Experimental Eye Research 149 (2016) 1–7

Contents lists available at ScienceDirect

Experimental Eye Research

journal homepage: www.elsevier.com/locate/yexer

Research article

The effect of triamcinolone acetonide or bevacizumab on the levels of proinflammatory cytokines after retinal laser photocoagulation in pigmented rabbits

Shogo Arimura, Yoshihiro Takamura*, Seiji Miyake, Makoto Gozawa, Kentaro Iwasaki, Takeshi Tomomatsu, Takehiro Matsumura, Masaru Inatani

Department of Ophthalmology, Faculty of Medical Sciences, University of Fukui, Eiheiji-cho, Yoshida-gun, Fukui-ken, 910-1193, Japan

ARTICLE INFO

Article history:
Received 11 October 2015
Received in revised form
29 March 2016
Accepted in revised form 8 June 2016
Available online 11 June 2016

Keywords:
Proinflammatory cytokine
VEGF
Bevacizumab
Triamcinolone acetonide
Retinal laser photocoagulation

ABSTRACT

Although laser photocoagulation is a gold standard for the treatment of retinal ischemic diseases, thermal burn induces the inflammation and the progression of macular edema. To prevent this complication, combination therapy using anti-vascular endothelial growth factor (VEGF) drugs or steroids is clinically utilized, however the mechanisms are still unclear. In this study, we aimed to evaluate the changes in inflammatory and angiogenic cytokine levels in aqueous humor and vitreous body after intravitreal injection of bevacizumab (IVB) or triamcinolone (IVTA), as well as sub-Tenon injection of triamcinolone (STTA) after retinal laser photocoagulation in rabbits. Pigmented rabbits were treated with retinal laser photocoagulation and divided into 4 groups, namely Control (no additional treatment), IVB, IVTA or STTA accordingly. Samples of vitreous and aqueous humor were collected on post-treatment days 0, 1, 7 and 14. The levels of intraocular VEGF, interleukin-6 (IL-6), intercellular adhesion molecule-1 (ICAM-1) and monocyte chemotactic protein-1 (MCP-1) were measured using an immunoassay. The levels of VEGF, IL-6, ICAM-1 and MCP-1 were significantly elevated 1 day after laser treatment. IVTA and STTA significantly reduced the increase in the levels of VEGF, IL-6, ICAM-1 and MCP-1, while IVB reduced that of VEGF only in aqueous humor and vitreous body. The protein amount in the aqueous humor transiently increased 1 day after laser, and was significantly prevented by IVTA or STTA but not IVB. Data showed that bevacizumab only reduced intraocular VEGF after laser, while triamcinolone suppressed both the expression of VEGF and proinflammatory cytokines. We propose that these cytokine profiles may play an important role in the pathogenesis of inflammation after photocoagulation and the underlying mechanism of treatment with anti-VEGF drug and steroids.

Retinal laser photocoagulation is known to be beneficial in the treatment of a variety of retinal ischemic diseases, including proliferative diabetic retinopathy (PDR), retinal vein occlusion and rubeosis. (The Diabetic Retinopathy Study Research Group, 1981), (Branch Vein Occlusion Study Group, 1986) Panretinal photocoagulation (PRP) for the treatment of ischemic lesions involves the purposeful destruction of a fraction of the photoreceptors, as well as other more superficial retinal layers(Paulus et al., 2008). Although it is recognized that PRP contributes to the reduction of the risks of vision loss in the patients with severe non-PDR and PDR, the decreased visual acuity with PRP-induced progression of diabetic macular edema (DME) is sometimes observed. Several studies that previously attempted to explain the underlying mechanism of macular edema after PRP have focused on ocular circulation, and the accumulation of leucocytes and cytokines(Oh et al., 2010; Shimura et al., 2009; Takahashi et al., 2008). Shimura et al. reported that the levels of proinflammatory cytokines in the vitreous body is higher in the PRP-treated eyes of the patients with high-risk PDR, which showed transient worsening of macular edema after PRP in comparison to the fellow eye with no PRP treatment(Shimura et al., 2009).

