Therapeutic Effects of Edaravone on Retinopathy in Streptozotocin-Induced Diabetic Rats

Streptozotosin ile İndüklenmiş Diyabetik Sıçanlarda Edaravonun Retinopati Üzerine Olan Terapötik Etkileri

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ABSTRACT

Purpose: To investigate the possible vascular endothelial growth factor inhibition effects of edaravone and to compare bevacizumab on diabetic retinopathy in streptozotocin-induced diabetic rats.

Materials and Methods: Thirty-six adult male Sprague Dawley rats were used in the study. Diabetes was induced with streptozotocin (60 mg/kg). After 4 weeks of treatment, the rats were euthanized and blood samples were collected for vascular endothelial growth factor assay. Additionally, enucleation was performed for vascular endothelial growth factor immunohistochemistry and outer nuclear layer measurements.

Results: Treatments with both bevacizumab and edaravone significantly decreased outer nuclear layer thickness and retinal vascular endothelial growth factor expression compared to serum physiologic groups. In addition, plasma vascular endothelial growth factor levels were significantly reduced in both bevacizumab and edaravone treated groups compared to the serum physiologic group.

Conclusions: Intraperitoneal and intravitreal bevacizumab and intraperitoneal ederavone could diminish the progression of diabetic retinopathy. These findings suggest that edaravone may become one of the therapeutic candidates of diabetic retinopathy in the future.

Keywords: Bevacizumab, diabetic retinopathy, edaravone, experimental, rat.

ÖZ

Amaç: Streptozotosin ile diabet oluşturulan sıçanlarda diyabetik retinopatide edaravonun olası vasküler endotelyal growth faktör inhibe edici etkisinin araştırılması ve bevacizumab ile karşılaştırılması.

Materyal ve Metod: Bu çalışmada 36 adet Sprague Dawley erkek sıçan kullanılmıştır. Diyabet streptozotosin (60 mg/kg) ile oluşturuldu. Sıçanlara 6 gruba ayrılarak intravitreal veya intraperitoneal olarak edaravone, bevacizumab ve serum fizyolojik uygulandı. Dört haftalık tedavinin ardından, sıçanlara ötenazi yapıldı ve vasküler endotelyal growth faktör değerlendirmesi için kan örnekleri alındı. Ek olarak, vasküler endotelyal growth faktörün immünhistokimyasal olarak değerlendirilmesi ve dış nükleer tabakanın değerlendirilmesi için enükleasyon yapıldı.

Bulgular: Bevacizumab ve edaravone tedavileri ile dış nükleer tabaka kalınlığında ve vasküler endotelyal growth faktör immünhistokimayasal değerlendirmesinde serum fizyolojik grubu ile karşılaştırıldığında belirgin azalma mevcuttu. Ayrıca, kan vasküler endotelyal growth faktör seviyelerinde bevacizumab ve edaravone ile tedavi edilen gruparda serum fizyolojik ile tedavi edilen grup ile karşılaştırıldığında belirgin azalma vardı.

Sonuç: İntravitreal ve intraperitoneal bevacizumab ile intraperitoneal edaravone diyabetik retinopatinin ilerlemesini yavaşlatabilirler. Bu bulgular edaravonun gelecekte diyabetik retinopati tedavisinde bir aday olabileceğini düşündürtmektedir.

Anahtar Sözcükler: Bevacizumab, diyabetik retinopati, edaravone, sıçan, streptozotosin.

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1. INTRODUCTION

Diabetes mellitus is a common health problem in the world and diabetic retinopathy (DR) is one of the leading causes of blindness worldwide.[1] Although the mechanisms of DR still remain unclear, retinopathy is considered to be an ischemic disorder that causes the development of neovascularization and blindness.

