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Lack of association between VAP-1/SSAO activity and corneal neovascularization in a rabbit model

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Abstract

The aim of this study is to determine the efficacy of a potent and specific vascular adhesive protein-1/ semicarbazide-sensitive amine oxidase (VAP-1/SSAO) inhibitor, LJP 1207, as a potential antiangiogenic and anti-inflammatory agent in the therapy of corneal neovascularization. Corneal neovascularization was induced with intrastromal suturing in rabbits ($n = 20$). Topical treatment with VAP-1/SSAO inhibitor LJP 1207 ($n = 5$, 4 times a day), bevacizumab ($n = 5$, daily), their combination ($n = 5$) and vehicle only ($n = 5$, 4 times a day) were applied postoperatively for 2 weeks. The development and extent of corneal neovascularization were evaluated by digital image analysis. At the end of the observation period, the level of corneal and serum VAP-1/SSAO activity was measured fluorometrically and radiochemically. The corneal

VAP-1/SSAO activity was significantly elevated in the suture-challenged vehicle-treated group ($3,075 \pm 1,009$ pmol/mg/h) as compared to unoperated controls (464.2 ± 135 pmol/mg/h, $p < 0.001$). Treatment with LJP 1207 resulted in slower early phase neovascularization compared to vehicle-treated animals (not significant). At days 7–14, there was no significant difference in the extent of corneal neovascularization between inhibitor- and vehicle-treated corneas, even though inhibitor treatment caused a normalization of corneal VAP-1/SSAO activity (885 ± 452 pmol/mg/h). Our results demonstrate that the significant elevation of VAP-1/SSAO activity due to corneal injury can be prevented with VAP-1/SSAO inhibitor LJP 1207 treatment. However, normalization of VAP-1/SSAO activity in this model does not prevent the development of corneal neovascularization.

Keywords

VAP-1/SSAO; LJP 1207; Cornea; Neovascularization

Introduction

The cornea is a transparent, avascular tissue that covers the anterior surface of the eye. In the case of a wide range of ocular pathologies, blood vessels originating from limbal stem cells may vascularize the corneal tissue (Zhang and Ma 2007). Corneal neovascularization (NV) occurs in a wide variety of immunologic, infectious and degenerative disorders. Furthermore, it may be related to trauma, chemical injury and contact lens wearing (Chang et al. 2001). In severe cases, corneal NV might account for loss of vision and necessitate replacement of the corneal surface by transplantation of avascular donor tissue.

Current therapeutic options (including steroid and non-steroidal drugs, laser photocoagulation, fine-needle diathermy, photodynamic therapy) exert limited clinical effects and often lead to severe adverse effects (Gupta and Illingworth 2011). Therefore, it is important to explore the molecular mechanisms leading to corneal angiogenesis to find suitable therapies. A number of promising therapeutic agents are under investigation according to literature data (Qazi et al. 2010). Vascular endothelial growth factor (VEGF) is thought to be one of the key mediators in the process of NV, and the effects of anti-VEGF antibodies have been demonstrated on various experimental corneal NV models (for review see Gupta and Illingworth 2011). So far, anti-VEGF antibodies have been licensed exclusively for vitreoretinal diseases, but they are off-label for corneal NV, as there is little evidence for their efficacy on the cornea (Regenfuss et al. 2009; Benayoun et al. 2012).

Vascular adhesive protein-1 (VAP-1), which is identical with the enzyme semicarbazide-sensitive amine oxidase (SSAO), has recently emerged as a new potential therapeutic target against inflammation (Dunkel et al. 2008). VAP-1/SSAO mediates leukocyte interactions with vascular endothelial cells during leukocyte extravasation. In addition, VAP-1/SSAO catalyzes the deamination of primary amines into aldehyde, producing ammonia and hydrogen peroxide as by-products. Given their potential toxicity, these enzymatic products are thought to contribute to vascular complications (O'Sullivan et al. 2004). However,

VAP-1/SSAO activity is reported to participate in vitro in the maintenance of organized vascular structure (Langford et al. 2002).

