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Angiostatins modulate ACE2 and GFAP levels in injured rat cornea and do not affect viability of retinal pigment epithelial cells

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Angiostatins (AS) are proteolytically derived fragments of plasminogen with the established antiangiogenic and anti-inflammatory capacities, which can be useful for ocular surface relief after injury. **Aim.** To investigate the effects of AS K1-3 and K5 on the protein levels of SARS-CoV-2 receptor, ACE2, and marker of activated satellite glia, GFAP, in the injured rat cornea, and to assess if AS could affect the viability of retinal pigment epithelial (RPE) cells. **Methods.** AS (K1-3 and K5) were produced by limited proteolysis of plasminogen isolated from human plasma followed by affine chromatography purification. AS (K1-3 0.1 or 1.0 μM, K5 0.1 μM) were applied topically as eye drops in rat model of alkali burn of cornea. The protein levels of ACE2 and GFAP were evaluated in corneal tissue lysates by western blot and expressed as arbitrary units (a.u.). The effects of AS on the viability of RPE cells were evaluated by MTT test. **Results.** The ocular injury caused by alkali burn induced overexpression of both ACE2 (by 7.7 fold vs. control, $P < 0.001$) and GFAP (by 62 folds vs. control, $P < 0.001$). Application of K5 or K1-3 (1.0 μM) reduced the expression level of ACE2 in injured corneas by two-folds ($P < 0.05$ vs. Burn group), whereas K1-3 (0.1 μM) lowered the content of ACE2 by 3.4-folds compared with the Burn group ($P < 0.05$) suggesting that AS can decrease a potential risk of SARS-CoV-2 entry due to the down-regulation of its receptor expression. Interestingly, K5 appeared to be more effective in suppressing the GFAP overexpression (by 10.9-fold vs. Burn group, $P < 0.01$) indicating that AS may act as neuroprotective substances via alleviating excessive response of satellite glia in injured cornea. AS had no cytotoxic effects on retinal

pigment endothelium in the range of concentrations 2-100 nM. **Conclusions.** Collectively, our data may pave the way for a possible application of AS in designing an ophthalmic drug as an effective and safe supplementary with combined protective effects.

Keywords: angiostatins, corneal injury, ACE2, GFAP, RPE cells

Introduction

The cornea is an optically transparent avascular tissue, which acts as a major refractive structure of the eye, provides mechanical stability and protects the eye against injury, infections, and other environmental impacts [1]. Corneal wounds caused by traumatic injuries, chemical burns, surgeries, contact lens wearing, keratitis, bacterial and viral infections may induce acute or chronic inflammation. Inflammation, if excessive, can delay wound healing and upregulate angiogenic cytokines, which activate and support corneal neovascularization as a non-specific response to chronic hypoxia in an attempt to enhance the oxygen supply to the cornea [2]. Thus, seeking for the treatment options to inhibit inflammation and limit neovascularization in the injured cornea is still an actual aspect for ophthalmology. During the inflammatory process, overexpression of angiotensin-converting enzyme 2 (ACE2) has been found in corneal epithelial cells. Previous studies have indicated that the ACE2 expression is highly upregulated by TNFα or IL-1β in human corneal epithelial cells and appeared to be also enhanced in inflamed mouse corneal epithelium after the injuries including alkali burn [3]. Since SARS-CoV-2 spike protein has essential binding affinity to ACE2, the inflammation-induced overexpression of ACE2 makes cornea a potential route of viral entry into an organism [4]. Several studies have revealed that SARS-CoV-2 is detectable by PCR-RT in conjunctival swabs and tears of patients with COVID-19 [5, 6]. Although the relationship between the ACE2 expression levels in corneal epithelium and SARS-CoV-2 spread is still poorly explored, it is apparent that reduction of the ACE2 expression in injured cornea can be a protective option against viral invasion through the ocular surface.