Diabetes leads to tissue damage through different pathways. The polyol pathway entails increased production of advanced glycation end products (AGEs), increased receptor expression of AGEs (RAGE), protein kinase C (PKC) activation and overactivity of the hexosamine pathway. PKC activation may play a role in increased vascular permeabilty, alterations in blood flow and stimulation of neovascularization. PKC may aso induce the expression of vascular endothelial growth factor (VEGF), an important molecule that triggers retinal neovascularization.[2] The hallmark of proliferative DR is neovascularization, and this occurs as a result of increased VEGF levels and expression. Therefore, anti-VEGF drugs such as bevacizumab were developed and are in use. Bevacizumab is a monocolonal antibody and prevents VEGF-A from binding to its receptor.[3] It has been used in the treatment of DR, choroidal neovascularization and macular edema.[4-6]

On the other hand, evidence indicates that most of the pathways are activated by a single upstream event: generation of reactive oxygen species (ROS) as a result of intracellular hyperglycemia. This may be because the retina has a high content of polyunsaturated fatty acids and utilizes more oxygen and glucose than any other tissues.[4-7] Oxidative stress, resulting from excessive generation of ROS, may cause functional and anatomical changes in retinal microvascular structure through the induction of apoptosis.[2] Therefore, many studies have focused on oxidative stress for the treatment of retinopathy.

Edaravone (3-methyl-1-phenyl-2-pyrazolin-5-one), a potent free radical scavenger, was approved for clinical use in the management of acute ischemic stroke in Japan in June 2001.[4-6,8] It exerts protective effects against ischemia-reperfusion injuries, such as lung injury after hepatic ischemia-reperfusion, peripheral nerve injury, myocardial infarction and acute intracerebral hemorrhage.[8-12] The protective effects of edaravone on diabetic retinopathy has also been shown, however, the mechanism is currently unclear.[4]

Considering the aforementioned facts, we aimed to investigate the therapeutic possible VEGF inhibition effects of intravitreal and intraperitoneal administration of edaravone and to compare bevacizumab on diabetic retinopathy (DR) in streptozotocin-induced diabetic rats.

2. MATERIALS AND METHODS

2.1 Animals

Thirty-six adult male Sprague Dawley rats, weighing 200-250 g, were used in the study. Animals were maintained under standard conditions with 12-h light/dark cycles in a temperature-controlled room (22± 2°C). All rats were fed ad libitum with standard diet and tap water during the study. The local ethics committee of animal experiments at Ege University approved the study protocol. All chemicals were obtained from Sigma-Aldrich Inc. unless otherwise noted.

2.2. Diabetes induction

The diabetes model was induced to rats with a single intraperitoneal injection of streptozotocin (STZ, 60 mg/kg in 100 mmol/L citrate buffer, pH 4,5). Diabetes was confirmed after 48 h by measuring the blood glucose levels with the use of glucose oxidase reagent strips (Plusmed). The rats showing blood glucose levels above 250 mg/dl were included in the study.

2.3. Experimental design

The diabetic rats were divided into six groups and treatments were performed as follows; Group I: intravitreal serum physiologic (10 µl/week), Group II: intravitreal edaravone (20 µg/µl/week), Group III: intravitreal bevacizumab (50 µg/µl/week), Group IV: intraperitoneal serum physiologic (1 ml/kg/day), Group V: intraperitoneal edaravone (10 mg/kg/day) and Group VI: intraperitoneal bevacizumab (50 mg/kg/week). For the administration of these drugs, 27 gauge needles were used for intraperitoneal injections and 31 gauge needles with Hamilton syringes were used for intravitreal injections. To perform intravitreal injections rats were anaesthetized with ketamine (80 mg/kg) and xylazine (8mg/kg). After applying a drop of 0.5% alcaine, a 31 gauge needle was used to puncture sclera at pars plana level and injection performed. After 4 weeks of treatment, the rats were euthanized and blood samples were collected by cardiac puncture to determine plasma VEGF levels in the intraperitoneal treatment groups. Following this, enucleation was performed for morphologic analysis, VEGF immunohistochemistry and ONL layer measurements.