The anti-inflammatory effect of VAP-1/SSAO blockade has been demonstrated by several recent reports (Jalkanen and Salmi 2008; Salter-Cid et al. 2005). Furthermore, VAP-1/SSAO blockade significantly reduced the extent of neovascularized area and the expression of inflammatory molecules TNF-alpha, monocyte chemoattractant protein-1, and intercellular adhesion molecule-1 in laser-induced choroidal NV (Noda et al. 2008). On the basis of extensive evidence that VAP-1/SSAO is associated with the process of inflammation, we endeavored to advance this line of research by testing whether VAP-1/SSAO could have a role in inflammation-associated angiogenesis.

In the present study, we measured VAP-1/SSAO activity in suture-challenged corneas and tested whether treatment of rabbits with a VAP-1/SSAO inhibitor (LJP 1207) affected the level of VAP-1/SSAO activity and the development of corneal neovascularization.

Materials and methods

Animals

New Zealand white adult male rabbits ($n = 25$) of the same body weight (2.5 kg) were used in this study. Rabbits had unlimited access to standard chow and water, and were caged separately.

Our experiments were carried out in accordance with the relevant local institutional, national regulations and legislations, and with the ARVO Statement for the Use of Animals in Ophthalmic and Visual Research.

Induction of NV

20 rabbits were anesthetized by intramuscular injection of a mixture of ketamine (30 mg/kg body weight) and xylazine (6 mg/kg body weight), and by topical administration of oxybuprocaine (0.4 %). NV was induced by three 7-0 polypropylene sutures placed at midstromal depth, radially, 1 mm from the limbal line at 11, 12 and 1 clock hour of the cornea, at 3 mm length, under stereomicroscope.

Treatments

Suture-challenged rabbits were randomly divided into four groups. From the day of suture placement VAP-1/SSAO inhibitor LJP 1207 alone, VAP-1/SSAO inhibitor LJP 1207 and bevacizumab in combination, bevacizumab alone and vehicle (0.9 % sterile sodium chloride) were administered as eye drops ($n = 5$ in each group). VAP-1/SSAO inhibitor and vehicle were administered four times a day (dose of LJP 1207 was 30 mg/kg), while bevacizumab (8 mg/kg) was applied once a day.

To avoid bacterial infection, ofloxacin (3 %) was applied as eye drop into the injured eyes twice a day for 3 days.

Detection of the neovascularized area

To determine the area of corneal NV, we captured digital photographs with a Canon EOS 30D digital camera on the 3rd, 7th, 10th, and 14th day after suture placement. ImageJ image analysis software (Research Services Branch, National Institutes of Mental Health, Bethesda, MD, USA) was used to quantify the vascularized corneal area. At a representative photograph of each animal at each checkpoint, the vascularized corneal area was measured in pixels, and these values were expressed as percent values of the entire corneal surface.

Determination of serum VAP-1/SSAO activity

At the end of the period of observation (at day 14) we obtained a specimen of venous blood from the ear of each rabbit. Serum samples were prepared by centrifugation at 2,500g for 10 min, at 4 °C, and were stored at –80 °C until further processing. The radiometric method of Yu and Zuo (1993) was adapted with slight modifications to determine enzyme activity in serum samples. Serum sample (40 µL) was preincubated with clorgyline (10^{-4} M) in 100 mM phosphate buffer pH 7.4 at room temperature for 20 min to inhibit MAO activity. The enzyme reaction was initiated by adding radiolabeled [14 C]-benzylamine (5×10^{-4} M final concentration; 0.02 µCi) as substrate, then incubated in a final volume of 200 µl for 40 min at 37 °C. The reaction was stopped by addition of equal volume of 2 M citric acid. The oxidized product ([14 C]-benzaldehyde) was extracted into 1 ml of toluene:ethylacetate (1:1), then 600 µL of the organic phase was transferred to a scintillation vial, containing 5 ml of optiphase scintillation fluid. Radioactivity was measured by liquid scintillation counting. Protein content of the samples was determined by standard Bradford method (Bradford 1976). The enzyme activity was expressed as pmol benzaldehyde formed by 1 mg serum protein in 1 h at 37 °C (pmol/mg/h).