The cornea is primarily innervated by the ophthalmic branch of the trigeminal nerves and is thought to be one of the most densely innervated structures of the whole body [7]. Corneal injury may cause a damage to corneal nerves because of their location close to the ocular surface. Disruption of corneal nerves results in disturbances of the regulation of epithelial cell proliferation and wound healing, as well as in a decrease in defensive reflex and tear flow, and loss of sensual perception. It has been earlier established that corneal trauma and inflammation can aggravate the dysfunction of corneal nerves and impair their recovery [8]. Also, corneal injury may affect satellite cells, which play trophic, supporting, and protective roles for the periphery nerves. Acting like glia in CNS, satellite glial cells may acquire a reactive phenotype in response to the injury, while their hyperactivity can be detrimental for neurons via producing surplus of pro-inflammatory cytokines and reactive oxygen/nitrogen species [9]. As well, the activated satellite glial cells are able to abundantly synthesize glial fibrillary acidic protein

(GFAP), a specific cytoskeletal protein of glial origin, which is used as a classical marker of glial reactivity [10]. Therefore, the pharmacological modulation of nerve-supporting cell response can be beneficial for eliciting neural regeneration after corneal injury.

During the last two decades, proteolytically derived fragments of plasminogen, which are referred to as angiostatins (AS), have been considered as promising candidates for the suppression of corneal neovascularization [11, 12]. AS comprise a group of polypeptides, which contain various number of plasminogen kringle (K) domains (K1-3, K1-4, K1-4.5, K5, *etc.*), exhibiting multiple biological activities. AS play a role of potent endogenous inhibitors of the vessel outgrowth via counteracting vascular endothelial growth factor (VEGF) to induce the apoptosis of endothelial cells and suppress their migration [13]. Anti-adhesive and anti-inflammatory activities of AS have also been described [14]. Past studies have shown that plasminogen and AS are produced in the corneal tissue and the latter is involved in preventing vascularization during injury and hypoxic conditions [15]. Here, we hypothesized that AS can be used as an effective therapeutic tool for restricting inflammation-driven processes in the injured cornea including overexpression of ACE2 and excessive glial reactivity. Since the substances used topically as an eye drop may reach posterior ocular structures and affect retinal cells, the examination of novel ophthalmological drugs for their potential cytotoxicity is required. In order to verify biosafety of AS tested in our study, we performed the viability assay using the retinal pigment epithelial (RPE) cells, which are critical for normal vision [16]. Thus, the aim of the present study was to investigate the effects of two angiostatin variants, K1-3 and K5, on the protein levels of ACE2 and GFAP in the rat cornea in the model of corneal alkali burn, and to verify if angiostatins may affect the viability of RPE cells.

Materials and Methods

Reagents. All chemicals were purchased from Sigma Aldrich Co. (St Louis, MO, USA) unless otherwise stated. All other reagents were of analytical grade and were provided by commercial suppliers.

Angiostatin production. Native (Glu-) form of human plasminogen was isolated by the affine chromatography on lysine-sepharose from the fresh citrated donor plasma as described earlier [17]. AS K1-3 was produced by limited proteolysis of plasminogen with the use of porcine pancreatic elastase followed by gel-filtration and affine chromatography steps as described elsewhere [18]. Mini-plasminogen, a by-product of plasminogen elastolysis, was used for producing AS K5 by means of fragmentation by pepsin followed by purification with the use of aminohexyl (AH)-sepharose affinity chromatography as reported elsewhere in details [19]. Prepared proteins were electrophoretically homogenous (Fig. 1) and did not contain proteolytically active impurities.