Triamcinolone acetonide (TA) is a long-acting corticosteroid effective against DME, at least for short-term duration (Massin et al., 2004; Martidis et al., 2002). Studies have demonstrated that the combination therapy of intravitreal injection of TA (IVTA) or sub-Tenon's capsule administration of TA (STTA) is effective in

* Corresponding author.
E-mail address: ytakamura@hotmail.com (Y. Takamura).

http://dx.doi.org/10.1016/j.exer.2016.06.004
0014-4835/

preventing PRP-induced macular swelling and visual dysfunction(Margolis et al., 2008; Mirshahi et al., 2010; Mitchell et al., 2011; Shimura et al., 2006; Unoki et al., 2009; Zacks and Johnson, 2005; Zein et al., 2006). These therapeutic effects of TA are due to the strong anti-inflammatory properties, which reduce capillary permeability, fibrin deposition and loss of endothelial tight junction.

Vascular endothelial growth factor (VEGF) was originally isolated as a vascular permeability factor involved in angiogenesis(Dvorak et al., 1995; Keck et al., 1989). A marked increase in VEGF expression has been found in the vitreous and aqueous fluids in eyes of patients with PDR, DME, retinal vein occlusion and retinopathy of prematurity(Aiello et al., 1994; Funatsu et al., 2003; Lashkari et al., 2000; Pe'er et al., 1998). It has been reported that the production of VEGF is enhanced by photocoagulation in the eyes of pigmented rabbits(Chen et al., 2012). Clinically, intravitreal injection of anti-VEGF agents, such as bevacizumab, ranibizumab and aflibercept, is another treatment option to inhibit macular swelling after PRP(Arevalo et al., 2007; Wells et al., 2015).

Photocoagulation causes thermal destruction of retina, which will probably enhance the levels of inflammation. The release of chemical mediators and leakage of proteins into the vitreous fluid after PRP have been reported to be indicative of a breakdown of the blood-retinal barrier(Lincoff et al., 1981; Naveh and Weissman, 1990). Besides this, in the anterior segment of the eye, a recent experimental study using pigmented rabbits showed that a significant increase in the aqueous flare intensity after retinal laser photocoagulation was observed, indicating a breakdown of the blood-aqueous barrier (BAB) (Chen et al., 2012). Although the efficacy of anti-VEGF drugs or TA therapy to prevent the progression of macular edema after PRP are reported, the effects of these therapies on the levels of proinflammatory cytokines induced by retinal lasers remain undetermined. Therefore, in this study, we analyzed the effects of IVTA, STTA or intravitreal injection of bevacizumab (IVB) on the temporal profiles of the levels of proinflammatory cytokines after laser treatment on normal pigmented rabbits.

1. Methods

1.1. Animals

We purchased pigmented male Rex rabbits weighing 2.0–2.5 kg (Japan SLC Co. Ltd, Shizuoka, Japan) and acclimated them to the surroundings for at least one week. All rabbits were kept under pathogen-free conditions at 23 °C ± 1 °C, 60% ± 10% humidity, and 12 h of light with 12 h of dark. Animals were housed with free access to water and food (RC4; oriental yeast Co. Ltd, Tokyo Japan) throughout the day. The maintenance and experimentation of animals conformed to the guidelines of the Association for Research in Vision and Ophthalmology Statement for the Use of Animals in Ophthalmic and Vision Research during all procedures. The rabbits were anesthetized with intramuscular injections of a mixture of ketamine hydrochloride (3.5 mg/kg) and xylazine hydrochloride (5 mg/kg) before photocoagulation and drug administration.

After photocoagulation for both eyes of 48 rabbits, rabbits were randomly divided into 4 groups: control group, IVTA group, STTA group and IVB group. Four rabbits were used in each group, and sacrificed at 1, 7 and 14 days. Four rabbits with no photocoagulation were used as the sample of Day 0, thus total 52 rabbits were used.