Determination of VAP-1/SSAO activity in the corneal tissue

After taking blood samples rabbits were euthanized by pentobarbital overdose. The entire corneas were excised from each animal, and were stored at –80 °C until further processing. The fluorometric method of Zhou and Panchuk-Voloshina (1997) was adapted to determine amine oxidase activity of corneal tissue samples. Briefly, samples were homogenized in 100 mM phosphate buffer pH 7.4 by a blade tissue homogenizer then centrifuged at 20,000g for 5 min at 4 °C. The clear supernatant was used for the enzyme assay. 50 µL tissue extract was preincubated in the presence of 1 mM pargyline or 1 mM pargyline and 1 mM 2-bromo-ethylamine to inhibit MAO and SSAO activity, respectively. The enzyme reaction was initiated by adding a mixture of 1 mM benzylamine, 100 µM Amplex red and 1 U/mL horseradish peroxidase, then incubated at 37 °C in the dark for 30 min. The fluorescence generated was detected with a fluorescent microplate reader (Fluorescen Ascent FL, Thermo Electron Corporation, Vintaa, Finland) using 530 and 590 nm excitation and emission filter, respectively. Hydrogen peroxide (0.625–10 µM) was used for calibration. SSAO activity was calculated from the 2-bromo-ethylamine inhibitable hydrogen peroxide formation. Protein content of corneal tissue samples was determined by standard Bradford method (Bradford 1976). The enzyme activity was expressed as pmol hydrogen peroxide formed by 1 mg sample protein in 1 h at 37 °C (pmol/mg/h).

Statistical analysis

For clinical evaluation and activity measurements, ANOVA test followed by Tukey's post hoc test was applied using SPSS 15.0 software. $p < 0.05$ was considered to be statistically significant.

Results

Clinical evaluation of NV

Digital photographs were captured four times during the two-week period of observation after intrastromal suture placement (Fig. 1). At each checkpoint, the area of neo-vascularized cornea was measured and expressed relative to the entire corneal surface (Fig. 2).

The first detectable vessels began to develop in all the 20 suture-challenged corneas at day 3. At this time, no significant changes in neovascularization was observed between the vehicle-treated and any other treated groups, although the vehicle-treated group trended toward being more affected. On day 7, both the bevacizumab and the LJP 1207 + bevacizumab-treated groups showed a significantly reduced (6.3 ± 1.9 and 4.8 ± 1.4 %, respectively) neovascularized area in comparison with the LJP 1207 treated group (12.5 ± 1.7 %) or the vehicle-treated group (12.6 ± 2.9 %). While neovascularization increased in all groups from day 7 until the end of the observation period, the difference remained obvious between the bevacizumab-treated and bevacizumab-untreated groups. There was no difference between the effects of LJP 1207 and vehicle treatments considering the area of NV (Figs. 1, 2).

VAP-1/SSAO activity in corneal tissue and serum samples

In the corneal tissue VAP-1/SSAO activity was measured fluorometrically in each rabbit (Fig. 3). In healthy, unoperated control rabbits corneal VAP-1/SSAO activity was measured to be 464.2 ± 135 pmol/mg/h. Corneal NV induced by sutures was associated with a significant elevation of corneal VAP-1/SSAO activity in the vehicle-treated animals ($3,075 \pm 1,009$ pmol/mg/h $p < 0.001$) compared to unsutured control corneas. However, topically applied LJP 1207 treatment alone and in combination with bevacizumab reduced the corneal VAP-1/SSAO activity back to normal level (885 ± 452 pmol/mg/h and $1,145 \pm 523$ pmol/mg/h), which were significantly lower than the suture-challenged, vehicle-treated controls ($p < 0.05$). As expected, bevacizumab treatment alone in suture-challenged corneas did not reduce tissue VAP-1/SSAO activity ($p > 0.05$).

We measured VAP-1/SSAO activity radiochemically in the serum of healthy control and all suture-challenged rabbits (Fig. 4). The serum VAP-1/SSAO activity of vehicle-treated suture-challenged rabbits did not differ significantly from the values of healthy controls (average values of the groups $19,758 \pm 4,475$ vs. $17,600 \pm 7,081$ pmol/mg/h, $p > 0.05$). Activity levels were similar in the bevacizumab-treated group ($20,189 \pm 7,788$ pmol/mg/h, $p > 0.05$). However, topically applied VAP-1/SSAO inhibitor LJP 1207 resulted in a significant reduction ($9,180 \pm 1,469$ pmol/mg/h) in the serum VAP-1/SSAO activity ($p < 0.05$ vs. vehicle treatment).