Experimental model of corneal alkali burn. Wistar rats (6-months-old males) were kept and handled in compliance with the guidelines of the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research. The experimental protocol was approved by the Institutional Bioethical Committee (protocol no. 4, 2021/06/08). The rats were anesthetized by intraperitoneal injection of ketamine (50–

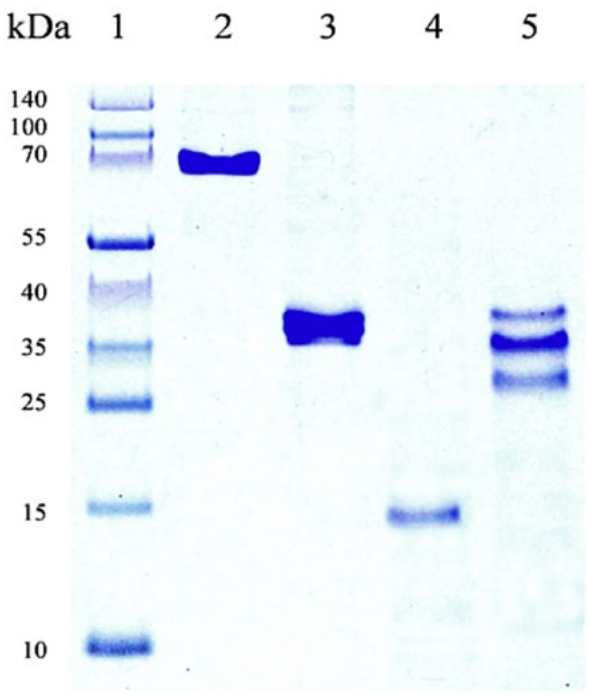

Fig. 1. Electrophoregram of Glu-plasminogen (2) and products of its limited proteolysis: mini-plasminogen (3), K5 (4), and K1-3 (5); 1 — markers of M_m.

100 mg/kg) and received topically a drop of tetracaine. A 4 mm diameter filter paper soaked in 1 N NaOH was placed on the center of the corneal surface of the right eye for 30 sec, and then the eye was thoroughly washed with 10 ml of saline. After injury, the rats were randomly divided into five treatment groups (n = 6 in each group): i) intact control (saline + + AS); ii) burn control (saline); iii) burn + K5 (1.0 μM); iv) burn + K1-3 (1.0 μM); v) burn + + K1-3 (0.1 μM). Topical administration (25 μl) of AS dissolved in sterile buffered saline to injured eye was done daily for 14 days. To determine if tested polypeptides have any side effects in the non-injured rat eye, the eye drops at the same concentrations were applied to the left eye daily for one week. K5 was used in one concentration because of its limited amount available. All rats were thereafter euthanatized.

Cornea isolation and protein sample preparation. After eye enucleation, the corneas were slightly rinsed for 5 minutes in the ice-cold phosphate-buffered saline (PBS). Both corneas of each rat were pooled, grinded in liquid nitrogen, and homogenized in lysed buffer (0.05 M Tris-HCl, pH 7.4, 0.15 M NaCl, 1 % Triton X-100, 0.1 % SDS) supplemented with the Pierce™ protease and phosphatase inhibitor cocktail (ThermoScientific, USA, cat. no. A32961). Tissue to buffer ratio was 1:5 (m/v). After additional sonification (ultrasound disintegrator Sartorius, Labsonic®M, Göttingen, Germany), homogenates were centrifuged at 16,000 g for 45 min at 4 °C. Total protein content in supernatants was measured spectrophotometrically, using the measurements of absorbance at the wavelengths of 280 and 260 nm as described earlier [20]. Supernatants were then mixed with an equal volume of 2 × reducing sample buffer.

Western blot. Aliquots were loaded onto 10 % SDS-PAGE (50 μg protein per track) and electrophoresed in a vertical gel electrophoresis chamber (BioRad, USA) and then the proteins were transferred from the gel onto nitrocellulose membrane (GE Healthcare, Amersham Bioscience, UK, RPN 203D) with 0.45 μm pore diameter by electroblot. After 1 h blocking in a 5 % solution of skimmed milk powder in PBS, the blots were probed with primary antibodies for: ACE2 (Millipore Inc., USA, cat. no. MABN59, clone 4G5.1, 1:1,000 diluted), GFAP (Santa Cruz Biotechnology, USA, cat. no. sc-9065, 1:2,500 diluted), and β-actin