1.2. Retinal laser photocoagulation

Transpupillary retinal laser photocoagulation was carried out on both eyes of the rabbits using a laser photocoagulator (OcuLight GLx, Iridex Corp., CA, USA) (Fig. 1). The laser power setting was 200 mW and duration of exposure was 200 ms in yellow wavelength (577 nm), with a space between each of the coagulation spot parts adjusted to approximately one coagulation spot. Photocoagulation was carefully carried out by one trained ophthalmologist (YT) to avoid hitting the iris, optic disc and vascular. The number of laser spots per eye was constantly 400.

1.3. IVB, IVTA and STTA treatment

Immediately after photocoagulation, IVB, IVTA, or STTA was carried out. After the eyes were prepared in a standard procedure using 5% povidone iodine, an eyelid speculum was used to stabilize the eyelids with topical anesthesia 0.4% oxybuprocaine hydrochloride (0.4% benoxyl ophthalmic solution, Santen Co. Ltd., Osaka, Japan), and the drugs were given to each group. TA (MaQaid® Wakamoto Pharmaceutical Co. Ltd., Tokyo, Japan) was dissolved in physiological saline (40 mg/ml). In the IVTA and IVB groups, 4.0 mg in 0.1 ml TA and 1.25 mg in 0.05 ml bevacizumab (Avastin™, Genentech, South San Francisco, CA, USA) were respectively injected into the vitreous body immediately after photocoagulation. TA or bevacizumab was injected 3.0 mm posteriorly from the corneal limbus at five o'clock using a 30-gauge needle. The injection site was compressed using a cotton stick for 20 s without anterior chamber paracentesis. In the STTA group, 20 mg in 0.5 ml TA was injected through the sub-Tenon's capsule space, reaching the posterior pole in both eyes.

1.4. Measurement of the levels of cytokines in aqueous humor and vitreous body

To study the effects of IVTA, STTA and IVB on the intraocular levels of VEGF, interleukin-6 (IL-6), monocyte chemotactic protein-1 (MCP-1) and ICAM-1 after photocoagulation, we measured their amounts in the aqueous humor and vitreous at 1, 7 and 14 days post-operation. Both eyes of 4 rabbits underwent the injection of same drug, and thus the number of eyes for each group at each time

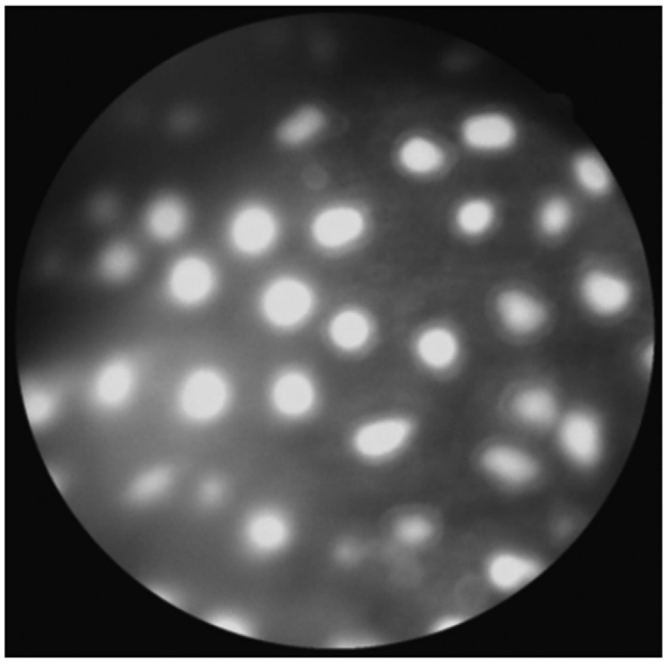

Fig. 1. Fundus photography of the eye of a rabbit immediately after retinal laser photocoagulation.