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Research article

Retinal proteome changes following experimental branch retinal vein occlusion and intervention with ranibizumab

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ABSTRACT

Animal models of experimental branch retinal vein occlusion (BRVO) provide a unique opportunity to study protein changes directly in retinal tissue. Results from these experimental models suggest that experimental BRVO is associated with an upregulation of extracellular matrix remodeling and adhesion signaling processes. To study whether these processes could be blocked by inhibition of VEGF-A, a porcine model of experimental BRVO was combined with proteomic analyses. In six Danish Landrace pigs experimental BRVO was induced with argon laser in both eyes. After 24 h an injection of 0.05 mL ranibizumab was given in the right eyes of the animals while left eyes received an injection of 0.05 mL 9 mg/mL sodium chloride water. Retinas were dissected three days after BRVO and the retinal samples were analyzed with label-free quantification as well as tandem mass tag based proteomics. In retinas treated with ranibizumab five proteins exhibited statistically significant changes in content with both proteomic techniques. These five proteins, which were all decreased in content, included integrin β-1, peroxisomal 3-ketoacyl-CoA thiolase, OCIA domain-containing protein 1, calnexin and 40S ribosomal protein S5. As anti-integrin therapies are under development for inhibition of angiogenesis in retinal diseases it is interesting that inhibition of VEGF-A in itself resulted in a small decrease in the content of integrin β-1. The decreased content of integrin β-1 indicates that extracellular matrix remodeling and adhesion processes associated with BRVO are at least partly reversed through inhibition of VEGF-A.

1. Introduction

Studies on protein changes following branch retinal vein occlusion (BRVO) have traditionally been conducted on aqueous humor and the vitreous body samples from patients with macular edema secondary to BRVO. These analyses have led to the identification of important proteins that are involved in the formation of macular edema (Campochiaro et al., 2009; Noma et al., 2011, 2012, 2014). Animal models of experimental BRVO allow for protein studies to be conducted directly on retinal tissue where major pathological changes are thought to take place (Cehofski et al., 2014, 2015a). We recently published a proteomic study on large-scale protein changes in porcine retinas 15 days after exposure to experimental BRVO (Fig 1A) and proposed the hypothesis that experimental BRVO was associated with an upregulation of proteins involved in extra cellular matrix (ECM) remodeling and focal adhesion processes (Cehofski et al., 2015b). Proteins involved in ECM remodeling and focal adhesion signaling included laminin subunit β-2, laminin subunit γ-1, lipocalin-7, nidogen-2, osteopontin, integrin β-1, isoform 2 of α-actinin-1, talin-2 and filamin C (Cehofski et al., 2015b). As these proteins have not previously been associated with BRVO, the present study was established to examine if the content of the proteins would change when subjected to an intervention with ranibizumab.

Ranibizumab is a humanized monoclonal antibody fragment that binds VEGF-A isoforms such as $VEGF_{110}$, $VEGF_{121}$ and $VEGF_{165}$