2.4. VEGF Immunohistochemistry

Two µm thick cross-sections were obtained with a microtome (Leica MR 2145) from paraformaldehyde-fixed paraffin-embedded eye tissues. They were floated in a sterile bath, picked up 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. Primary antibodies were applied in an incubator at 57°C and washed with PBS. Afterwards, a biotinylated secondary IgG antibody was applied and washed with PBS before incubating with a 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 photographed with an Olympus C-5050 digital camera mounted on an Olympus BX51 microscope. A specialist blinded to the study groups performed the immunohistochemical evaluations. All comparisions of staining intensities were scored at X40 magnification over 10 different sections. According to the score; 0 represented no expression, while 1, 2, 3 and 4 represented 0-24%, 25-49%, 50-75% and >75% expressions, respectively.

2.5. Measurement of plasma VEGF levels

Plasma VEGF levels were determined using enzyme linked immunosorbent assay (ELISA) according to the manufacturer's instructions (RayBiotech, Inc., GA, USA). The results were expressed as pg/ml. The detection limit was less than 2 pg/ml, and intraassay and interassay coefficients of variation were less than 10%.

2.6. Statistical analysis

Data analyses were performed using statistical software (SPSS for Windows Version 16, Statistical Package for Social Science, Worldwide Headquarters SPSS Inc., Chicago, IL, USA). Bevacizumab and edaravone groups were compared with the serum physiologic group. Statistical comparisons were made by Mann Whitney U test. Results are given as mean ± SEM. A value of p< 0,05 was considered as statistically significant.

3. RESULTS

3.1. Evaluation of VEGF expression by immunohistochemistry

Figure 1 shows the level of VEGF expression after cessation of treatment in the rat retina. The mean VEGF expression scores were 1,6 ± 0,2, 0,8 ± 0,1, and 0,5 ± 0,2 in the intraperitoneal serum physiologic, bevacizumab and edaravone groups, respectively. Intraperitoneal bevacizumab and edaravone treatments showed significantly decreased VEGF expression compared to the serum physiologic-treated group (p<0.05). On the other hand, these scores

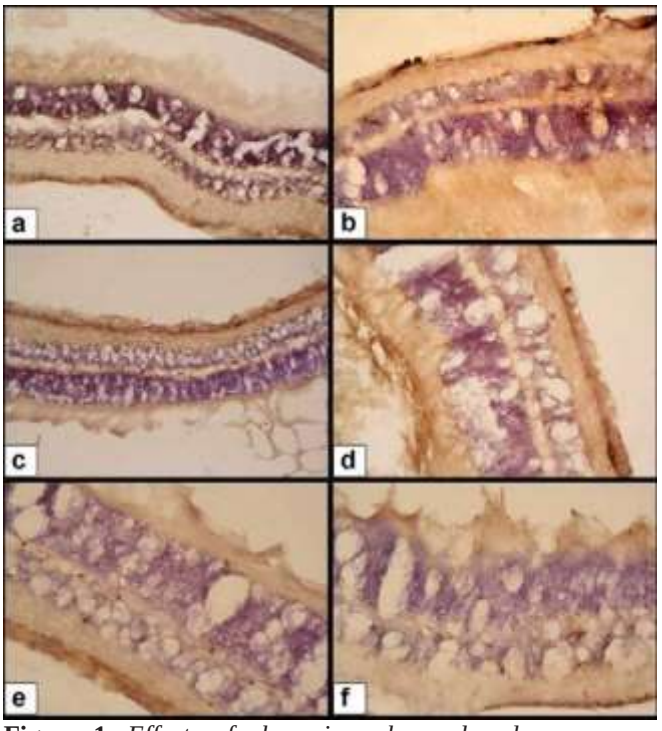

Figure 1. *Effects of bevacizumab and edaravone on retinal VEGF immunoexpression in diabetic rats. a,b are groups which treated with intraperitoneal and intravitreal serum physiologic; c,d are groups which treated with intraperitoneal and intravitreal bevacizumab and; e,f are groups which treated with intraperitoneal and intravitreal edaravone.*