(Invitrogen, USA, cat. no. MA5-15739, 1:5,000 diluted) as a loading control. After washing each membrane five times with PBS containing 0.05 % Triton X-100 (PBST), they were incubated with HRP-conjugated goat anti-rabbit IgG (1:8,000 diluted) or HRP-conjugated goat anti-mouse IgG (1:10,000 diluted) for 2 h at 37 °C. After washing with PBST, the specific immunoreactivity was visualized by enhanced chemiluminescence (ECL) and the band intensities were calculated with the use of densitometry software TotalLab TL120 (Nonlinear Inc., USA). The molecular weights of proteins in the samples were determined by comparing their migration with the location of coloured markers Ruler™ Plus Prestained Protein Ladder 10–230 kDa (ThermoScientific, Lithuania, cat. no. 26619). Signal intensities of the studied proteins in each sample were normalized to the actin levels and expressed as arbitrary units.

RPE-1 cell culture. Human retinal pigment epithelial-1 (RPE-1 hTERT) cells (ATCC, Manassas, VA, USA) were seeded in plastic 96-well culture plates at 37 °C and 5 % CO_2 until reaching ~ 90 % confluency (1×10^5 cells per well). Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10 % fetal calf serum (Invitrogen, Carlsbad, CA, USA) and penicillin/streptomycin were replaced with fresh medium every three days. Then, RPE-1 cells were extensively washed with PBS, and serum-free DMEM without additives was added overnight before exposure to AS. K1-3 or K5 dissolved in serum-free DMEM in the range of concentrations 2-100 nM was added to RPE-1 cells for 24-h incubation. The RPE-1 cells cultivated during indicated period and processed the same washing procedures like AS-exposed cells served as a control.

Activity of RPE cell metabolism (MTT test). The MTT assay is based on the cellular conversion of tetrazolium salt (3-(4,5-dimethyl thiazol-2-yl)-2,5-diphenyl tetrazolium bromide) into a formazan product that can be easily detected spectrophotometrically. In the present study, MTT assay was used in order to assess possible cytotoxic effects of AS on RPE cells and was performed as described below. After incubation with tested polypeptides, the cells were washed with PBS and then 0.5 mg/ml MTT reactive in serum free medium were added to the culture and incubated at 37°C for 4 hours. After washing out excessive MTT reagent, precipitated formazan crystals in each well were dissolved in 200 µl of dimethyl sulfoxide at room temperature for 15 min. The absorbance intensities were measured at wavelength 570 nm with reference 650 nm by a plate reader (SpectraMax Gemini™ XPS/EM Microplate Readers, Molecular Devices LLC, CA, USA). For each group, four individual samples were collected and measured. The percentage of cell viability was determined as OD_{570} sample/OD_{570} control × 100 %.

Statistical analysis. The results are given as mean ± SEM. Analysis of variances (ANOVA) followed by post-hoc Tukey's Multiple Comparison was used to verify significant difference between group means. The *P* value of less than 0.05 was considered significant.

Results and Discussion

The blotograms of ACE2 and GFAP from freshly harvested rat corneas at the 14th day after injury are presented in Fig. 2A. The results of correspondent densitometric analysis of the signal intensities in the blots are illustrated in Fig. 2B. Western blot analysis showed

that ACE2 immunoreactivity was revealed as a single polypeptide band of 110 kDa. As expected, the controls showed a non-significant basal expression level of ACE2. Densitometry analysis showed that the ACE2 level in injured cornea was 7.7-fold higher when compared with the control ($P < 0.01$), thus indicating the overexpression of ACE2 in corneas after alkali burn. Treatment of injured cornea with either K5 or K1-3 (1.0 μM) caused double reduction of ACE2 as compared with the Burn group ($P < 0.05$). Among all variants of AS-based treatment, K1-3 (0.1 μM) caused the most noticeable effects on the ACE2 expression reducing its expression by 3.4 folds in comparison with the Burn group ($P < 0.01$).

