (200 μg dissolved in carboxymethylcellulose 0.5%) were injected periocularly. The ocular level of VEGF was measured in all the study groups using enzyme-linked immuno sorbent assay (ELISA) method.

• RESULTS: Ocular VEGF level was significantly increased (1.25 fold) in the diabetic control group when compared to the non-diabetic group one week after induction with streptozotocin (P=0.002). Both periocular propranolol and celecoxib significantly reduced ocular VEGF levels (P=0.047 and P<0.001, respectively). The effect was more pronounced with celecoxib.

• CONCLUSION: The periocular administration of propranolol and celecoxib can significantly reduce ocular VEGF levels in a diabetic mouse model.

• KEYWORDS: diabetic retinopathy; celecoxib; propranolol; vascular endothelial growth factor; neovascularization; diabetic mouse model
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INTRODUCTION

Diabetic retinopathy is one of the leading causes of blindness among adults [1]. Several growth factors have been identified to be involved in the progression of diabetic retinopathy. Among them, vascular endothelial growth factor (VEGF), which has the highest potency, is up-regulated in retina during the early stages of diabetic retinopathy [2] and induces hyper-permeability of vessels and neovascularization[3]. It has been shown that prostaglandins and in particular prostaglandin E2, which are increased in diabetic rat retina, play an important role in the pathogenesis of diabetic retinopathy by inducing VEGF expression [4-5]. There are two distinct enzymes for prostaglandins synthesis (cyclooxygenase 1 and 2) [4]. Cyclooxygenase-2, which is usually induced ininflammatory conditions, has been shown to have a more prominent role in diabetes [5-6]. A non-selective cyclooxygenase inhibitor such as aspirin has been shown to significantly inhibit the development of retinal hemorrhages and acellular capillaries in adiabetic dog model [7]. This indicates that cyclooxygenase inhibitors may play a role in the treatment of diabetic retinopathy. In this concept, the role of oral celecoxib as a selective cyclooxygenase-2 inhibitor in reducing ocular VEGF expression has been documented[8].

Propranolol, a beta-adrenergic blocking agent, has been shown to have beneficial treatment effects in infantile hemangiomas and oxygen-induced retinopathy [9-10]. These effects are believed to be due to its anti-angiogenesis properties. In this study, we aim to investigate the effect of periocular injection of celecoxib andpropranolol on ocular levels of VEGF in a diabetic mouse model.

MATERIALS AND METHODS

Forty 4-6wk BALB-C male mice weighing 20-25 g were used in this study. They were kept for acclimatization for a period of 7d before starting the experiment, housed in polycarbonate cages under standard condition (12h light/dark cycle, relative humidity of 45% to 55%, temperature 23℃±2℃) and allowed free access to feed and clean drinking water during the period. Animal procedures were in accordance with the guidelines for animal care prepared by Committee on Care and Use of Laboratory Animal resources, National Research Council, USA, and were approved by the Institute Animal Ethics Committee (IAEC) of AJUMS for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) (Reg. No. PRC-9354). Every effort was made to minimize the animal suffering and decrease the number of animals used.

To induce type 1 diabetes, thirty animals were injected with asingle intraperitoneal dose of streptozotocin (200 mg/kg; SIGMA-ALDRICH, Bangalore, India) dissolved in 10 mmol/L citrate buffer. Following the injections, animals were given free access to food and water. The blood glucose levels were measured daily using a glucometer (Glucometer Elite XL, Bayer, Laubach, Germany). All our animals responded to this dose of streptozotocin within the first 2-5d after injection. We did not conduct any pathologic study on the specimens to confirm the presence of clinical manifestations of diabetic retinopathy (*i.e.* neovascularization). However, it is not expected to see those findings in such an early stage of inducing acute diabetes. Animals with blood glucose >250 mg/dL were considered to be diabetic [8] and those with blood glucose level >120 mg/dL and <250 mg/dL were excluded from the study. Animals with no injection and blood glucose level <120 mg/dL were considered as the non-diabetic control group (group 1; n=10); diabetic animals were then grouped as diabetic control (group 2; n=10), diabetic propranolol (group 3; n=10) and diabetic celecoxib (group 4; n=10). We considered 10 mice in each of the groups. After induction of diabetes, animals were injected with propranolol (10 μg) (group 3) and celecoxib (200 μg) dissolved in carboxymethylcellulose 0.5% (group 4). All the injections were performed in right eyes and in the periocular tissues (transconjunctival peribulbar injections in inferotemporal quadrant). Anesthesia was performed by intraperitoneal injection of ketamine (80 mg/kg) with xylazine (7 mg/kg). The injections were repeated every other day for 4 consecutive doses. Two days after the last dose, the animals were sacrificed, eyes were enucleated, the intraocular lenses were separated and the remaining ocular tissues were frozen for further analysis. All the animals were treated in accordance with the Association for Research in Vision and Ophthalmology (ARVO) statement for the use of animals in ophthalmic and vision research.