were 1,3 ± 0,3, 0,3 ± 0,2, and 0,7 ± 0,2, respectively in the intravitreal serum physiologic, bevacizumab and edaravone treatment groups. In intravitreal treatments, while VEGF expression was significantly decreased in the bevacizumab treated group (p<0,05), no statistically significant difference was found between the edaravone and serum physiologic-treated groups.(Table 1)

Table 1. *VEGF expressions of rats in all groups*

Route	Groups	
	Intraperitoneal	Intravitreal
Serum physiologic	1,6 ± 0,2	1,3 ± 0,3
Bevacizumab	0,8 ± 0,1 *	0,3 ± 0,2 *
Edaravone	0,5 ± 0,2 *	0,7 ± 0,2
*Statistically significant P<0,005		

3.2. Evaluation of thickness of the outer nuclear layer

Figure 2 represents the alterations in the thickness of the outer nuclear layer of retina (ONL) of the study groups. The thickness ONL was compared between the groups. (Table 2) Morphometric analysis of retinal cross sections of rats

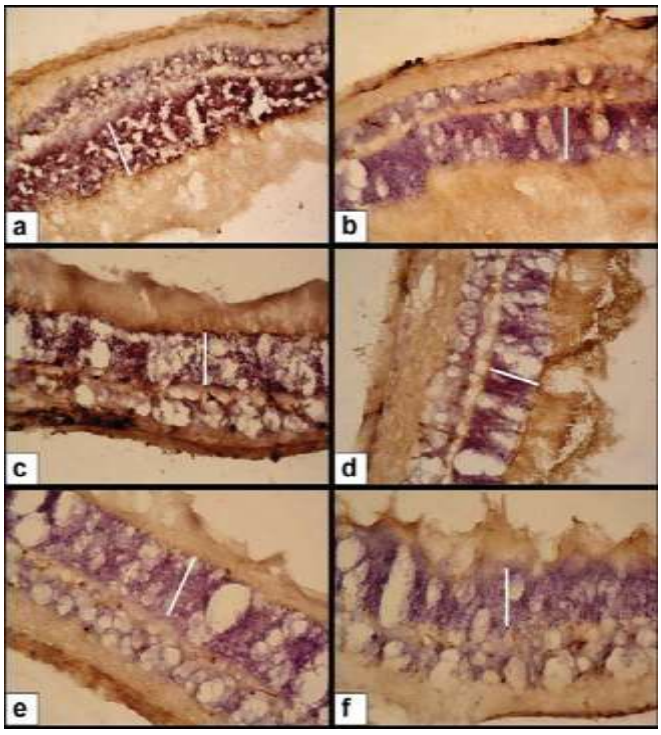

Figure 2. *Effects of bevacizumab and edaravone on outer nuclear layer thickness in diabetic rats. a,b are groups which treated with intraperitoneal and intravitreal SF; c,d are groups which treated with intraperitoneal and intravitreal bevacizumab and; e,f are groups which treated with intraperitoneal and intravitreal edaravone.*

Table 2. *Outer nuclear layer thickness evaluation according to serum physiologic group (%)*

Route	Groups	
	Intraperitoneal	Intravitreal
Serum physiologic	100 ± 5,0	100 ± 4,7
Bevacizumab	101,5 ± 5,3	123,5 ± 6,1 *
Edaravone	125,5 ± 6,6 *	105,3 ± 4,6
*Statistically significant P<0,005		

showed that intravitreal bevacizumab and intraperitoneal edaravone significantly improved ONL thickness compared to serum physiologic-treated group (% 23 and % 25, respectively, p<0.001).