Western blot of GFAP revealed a heterogenicity of immunoreactive bands of this glial marker. In control, intact polypeptide 49 kDa was found in trace amounts that suggests non-activated state of the satellite glial cells in the healthy cornea. However, injury induced drastic increase in the GFAP expression in corneal tissue that was 62-fold higher than the control value ($P < 0.001$). Moreover, in the corneas after alkali burn, along with the major 49 kDa GFAP band, several truncated polypeptides were observed, including the most intense band with M_m 38 kDa. Overexpression of GFAP along with an increased content of its proteolytically cleaved fragments in injured tissue are the indications of the reactive gliosis. Among all AS tested, K5 exerted the most pronounced protective effect against the glial reactivity via reducing the GFAP level by 10.9 folds in comparison with the Burn group ($P < 0.01$). K1-3 in both concentrations appeared to be twice less effective than K5 to suppress the glial reactivity in alkali-burned cornea.

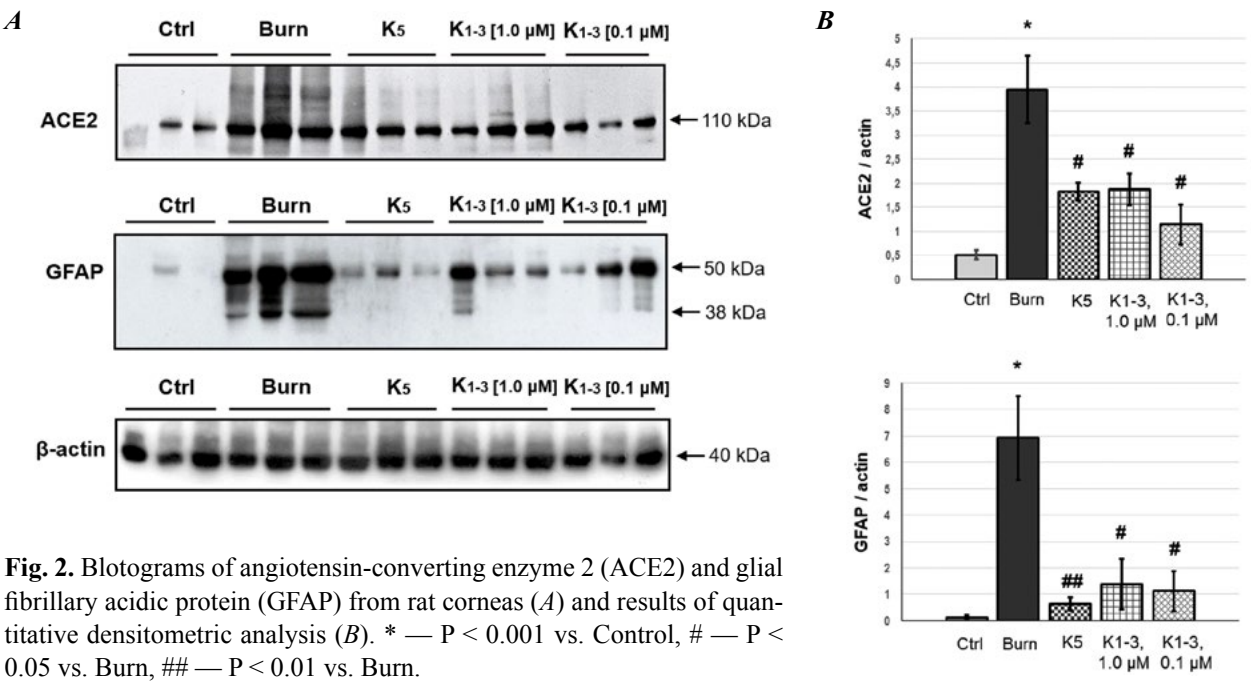

Fig. 2. Blotograms of angiotensin-converting enzyme 2 (ACE2) and glial fibrillary acidic protein (GFAP) from rat corneas (*A*) and results of quantitative densitometric analysis (*B*). * — $P < 0.001$ vs. Control, # — $P < 0.05$ vs. Burn, ## — $P < 0.01$ vs. Burn.