Discussion

The involvement of VAP-1/SSAO in certain inflammation-associated diseases such as diabetes mellitus, atherosclerosis, rheumatoid arthritis, chronic heart failure (for review see O'Sullivan et al. 2004) and also in angiogenesis (Énzsöly et al. 2011a; Noda et al. 2008; Garpenstrand et al. 2004) has been convincingly established. However, a possible role for VAP-1/SSAO in the pathogenesis of these diseases is still poorly understood. In the present study, we investigated whether VAP-1/SSAO may play a role in mechanisms leading to angiogenesis, and tested the possibility that an inhibitor of VAP-1/SSAO may have efficacy against neovascularization using the well-established suture model in experimental rabbits.

VAP-1/SSAO is reported to be found in most organs (Boomsma et al. 2005). As to ocular tissues, Almulki et al. (2010) reported that VAP-1/SSAO expression is limited to the endothelial cells of retinal and choroidal vessels. In marked contrast to that, VAP-1/SSAO activity was detected by us in the normal, avascular corneal tissue, in a level one magnitude lower than in the serum. We hypothesize that the source of VAP-1/SSAO in normal cornea might be the endothelium of the cornea.

In the current study, we demonstrate that the induction of corneal NV is associated with a significant increase in VAP-1/SSAO activity in corneal tissue, suggesting an association between VAP-1/SSAO activity and angiogenesis. This observation is in agreement with the study of Noda et al. (2008), who found a higher expression of VAP-1 in the choroid of a rat model of choroidal NV. However, the study of Noda et al. (2008) did not include VAP-1/SSAO activity data. Previous observations of a correlation between serum VAP-1/SSAO activity and circulating VEGF in patients with lung cancer are consistent with our hypothesis that there should be an association between VAP-1/SSAO activity and angiogenesis (Garpenstrand et al. 2004). It is still unclear whether VAP-1/SSAO activity has either causative or consequential role in the process of angiogenesis.

The increase in corneal VAP-1/SSAO activity can either be derived from the circulating form or the locally produced membrane-bound form of the enzyme. Vascular endothelial cells are considered to be the main source of circulating VAP-1/SSAO (Stolen et al. 2004). Based on our observations, we suspect that pathological vessels may contribute, along with normal vessels, as the source of VAP-1/SSAO. Lack of elevated serum SSAO activity due to corneal NV induction suggests that intracorneal sutures would represent a local injury without exerting a systemic effect on the level of circulating form of VAP-1/SSAO. The other hypothesis for the origin of elevated VAP-1/SSAO level in corneal NV might be the increase of local basal production. This hypothesis might explain our observation that an elevation in corneal VAP-1/SSAO activity is not accompanied by an elevated serum activity.

The VAP-1/SSAO inhibitor we administered here was LJP 1207 [N'-(2-phenyl-allyl)-hydrazine hydrochloride], which is a potent (human SSAO IC_{50} = 17nM, rat SSAO IC_{50} = 7.5 nM, no available data for rabbits) and selective inhibitor that blocks both the enzymatic and adhesive functions of VAP-1/SSAO (O'Rourke et al. 2007; Xu et al. 2006; Salter-Cid et al. 2005). Anti-VEGF antibodies have a well-documented effect on corneal angiogenesis

(Hosseini et al. 2012; Gupta and Illingworth 2011), which led us to consider bevacizumab as a positive control for inhibition of corneal NV.

LJP 1207 is known as an orally active drug (Salter-Cid et al. 2005). The dose we applied (30 mg/kg) was chosen on the basis of literature data, where beneficial effects have been reported on different rat models (Yang et al. 2011; Xu et al. 2006; Yu and Zuo 1993). The dramatic reduction of the corneal VAP-1/SSAO activity by inhibitor treatment strongly supports the use of LJP 1207 formulated as an eye drop. Therefore, the lack of NV inhibition in our studies is unlikely due to inadequate dosing or tissue penetration of LJP 1207. Instead, we conclude that the extent of NV is unrelated to VAP-1/SSAO activity.

Intrastromally placed sutures mediate a chronic trauma for corneal tissue resulting in formation of NV. In this study, intrastromal sutures were not removed until the end of the observation period to model long-lasting treatment. At the first checkpoint of the study (at day 3), both LJP 1207 and bevacizumab treatment trended toward a delay in the development of corneal NV compared to vehicle treatment. We have shown earlier in a less severe model of NV that both bevacizumab and LJP 1207 had roughly equal effects in slowing the development of NV (Énzsöly et al. 2011b). We report here that from day 7, the effect of LJP 1207 was essentially equal to vehicle treatment in their effects on the extent of NV. As far as we know, this is the first report to demonstrate that VAP-1/SSAO inhibition is ineffective on a suture-induced corneal NV model. The possible synergistic effects of VAP-1/SSAO inhibitor LJP 1207 and anti-VEGF antibody suggested by our results need further investigations.