3.3. Evaluation of plasma VEGF levels

Table 3 shows the effects of systemic treatment of bevazicumab and edaravone on plasma VEGF levels as evaluated by ELISA. According to these results, intraperitoneal administration of bevacizumab and edaravone significantly decreased the plasma VEGF levels compared to the serum physiologic-treated group (p<0,0005 and p<0,05, respectively).

4. DISCUSSION

In the present study, we aimed to compare the effects of intravitreal and intraperitoneal applications of bevacizumab, an anti-VEGF agent, and edaravone, a free radical scavenger, in retinal damage in streptozosin-induced diabetic rats.

Several molecular pathways have been suggested toclarifythe underlying mechanisms for diabetes related complications, including DR. Oxidative stress, which may occur because of an imbalance between the generation and elimination of ROS, can be critical for the development and progression of diabetic vascular complications. ROS generation and lipid peroxidation are elevated in diabetic patients, especially in those with poor glycemic control.[13] Moreover, the retina is known to be more susceptible to oxidative damage than any other tissue due to its high content of polyunsaturated fatty acids and the highest oxygen uptake and glucose oxidation.[1] Besides, there are several lines of evidence that suggest that antioxidant defense mechanisms may be significantly weakened in diabetes. For instance, the activities of antioxidant enzymes such as superoxide dismutase (SOD), glutathione reductase, glutathione peroxidase, and catalase are reduced in the retina. The level of glutathione (GSH), an intracellular antioxidant that can act as a potent radical scavenger, is also diminished in the diabetic retina.[13] Apart from these, other specific antioxidants such as vitamin C, vitamin E, and β-carotene are also diminished in diabetes-induced oxidative stress.[14]

The reduction in retinal perfusion triggered by vasoconstriction induces a series of biochemical and metabolic alterations. Progressive thickening of the basement membrane and hypertension disrupts the tight link between pericytes and endothelial cells, causing pericyte loss. Accordingly, these microvascular changes cause retinal

Table 3. *The effects of bevacizumab and edaravone on plasma VEGF levels of diabetic rats (pg/ml).*

	Intraperitoneal Serum physiologic	Intraperitoneal Edaravone	Intraperitoneal Bevacizumab
Mean ± SD	161,29 ± 47,36	7,35 ± 4,28 *	24,62 ± 12,41*
Min- Max	115,75 - 225,25	1,65 - 12,70	13,23 - 48,45
SD: Standard deviation * p<0,005, different from DM with serum physiologic group			

ischemia, with a release of pro-angiogenic factors (VEGF) and various pro-inflammatory cytokines (TNF-α, IL-6, and IL-1β) that result in the development of abnormal vessels on the cortical vitreous surface.[15]

VEGF, aneffective pro-angiogenic factor, has been implicated as the mediator of various pathological conditions including proliferative and non-proliferative retinopathies. Oxidative stress mediates the hyperglycemia-induced pathological effects of VEGF on microvascular complications of diabetes.[16] It has been shown that retinal expression of VEGF is increased by ROS. and activation of PKC can induce the expression of VEGF and VEGF- mediated endothelial cell migration and replication.[17,18] Besides, AGE-modified proteins can interact with endothelial cells through AGE receptors (RAGE) and lead to overexpression and secretion of VEGF and pro-inflammatory cytokines.[19-20]

In the present study, we demonstrated that both intraperitoneal and intravitreal bevacizumab treatment significantly reduced both retinal VEGF expression and plasma VEGF levels. Similarly, intraperitoneal edaravone treatment (10 mg/kg) succesfully decreased both retinal VEGF expression and plasma VEGF levels.