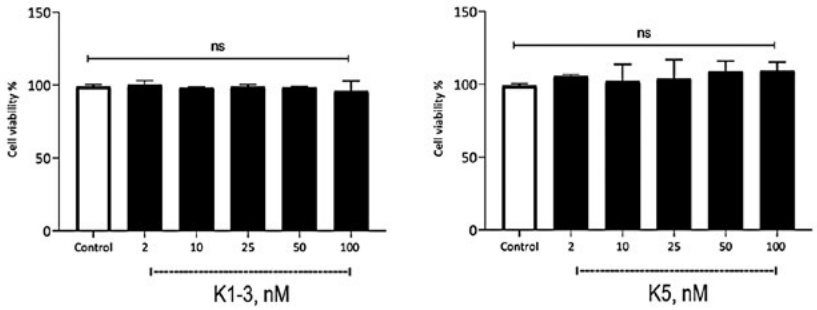

Fig. 3. Results of MTT-assay of the effects of angiostatins K1-3 and K5 on survival of human retinal pigment epithelium (RPE) cells: ns — non-significant changes ($P > 0.05$).

We decided to check if the tested AS could affect one of the main types of retina, RPE cells, in the *in vitro* experiment. As shown by the results of MTT test presented in Fig. 3, there were no significant differences between the intact retinal cells and the cells treated with AS in their capacity to enzymatically reduce the MTT reactive. This means that neither K1-3 nor K5 had any effect on the metabolic activity of RPE cells in the range of concentrations 2 – 100 nM. Earlier, AS have been reported to specifically induce apoptosis and interfere the migration activity of endothelial cells by binding with the molecular targets exposed on the plasma or mitochondrial membranes (F_1F_oATP-synthase, angiomotin, integrin $\alpha_v\beta_3$, annexin II, HGF-receptor c-met, proteoglycan NG2, voltage-dependent anion channel, or VDAC, and exposed β-actin) [21–24]. Anti-proliferative efficacy for K5 and K1-3 toward the endothelial cells has been evaluated as IC_{50} 50 and 70 nM, respectively [21]. The absence of cytotoxic effects of the tested AS on RPE cells can be explained by the lack of specific targets expressed by this type of retinal cells.

As far as it is known, the present study provides the first evidence that the AS topical application is associated with the attenuation of expression levels of ACE2 and GFAP in the cornea injured by alkali burn. The importance of our observation is linked with the ability of AS (containing K-domains 1-3 or K-domain 5 of plasminogen) to be potentially beneficial for ocular surface via reducing the inflammatory-related processes, including neurodegeneration.

AS are naturally occurring polypeptides, which were originally reported as anti-angiogenic and anti-metastatic regulators acting as the apoptosis inductors and migration inhibitors for endotheliocytes [25]. Lately, it has been evidenced that AS are the potent anti-inflammatory factors that explains the multifacial roles of these polypeptides in various pathological states. For example, Chavakis *et al.* [14] have shown that AS K1-4 acts as an anti-inflammatory factor by inhibiting β1- and β2-integrin-mediated adhesion of leukocytes to extracellular matrix proteins and the endothelium and blocking neutrophil migration *in vivo*. Moreover, AS reduced an activation of the pro-inflammatory transcription nuclear factor kappa B (NF-κB) and blocked its nuclear translocation induced by IL-1β [26]. Perri *et al.* [27] have demonstrated that AS K1-3 suppresses the monocyte/macrophage migration via disruption of actin filopodia/lamelli-

podia. The authors argued that the K1-3-induced macrophage immobilization can determine the beneficial effects of AS during hypoxia-mediated pathological conditions along with decreasing cellular levels of reactive oxygen species (ROS). The K5-containing plasminogen derivates, K1-5 and free K5, have also been shown to exert the anti-inflammatory effects by inhibiting the expression of TNF-α-induced intercellular adhesion molecule-1 and vascular cell adhesion molecule-1, decreasing the HIF-1α level and impairing the nuclear HIF-1α accumulation [28, 29].