Surprisingly, despite the lack of a reducing effect on the development of NV, VAP-1/SSAO inhibitor LJP 1207 treatment prevented the elevation of VAP-1/SSAO activity due to NV in the cornea. As for the serum VAP-1/SSAO activity, it is remarkable that topical inhibitor treatment reduced activity levels to the lower limit of normal range. These observations support that LJP 1207 has a strong VAP-1/SSAO inhibitor potency. However, the reduction of tissue VAP-1/SSAO activity seems to be unrelated to corneal NV.

The underlying mechanisms of suture-induced NV might include VEGF, as demonstrated by the antiangiogenic effect of bevacizumab, an anti-VEGF antibody, in the current studies. However, our studies do not support the hypothesis that VAP-1/SSAO is involved in the pathogenesis of NV.

We demonstrate here on a suture-induced corneal NV model that VAP-1/SSAO blockade can slow the initial process of the development of NV, as we reported earlier (Énzsöly et al. 2011a). However, inhibition of VAP-1/SSAO is not sufficient to inhibit the development of NV on a long-term basis. Future studies are planned to test our hypothesis that VAP-1/SSAO blockade might be effective in inflammatory pathologies, and also in certain inflammatory-associated neovascularization diseases.

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References

- Almulki L, Noda K, Nakao S, et al. Localization of vascular adhesion protein-1 (VAP-1) in the human eye. *Exp Eye Res.* 2010; 90:26–32. [PubMed: 19761765]
- Benayoun Y, Adenis JP, Casse G, et al. Effects of subcon-junctival bevacizumab on corneal neovascularization: results of a prospective study. *Cornea.* 2012; 31:937–944. [PubMed: 22357391]
- Boomsma F, Hut H, Bagghoe U, et al. Semicarbazide-sensitive amine oxidase (SSAO): from cell to circulation. *Med Sci Monit.* 2005; 11:RA122–RA126. [PubMed: 15795708]
- Bradford MM. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal Biochem.* 1976; 7:248–254. [PubMed: 942051]
- Chang JH, Gabison EE, Kato T, et al. Corneal neovascularization. *Curr Opin Ophthalmol.* 2001; 12:242–249. [PubMed: 11507336]
- Dunkel P, Gelain A, Barlocco D, et al. Semicarbazide-sensitive amine oxidase/vascular adhesion protein 1: recent developments concerning substrates and inhibitors of a promising therapeutic target. *Curr Med Chem.* 2008; 18:1827–1839. [PubMed: 18691041]
- Énzsöly A, Dunkel P, Récsán Z, et al. Preliminary studies of the effects of vascular adhesion protein-1 inhibitors on experimental corneal neovascularization. *J Neural Transm.* 2011a; 118:1065–1069. [PubMed: 21331460]
- Énzsöly A, Recsan Z, Marko K, et al. The effect of a VAP-1 inhibitor on corneal neovascularization. *Invest Ophthalmol Vis Sci.* 2011b; 52:E-Abstract 6398.
- Garpenstrand H, Bergqvist M, Brattström D, et al. Serum semicarbazide-sensitive amine oxidase (SSAO) activity correlates with VEGF in non-small-cell lung cancer patients. *Med Oncol.* 2004; 21:241–250. [PubMed: 15456951]
- Gupta D, Illingworth C. Treatments for corneal neovascularization: a review. *Cornea.* 2011; 30:927–938. [PubMed: 21389854]
- Hosseini H, Nowroozzadeh MH, Salouti R, et al. Anti-VEGF therapy with bevacizumab for anterior segment eye disease. *Cornea.* 2012; 31:322–334. [PubMed: 22157572]
- Jalkanen S, Salmi M. VAP-1 and CD73, endothelial cell surface enzymes in leukocyte extravasation. *Arterioscler Thromb Vasc Biol.* 2008; 28:18–26. [PubMed: 17962625]
- Langford SD, Trent MB, Boor PJ. Semicarbazide-sensitive amine oxidase and extracellular matrix deposition by smooth-muscle cells. *Cardiovasc Toxicol.* 2002; 2:141–150. [PubMed: 12271157]
- Noda K, She H, Nakazawa T, et al. Vascular adhesion protein-1 blockade suppresses choroidal neovascularization. *FASEB J.* 2008; 22:2928–2935. [PubMed: 18436961]
- O'Rourke AM, Wang EY, Salter-Cid L, et al. Benefit of inhibiting SSAO in relapsing experimental autoimmune encephalomyelitis. *J Neural Transm.* 2007; 114:845–849. [PubMed: 17393060]
- O'Sullivan J, Unzeta M, Healy J, et al. Semicarbazide-sensitive amine oxidases: enzymes with quite a lot to do. *Neurotoxicology.* 2004; 25:303–315. [PubMed: 14697905]
- Qazi Y, Wong G, Monson B, et al. Corneal transparency: genesis, maintenance and dysfunction. *Brain Res Bull.* 2010; 81:198–210. [PubMed: 19481138]
- Regenfuss B, Bock F, Bachmann B, et al. Topical inhibition of angiogenesis at the cornea safety and efficacy. *Ophthalmologe.* 2009; 106:399–406. [PubMed: 19396447]
- Salter-Cid LM, Wang E, O'Rourke AM, et al. Anti-inflammatory effects of inhibiting the amine oxidase activity of semicarbazide-sensitive amine oxidase. *J Pharmacol Exp Ther.* 2005; 315:553–562. [PubMed: 16081681]
- Stolen CM, Yegutkin GG, Kurkijärvi R, et al. Origins of serum semicarbazide-sensitive amine oxidase. *Circ Res.* 2004; 95:50–57. [PubMed: 15178639]
- Xu HL, Salter-Cid L, Linnik MD, et al. Vascular adhesion protein-1 plays an important role in postischemic inflammation and neuropathology in diabetic, estrogen-treated ovariectomized female rats subjected to transient forebrain ischemia. *J Pharmacol Exp Ther.* 2006; 317:19–29. [PubMed: 16339390]