Edaravone, a lipophilic low molecular weight free-radical scavenger, has been studied not only in pre-clinical in vivo experimental settings but also in clinical practice. Anti-oxidative and anti-apoptotic effects of edaravone against cellular injury have been demonstrated in a variety of experimental animal models.[21] For instance, in a transient focal ischemia in mice brain, edaravone was found to reduce neuronal cell death by inhibiting the accumulation of 4-hydroxy-2-nonenal (4-HNE) and 8-hydroxy-deoxyguanosine (8-OHdG), which are important oxidative biomarkers.[22] It has also been reported that edaravone could reduce MDA levels, raise SOD activity, and decrease I/R-induced apoptosis of retinal neurons in the inner nuclear, ganglion cell, and outer nuclear layers of the rat retina in an ischemia-reperfusion injury model.[23] Recently, Niiya et al. have demonstrated that edaravone could inhibit AGE-induced vascular permeability, ROS overproduction and VEGF mRNA upregulation in bovine brain microvascular endothelial cells (BBMECs).[24] In a rat cerebral ischemia-reperfusion injury model, it has been found that treatment with edaravone not only diminished cerebral infarct size and neurological defects, but also effectively reduced ROS generation and HIF-1α as well as VEGF protein levels in the ischemic ipsilateral subventricular zone.[25] Also Masuda et al.[26] suggested that in their study intraperitoneal Edaravone significantly decreased diabetes induced retinal ganglion cell death and Inokuchi et al.[27] demonstrated protective effects of Edaravone against oxidative stress related cell damage.

In conclusion, in the current study, we demonstrated that intravitreal and systemic administration of edaravone may prevent the progression of diabetic retinopathy by VEGF inhibition. But Similarly, systemic administration of edaravone reduced the progression of retinal degeneration in diabetic rats. These findings suggest that edaravone may become a potential treatment for DR in the future. To the best of our knowledge, this is the first report that reveals the beneficial effects of intravitreal edaravone on diabetic retinopathy in an experimental model. However, this study is limited by a lack of dose-response experiments and biochemical determination of oxidative stress markers. Therefore, future studies will be required to clarify the underlying mechanisms of therapeutic effects of edaravone in diabetic retinopathy.

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REFERENCES / KAYNAKLAR

1. Kowluru RA, Chan P-S. Oxidative stress and diabetic retinopathy. Exp Diabetes Res. 2007;2007:43603.
2. Madsen-Bouterse SA, Kowluru RA. Oxidative stress and diabetic retinopathy: pathophysiological mechanisms and treatment perspectives. Rev Endocr Metab Disord. 2008; 9:315-27.
3. Scott IU, Edwards AR, Beck RW et al. Network DRCR. A phase II randomized clinical trial of intravitreal bevacizumab for diabetic macular edema. Ophthalmology. 2007; 114:1860-7.
4. Yuan D, Xu Y, Hang H et al. Edaravone protect against retinal damage in streptozotocin-induced diabetic mice. PLoS One. 2014; 9:e99219.
5. Lu F, Adelman RA. Are intravitreal bevacizumab and ranibizumab effective in a rat model of choroidal neovascularization? Graefes Arch Clin Exp Ophthalmol. 2009; 247:171-7.
6. Maeno T, Tano R, Takenaka H et al. Edaravone (MCI-186) is effective as a free radical scavenger following arteriovenous sheathotomy for treatment of macular oedema associated with branch retinal vein occlusion. Br J of Ophthalmol. 2009; 93:1479-82.
7. Giacco F, Brownlee M. Oxidative stress and diabetic complications. Circ Res. 2010; 107:1058-70.
8. Toyoda K, Fujii K, Kamouchi M et al.Free radical scavenger, edaravone, in stroke with internal carotid artery occlusion. J Neurol Sci. 2004; 221:11-17.
9. Uchiyama M, Tojo K, Yazawa T et al. Edaravone prevents lung injury induced by hepatic ischemia-reperfusion. J Surg Res. 2015; 194:551-7.
10. Shang H, Cui D, Yang D et al. The radical scavenger edaravone improves neurologic function and perihematomal glucose metabolism after acute intracerebral hemorrhage. J Stroke Cerebrovasc Dis. 2015; 24:215-22.
11. Zhang T, Li Z, Dong J et al. Edaravone promotes functional recovery after mechanical peripheral nerve injury. Neural Regen Res. 2014;9:1709-15.
12. Higashi Y, Jitsuiki D, Chayama K et al. Edaravone (3-methyl- 1-phenyl-2-pyrazolin-5-one), a novel free radical scavenger, for

treatment of cardiovascular diseases. Recent Pat Cardiovasc Drug Discov. 2006; 1:85-93.