AS are produced by cathepsins and matrix metalloproteinase digestion of plasminogen in the corneal epithelium, where these biologically active polypeptides are implicated in the maintenance of the avascularity of the normal cornea [30, 31]. The endogenous AS (K1-3, K1-4, K5) are extensively released to the human tear fluid during overnight eye closure [32]. It is believed that AS may contribute to the regulation of inflammatory reactions and restriction of neovascularization in the hypoxic closed eye environment via counteracting VEGF-mediated proangiogenic signalling. AS are abundantly produced in inflamed and vascularized corneas of experimental animals and play a vital role in regression of excessive neovascularization [11]. Currently, we have found that human tear fluid collected from the eye after non-penetrating corneal injury contains higher concentrations of AS than that of the healthy eye [33]. One of the most intriguing observations made in the present study is the AS-mediated down-regulation of ACE2 in alkali-burned rat cornea. Since ACE2 has been clearly identified as a receptor, which enables the infection of human cells with SARS-CoV-2, the enhanced expression of ACE2 in the inflamed cornea increases tissue tropism to the virus and a risk of its possible transmission through the ocular surface [34]. Currently, the available literature has described a high prevalence of ocular manifestations in patients with COVID-19 [5, 6, 35]. As it was mentioned above, the ACE2 overexpression in the cornea is an inflammation-governed process, so it can be hypothesized that AS may down-regulate ACE2 in the injured cornea via inhibiting the macrophage activation and leucocyte adhesion. Our data are in agreement with the latest study, in which upregulated ACE2 in inflammatory corneal epithelial cells was attenuated by resveratrol, an anti-oxidant and anti-inflammatory compound [4]. Although the precise mechanism by which AS can diminish the amount of ACE2 expressed in corneal cells after injury is still uncertain, these plasminogen fragments can be used as potential drugs to reduce the infection rate of SARS-CoV-2.

The satellite glial cells, which represent a principle glial cell population in the cornea, play a significant role in regulating sensory nerve activities and are crucial for neuroprotection. The intensity of glial activation correlates with the extent of harmful impact, while the exceeding glial reactivity can be deleterious for nerve regeneration via enormous releasing pro-inflammatory cytokines and ROS by reactive gliocytes [36]. It is known that GFAP, a specific glial intermediate filament protein, is not readily detectable in satellite glia in a resting state or under normal physiological conditions that was confirmed by our western blot result. Following nerve injury, the activation of GFAP-positive satellite glial cells takes place around the damaged neurons. Thus, the

upregulation of GFAP is commonly used as a marker of the satellite glial cell activation in periphery nervous system related to neural damage [37]. Since the satellite glial cell activation and inflammation in the injured cornea are intertwined, we tested a hypothesis that the modulation of inflammatory-related pathways in alkali-burned cornea by AS could alleviate excessive glial response. In the present study, we report for the first time that AS decreased the expression level of GFAP in the injured rat cornea. There is lack of information in the current literature concerning the modulation of glial activity in the injured cornea. Though, our data corroborate with the most recent research, in which GFAP has been reported as a sensitive marker of corneal damage by ethyl alcohol intoxication [38]. The intense GFAP overexpression has been shown as a result of satellite glia reactivation induced by metabolic insult and considered as a sign of ethanol corneal neurotoxicity. In the cited paper, the application of thiamine as a protector normalized GFAP levels, which was correlated with anti-apoptotic effects and improvement of neurological outcomes during the corneal wound reparation. In the present study, we showed that K5 is more effective suppressor of glial reactivity in the cornea than K1-3. Considering the surface location of the satellite glial cells in the eye, K5 is able to target these cells more readily in comparison with K1-3 because of a relatively small size of the molecule. It is known that the drugs instilled onto the ocular surface during topical ophthalmic administration can reach the posterior segment of the eye and affect the retinal cells even in small concentrations [39]. The results of MTT assay with the use of RPE cells confirm non-toxicity of both tested AS types for the retinal epithelium. The latter is an important observation in scope of drug design for ophthalmology because RPE cells have a remarkable importance in the maintenance of outer retina and function of photoreceptors, while their dysfunction can drive the pathophysiology of retinal diseases.