Table 1 The level of VEGF in ocular tissues in different study groups $\bar{x} \pm s$

Study groups	VEGF level (pg/mgpr)
Non-diabetic control, n=10	319.4±51.6
Diabetic control, n=10	399.9±52.8
Propranolol, n=10	339.8±50.0
Celecoxib, n=10	204.0±26.8

After irrigating ocular specimens with phosphate buffered saline (PBS) and protease inhibitor, 150 μL of RIPA buffer (50 mmol/L Tris, 150 mmol/L NaCl, 5 mmol/L EDTA, 1% Triton-X 100, 0.1% SDS, 0.5% deoxycholate) containing a protease inhibitor was added to them. They were then homogenized by sonicator (Hielsche, UP50F, Germany) and taken for VEGF measurement by VEGF-Aenzyme-linked immuno sorbent assay (ELISA) kits (Bender Medsystems, Vienna, Austria). In fact, the ocular specimen that the VEGF was measured included the whole eye without intraocular lens.

Statistical Analysis Data are expressed as mean±SD. SPSS 20 was used for data analysis. Shapiro-Wilk test was used for the test of normality. Comparison between groups was done by One-way ANOVA, post hoc Tukey and Scheffe tests. Differences were considered statistically significant at P<0.05.

RESULTS

Each study group included 10 animals. The mean±SD values for blood glucose levels of control and diabetic mice were 94±10 mg/dL and 433±79 mg/dL, respectively. Shapiro-Wilk test showed normal distribution of VEGF levels in all the study groups. The means' values for VEGF level were 319.4±51.6, 399.9±52.8, 339.8±50.0 and 204.0±26.8 pg/mgpr in groups 1-4, respectively (Table 1, Figure 1).

There was a statistically significant difference between the study groups in the level of VEGF (One-way ANOVA; P<0.001). Ocular VEGF level was significantly increased in the diabetic control group compared to the non-diabetic group (Tukey test; P=0.002). Periocular injection of both propranolol (Tukey test; P=0.047) and celecoxib (Tukey test; P<0.001) statistically significantly reduced the ocular levels of VEGF. This effect was more pronounced with celecoxib (Tukey test; P<0.001). The difference between non-diabetic group and propranolol group was not statistically significant (P=0.791). Post hoc Scheffe test was compatible with Tukey test. In our study, we did not observe any side effect (*i.e.* respiratory and cardiovascular failure) following injections in any of animals.

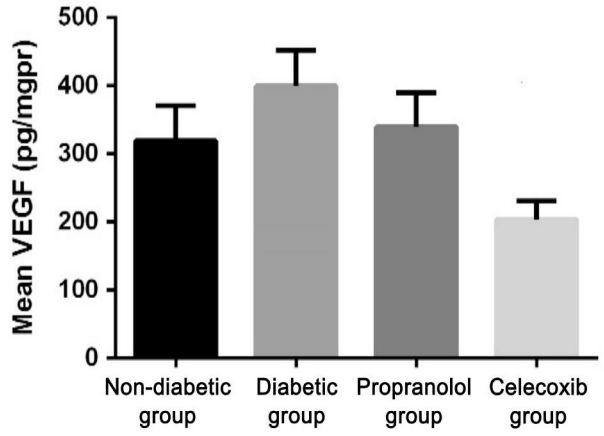

Figure 1 The mean vascular endothelial growth factor level in ocular tissues in different study groups.

