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Evaluation of the effects of resveratrol and bevacizumab on experimental corneal alkali burn

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ABSTRACT

Purpose: To evaluate the effects of resveratrol and bevacizumab on experimental corneal neovascularization.
Method: A corneal alkali burn was performed in 62 eyes of 31 male white Vienna rabbits. Resveratrol (group 1), dimethyl sulfoxide (group 2), bevacizumab (group 3) and 0.9% NaCl (group 4) were administered to both eyes of the rabbits by subconjunctival injection for 7 days. Corneal photos were taken at 15 days after alkali injury. Inflammatory index scores and neovascularization areas were calculated.
Results: In bevacizumab group both inflammatory index scores and the calculation of the corneal neovascularization area was significantly less than the groups.
Conclusion: The subconjunctival administration of bevacizumab inhibits corneal neovascularization effectively in the rabbit corneal alkali burn model. No effect of resveratrol to the corneal neovascularization on experimental model of the corneal alkali burn was seen at the doses of usage.

1. Introduction

Corneal neovascularization represents a major public health problem worldwide: it is seen in common blinding diseases such as trachoma [1]. Inflammatory, infectious, degenerative, and traumatic disorders may include corneal neovascularisation [2]. Corneal neovascularization is often accompanied by decreased visual acuity caused by stromal edema, scar formation, and lipid deposits [3]. Also, corneas vascularized after ulceration, viral keratitis, chemical burns, rejected transplants, and other causes are at high risk of allograft rejection after penetrating keratoplasty.

It has been shown that various chemicals and drugs inhibit corneal neovascularization in clinical or experimental studies such as steroids, methotrexate, and rapamycin [4–6]. However; there is no ideal treatment choice for corneal neovascularization.

It has been shown that the vascular endothelial growth factor (VEGF) stimulates corneal neovascularization in animal models [7]. In a clinical study, VEGF and its receptors were found in higher concentrations in the neovascular human corneas than in normal corneas [8]. Also, the inhibition of VEGF has also been shown to inhibit corneal neovascularisation [9].

Bevacizumab is a recombinant humanized murine monoclonal antibody that binds to and inhibits the biological activity of all human VEGF-A isoforms. Recently, it has been reported as a potential treatment of choroidal neovascularization, and other vascular retinal diseases such as proliferative diabetic retinopathy, diabetic macular edeme, premature retinopathy, and venous occlusive retinal diseases [10–14].

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Resveratrol is an agent which directly stops the tumour growing in an unknown mechanism. It has been shown that resveratrol has anti-angiogenic properties [15]. Also, in an experimental study; it has been shown that resveratrol inhibits corneal neovascularization with oral administration [16].

In this study, our aim was to evaluate the effects of subconjunctival administered resveratrol and bevacizumab on corneal neovascularization in the experimental corneal alkali burn model.

2. Materials and methods

31 male white Vienna rabbits weighing 800–1200 g were used in the study. The care, use, and treatment of all animals in this study was under strict agreement with the ARVO Statement for the use of Animals in Ophthalmic and Vision Research. The animals were divided randomly into four groups.

All of the rabbits were anesthetized using ketamine (30 mg/kg) and xylazine (6 mg/kg) intramuscularly, and 0.5% proparacaine hydrochloride (Alcaine, Alcon Fort Worth, TX) was applied topically. Corneal neovascularization was induced by 1 N NaOH soaked 7 mm Whatmann 3 filter paper to the central cornea for two minutes. After waiting for 7 days for alkali burn effects to appear subconjunctival injections superiorly of the all drugs performed. In all of the groups, the rabbits received the drugs to both of their eyes.

Subconjunctival injections were performed under general anesthesia induced by ketamine (30 mg/kg) and xylazine (6 mg/kg) intramuscularly, and 0.5% proparacaine hydrochloride was applied topically. The injections were performed to the 1–2 mm posterior to the limbus superiorly with a 30-gauge needle attached to a 1 ml tuberculin syringe. All injections were performed by the same investigator (P.F.).

Group 1 received 10 mg/ml of resveratrol (1%), 0.05 ml of which was dissolved in dimethyl sulfoxide (DMS). Group 2 received 0.05 ml of DMS as a control. Rabbits in group 3 received 10 mg/ml of bevacizumab (1%), which was dissolved in 0.9% NaCl, and as a control group 4 received 0.05 ml of 0.9% NaCl. All of the groups received the drugs for 7 days.