Conclusions

The presented results suggest that the topical administration of angiostatins AS K1-3 and K5 can be beneficial for the treatment of alkali-burn-induced corneal injury. AS reduced the expression levels of ACE2 in injured corneal tissue that potentially can minimize risks of the SARS-CoV-2 invasion through the ocular surface. AS diminished the expression levels of GFAP in injured corneal tissue indicating limitation of excessive glial response that is an important sign of neuroprotection. Since AS appeared to be non-cytotoxic for retinal pigment epithelium, these polypeptides can be regarded as an effective and safe supplementary for ophthalmic medication design, though the molecular mechanisms of AS effects deserve further in-depth study.

Acknowledgment

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or

financial relationships that could be construed as a potential conflict of interest.

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Ангіостатини модулюють рівень ACE2 і GFAP у пошкодженій рогівці щурів та не впливають на життєздатність клітин пігментного епітелію сітківки

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Ангіостатини (AS) є протеолітичними фрагментами плазміногену з відомою антиангіогенною та протизапальною активністю, які можуть бути застосовані для відновлення тканини поверхні ока після травми.

Мета. Дослідити вплив AS K1-3 і K5 на рівень рецептору SARS-CoV-2 протеїну ACE2 та маркеру активованої сателітної глії GFAP у пошкодженій рогівці щурів і визначити потенційні ефекти AS на життєздатність клітин пігментного епітелію сітківки (RPE). **Методи.** AS K1-3 і K5 були отримані шляхом обмеженого протеолізу плазміногену, ізольованого з плазми людини, з подальшим очищенням за допомогою афінної хроматографії. AS (K1-3 0,1 або 1,0 мкМ, K5 0,1 мкМ) застосовували місцево у вигляді очних крапель у створеній моделі лужного опіку рогівки щурів. Рівні протеїнів ACE2 і GFAP визначали в лізатах тканини рогівки за допомогою вестерн-блоту та виражали в умовних одиницях (у.о.). Вплив AS на життєздатність клітин RPE оцінювали за допомогою МТТ-тесту. **Результати.** Пошкодження поверхні ока, спричинене лужним опіком, призвело до надмірної експресії як ACE2 (у 7,7 разів порівняно з контролем, $P < 0,001$), так і GFAP (у 62 рази порівняно з контролем, $P < 0,001$). Застосування K5 або K1-3 (1,0 мкМ) сприяло зниженню вмісту ACE2 в ушкодженій рогівці вдвічі ($P < 0,05$ порівняно з групою Опік), тоді як K1-3 (0,1 мкМ) знижував вміст ACE2 у 3,4 рази порівняно з групою Опік ($P < 0,05$), що свідчить про здатність AS зменшувати потенційний ризик проникнення SARS-CoV-2 завдяки зниженню експресії його рецептору. Слід зазначити, що K5 виявив більш виражену здатність інгібувати надекспресію GFAP (у 10,9 разів порівняно з групою Опік, $P < 0,01$), що свідчить на користь реалізації AS нейропротекторних властивостей через пригнічення надмірної активації сателітної глії в пошкодженій рогівці. AS не чинили цитотоксичної дії на клітини пігментного ендотелію сітківки в діапазоні концентрацій 2-100 нМ. **Висновки.** Дані, отримані у представленій роботі, вказують на перспективи застосування AS для розробки ефективних та безпечних офтальмологічних препаратів з комплексними захисними властивостями.

Ключові слова: ангіостатини, пошкодження рогівки, ACE2, GFAP, клітини RPE

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