DISCUSSION

During early onset of diabetic retinopathy, up-regulation of cyclooxygenase-2 occurs in retinal cells which results in prostaglandin E2 secretion [11]. It has been shown that prostaglandin E2 stimulates VEGF and basic fibroblast growth factor (bFGF) expression in cultured rat Müller cells[4]. Celecoxib, as elective cyclooxygenase-2 antagonist inhibits prostaglandin E2 secretion in diabetic ratretina [11]. Ayalasomayajula and Kompella[8] reported that oral celecoxib (50 mg/kg *b.i.d.*) inhibited retinal VEGF mRNA expression and decreased retinal vascular leakage in the diabetic rat model. In another study, they reported that celecoxib-PLGA microparticles could sustain retinal celecoxib delivery and inhibit diabetic-induced retinal oxidative damage in a rat model [12]. In our study, we similarly found that celecoxib significantly reduced ocular VEGF levels. However, celecoxib was administered through periocular injection in our study.

Topical and systemic routes of drug administration are believed to be less effective in delivery of therapeutic amounts of a drug into intraocular tissues. Intravitreal injection is accompanied with several side effects, such as cataracts, endophthalmitis, and retinal detachment [13]. Ayalasomayajula and Kompella [14] showed that retinal delivery of celecoxib was substantially higher following subconjunctival administration compared to intraperitoneal route. Therefore, periocular drug administration could be a promising alternative to enhance drug delivery into the eye[15]. We also found that periocular injection of propranolol significantly decreased ocular level of VEGF. This is in contrast to the result by Zheng *et al* [16] where they showed that oral propranolol (*i.e.* through drinking water containing propranolol) had no significant effect on retinal VEGF expression. To our knowledge, no study has investigated the effect of periocular propranolol on ocular level of VEGF. In a retrospective case series, Montero *et al* [17] showed that concomitant systemic beta-adrenergic blocking agents may reduce the need for repeated intravitreal injections of bevacizumab in patients with choroidal neovascularization associated with age-related macular degeneration. The therapeutic effect of propranolol has been also documented in oxygen-induced retinopathy [10] and hemangiomas [18]. The mechanism involved in anti-angiogenesis effects of propranolol is still not well understood. Lamy *et al* [19] demonstrated that propranolol inhibited growth factor-induced proliferation of cultured human umbilical vein endothelial cells in a dose-dependent fashion through a G0/G1 phase cell cycle arrest. Storch and Hoeger[9] reviewed the mechanism of propranolol on infantile hemangioma and indicated that early, intermediate and long-term effects of propranolol on infantile hemangioma can be attributed to three different pharmacological targets. Early effects, which are accompanied by brightening of hemangioma surface within 1-3d after starting the therapy, are attributable to vasoconstriction secondary to decreased release of nitric oxide. Intermediate effects are due to the blockage of proangiogenic signals (VEGF, bFGF, metal matrix proteinase-2/9), which results in growth arrest. Long-term effects of propranolol are characterized by induction of apoptosis in proliferating endothelial cells, which results in tumor regression[9].

One of the shortcomings of this study is that we did not measure ocular tissue VEGF level after single dose periocular injection of celecoxib and propranolol. Further studies are needed to investigate the dose-response relationship of these drugs. In addition, we did not measure VEGF levels in different ocular tissues separately. Further studies are needed to investigate VEGF levels in different ocular tissues following periocular versus systemic administration of these drugs.

In conclusion, we observed that ocular VEGF level was significantly increased during the first week of streptozotocin induction of the diabetic mouse model. Periocular injections of celecoxib and propranolol reduced the ocular levels of VEGF considerably, and this effect was more pronounced with celecoxib. This may implicate the possible role of cyclooxygenase-2 enzyme and β-adrenoceptor in modulation of VEGF expression. To our knowledge, the effect of periocular propranolol and celecoxib on ocular VEGF level has not been documented in the literature. These agents may be considered as an alternative treatment for neovascular disorders such as diabetic retinopathy and age-related macular degeneration. The mechanisms by which these drugs decrease ocular VEGF level should be further elucidated. We also showed that periocular injection is a safe route for drug delivery, avoiding potential side effects of these agents following systemic or intraocular drug administration.

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