On day 15, the animals were euthanized by 75 mg/ml of ketamine hydrochloride (Ketalar, Park Davis CO, Morris Planis, NJ), and 5 mg/ml of xylazine hydrochloride (Rompun, Mobay.-Corp, Shownee, Kan) by intramuscular injection. Slit-lamp photographs were taken at a standardized magnification. The inflammatory index was analysed as previously described [17]. Briefly, the inflammatory index was analysed, based on the following parameters: ciliary hyperemia (absent, 0; present but less than 1 mm, 1; present between 1 and 2 mm, 2; present and more than 2 mm, 3); central corneal edema (absent, 0; present with visible iris details, 1 + ; present without visible iris details, 2+; present without visible pupil, 3+); peripheral corneal edema (absent, 0; present with visible iris details, 1+; present without visible iris details, 2+; present with no visible iris, 3+).

Corneal neovascularization areas were calculated on photographs using the Auto CAD-2005 program. Central corneal burned areas and total corneal areas were calculated in the same manner (Figs. 2–5).

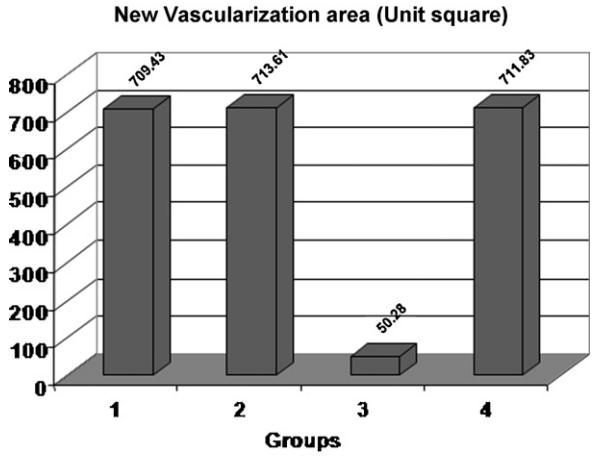
Statistical analyses were performed using SPSS 15.0 (SPSS for Windows Chicago, IL, USA). All values were presented as the mean ± SD. The Bonferoni Mann–Whitney *U* test was used for statistical calculations among the groups. A *P* value of <0.05 was considered statistically significant.

3. Results

The inflammatory index scores of the groups (ciliary hyperemia, central corneal edema and peripheral corneal edema) were calculated as [1.25 ± 0.68, 2.75 ± 0.45, 0.63 ± 0.5], [2.0 ± 0.73, 3.0 ± 0.00, 1.13 ± 0.62], [1.06 ± 0.44, 2.19 ± 0.4, 0 ± 0], [1.71 ± 0.47, 2.86 ± 0.36, 1 ± 0.39], respectively, in the groups (Fig. 1).

Neovascularization areas in all groups were calculated in unit square as [709.44 ± 156.98, 713.62 ± 229.22, 50.29 ± 110.28, 711.84 ± 114.22] respectively, in the groups (Graphic 1). The corneal areas of all groups were calculated in unit square as [2528.63 ± 149.97, 2512.63 ± 120.50, 2470.62 ± 78.49, 2433.15 ± 191.13] respectively, in the groups. The central burn areas of the groups were calculated in unit square as [446.88 ± 69.29, 431.97 ± 15.80, 427.10 ± 13.39, 434.10 ± 34.97] respectively, in the groups.

When the groups were compared in terms of inflammatory index scores (ciliary hyperemia, central corneal edema and peripheral corneal edema), there were no significant differences either between group 1 and group 2 (Fig. 1), or between group 2 and group 4 (Fig. 1) ($p > 0.008$ Bonferoni Mann–Whitney *U* test). There were significant differences between group 1 and 3 in terms of peripheral corneal edema and central corneal edema scoring (Fig. 1), between group 2 and 3 in terms of ciliary hyperemia, central corneal edema and peripheral corneal edema (Fig. 1), and between group 3 and 4 in terms of ciliary hyperemia, central corneal edema and peripheral corneal edema (Fig. 1) ($p < 0.008$). In the calculation of corneal neovascularization areas, vascularization was significantly poorer in group 3 than the other groups (Graphic 1). There were no significant differences between group 1 and group 2, and between group 2 and group 4 in the calculations of corneal neovascularization areas (Graphic 1) ($p > 0.008$).



Graphic 1 – Corneal neovascularization areas.