- Yang W, Li H, Luo H, et al. Inhibition of semicarbazide-sensitive amine oxidase attenuates myocardial ischemia-reperfusion injury in an in vivo rat model. *Life Sci.* 2011; 88:302–306. [PubMed: 21146547]
- Yu PH, Zuo DM. Oxidative deamination of methylamine by semicarbazide-sensitive amine oxidase leads to cytotoxic damage in endothelial cells. Possible consequences for diabetes. *Diabetes.* 1993; 42:594–603. [PubMed: 8454111]
- Zhang SX, Ma JX. Ocular neovascularization: implication of endogenous angiogenic inhibitors and potential therapy. *Prog Retin Eye Res.* 2007; 26:1–37. [PubMed: 17074526]
- Zhou M, Panchuk-Voloshina N. A one-step fluorometric method for the continuous measurement of monoamine oxidase activity. *Anal Biochem.* 1997; 253:169–174. [PubMed: 9367499]

Fig. 1. Representative *photographs* of differently treated $n = 5$ groups (**a–d** VAP-1/SSAO inhibitor LJP 1207 treatment, **e–h** LJP 1207 in combination with bevacizumab treatment, **i–l** bevacizumab treatment, **m–p** vehicle treatment) of rabbits at days 3, 7, 10 and 14 (**a, e, i, m** day 3; **b, f, j, n** day 7; **c, g, k, o** day 10; **d, h, l, p** day 14), after placement of intrastromal corneal sutures

Fig. 2.

Neovascularized corneal area in the proportion of the entire corneal surface ($n = 5$ in each group, data are mean $\pm$ SD, * $p < 0.05$, ** $p < 0.001$)

Fig. 3.

SSAO activity in corneal tissue of suture-challenged animals receiving different types of treatments for 14 days (LJP 1207, LJP 1207 combined with bevacizumab, bevacizumab, vehicle) and healthy control animals ($n = 5$ in each group, data are mean $\pm$ SD, $*p < 0.05$, $**p < 0.005$)

Fig. 4. Serum SSAO activity in suture-challenged animals receiving different types of treatments for 14 days (LJP 1207, LJP 1207 combined with bevacizumab, bevacizumab, vehicle) and healthy control animals ($n = 5$ in each group, data are mean $\pm$ SD, $*p < 0.05$)