13. Mancino R, Di Pierro D, Varesi C et al. Lipid peroxidation and total antioxidant capacity in vitreous, aqueous humor, and blood samples from patients with diabetic retinopathy. Mol Vis. 2011; 17:1298-304.
14. Kowluru RA, Kanwar M, Chan PS et al. Inhibition of retinopathy and retinal metabolic abnormalities in diabetic rats with AREDS-based micronutrients. Arch Ophthalmol. 2008; 126:1266-72.
15. Semeraro F, Cancarini A, dell'Omo R et al. Diabetic Retinopathy: Vascular and Inflammatory Disease. J Diabetes Res. 2015; 2015:582060.
16. Caldwell RB, Bartoli M, Behzadian MA et al. Vascular endothelial growth factor and diabetic retinopathy: role of oxidative stress. Curr Drug Targets. 2005; 6:511-24.
17. Lu M, Kuroki M, Amano S et al. Advanced glycation end products increase retinal vascular endothelial growth factor expression. J Clin Invest. 1998; 101:1219-24.
18. Clarke M, Dodson PM. PKC inhibition and diabetic microvascular complications. Best Pract Res Clin Endocrinol Metab. 2007; 21:573-86.
19. Stitt AW, Bhaduri T, McMullen CB et al. Advanced glycation end products induce blood-retinal barrier dysfunction in normoglycemic rats. Mol Cell Biol Res Commun. 2000;3:380-8.
20. Yamagishi S, Nakamura K, Matsui T et al. Pigment epithelium-derived factor inhibits advanced glycation end product-induced retinal vascular hyperpermeability by blocking reactive oxygen species-mediated vascular endothelial growth factor expression. J Biol Chem. 2006;281:20213-20.
21. Kikuchi K, Tancharoen S, Takeshige N et al. The efficacy of edaravone (radicut), a free radical scavenger, for cardiovascular disease. Int J Mol Sci. 2013;14:13909-30.
22. Zhang N, Komine-Kobayashi M, Tanaka R et al. Edaravone reduces early accumulation of oxidative products and sequential inflammatory responses after transient focal ischemia in mice brain. Stroke. 2005;36:2220-5.
23. Song Y, Gong YY, Xie ZG et al. Edaravone (MCI-186), a free radical scavenger, attenuates retinal ischemia/reperfusion injury in rats. Acta Pharmacol Sin. 2008;29:823-8.
24. Niiya Y, Abumiya T, Yamagishi S et al. Advanced glycation end products increase permeability of brain microvascular endothelial cells through reactive oxygen species-induced vascular endothelial growth factor expression. J Stroke Cerebrovasc Dis. 2012; 21:293-8.
25. Zhang P, Li W, Li L et al. Treatment with edaravone attenuates ischemic brain injury and inhibits neurogenesis in the subventricular zone of adult rats after focal cerebral ischemia and reperfusion injury. Neuroscience. 2012; 201:297-306.
26. Masuda T, Shimazawa M, Hara H. Retinal Diseases Associated with Oxidative Stress and the Effects of a Free Radical Scavenger (Edaravone). Oxid Med Cell Longev. 2017;2017:9208489.
27. Inokuchi Y, Imai S, Nakajima Yet al. Edaravone, a free radical scavenger, protects against retinal damage in vitro and in vivo. J Pharmacol Exp Ther. 2009;329:687-98.