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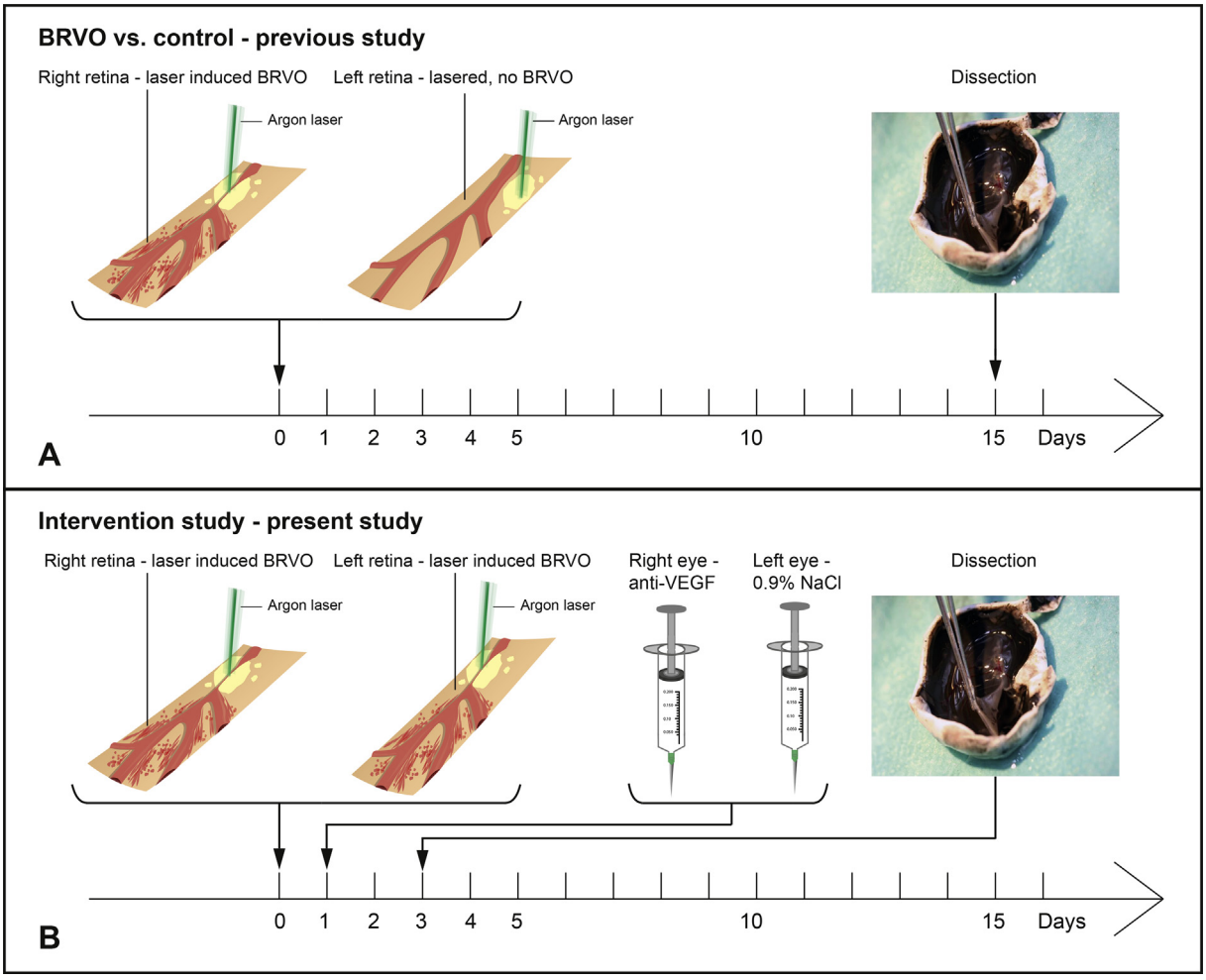

Fig. 1. **(A)** Experimental set-up from previous study (Cehofski et al., 2015b). In this previous work BRVO was induced in the right eyes of the animals by applying argon green laser directly onto a vein in the inferior retina. In the left eyes that served as controls a similar area of laser burns was created in an area devoid of vessels. The retinas were dissected after 15 days followed by proteomic analysis. **(B)** In the present intervention study BRVO was induced in both eyes of the animals. After 24 h VEGF-A was inhibited in all right eyes with an intravitreal injection of ranibizumab while all left eyes received an intravitreal injection of 0.9% sodium chloride. The retinas were dissected after 3 days. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

(Gaudreault et al., 2005). The drug is widely used for the treatment of macular edema secondary to BRVO (Campochiaro et al., 2010). However, the effects of ranibizumab on the large-scale retinal protein expression remain largely unstudied. By using two different proteomic techniques this study aimed at identifying retinal proteins and biological processes that had not been associated with sensitivity to ranibizumab previously.

2. Materials and methods

2.1. Animal preparation

The study was approved by the Danish Animal Experiments Inspectorate, permission no. 2013-15-2934-00775.

Six Danish Landrace pigs of approximately 30–40 kg were used in the present study. The animals were anesthetized by using tiletamine-zolazepam (Zoletil), a mixture of 2 dissociative anesthetics (ketamine 6.25 mg/mL, and tiletamine 6.25 mg/mL), a benzodiazepine (Zolazepam 6.25 mg/mL), a synthetic opioid (butorphanol, 1.25 mg/mL), and xylazine (6.5 mg/mL). This mixture was administered as an intramuscular injection at 1 mL/10 kg.

2.2. Experimental branch retinal vein occlusion

Experimental BRVO was induced as earlier described (Fig. 1A) (Cehofski et al., 2015a, 2015b). The eyes were anesthetized with Oxybuprocaine Hydro 0.4% (Bausch & Lomb) and Tetracaine 1% (Bausch & Lomb) followed by dilatation with Tropicamide 0.5% (Mydriacyl; Bausch & Lomb) and Phenylephrine 10% (Metaoxidrin; Bausch & Lomb). Systane Ultra eye drops (Polyethylene Glycol 400, Propylene Glycol; Alcon Copenhagen Denmark) were used to lubricate the eyes.

Experimental BRVO was induced in both eyes of the pigs (Fig. 1B). A standard argon laser (532 nm) given by indirect ophthalmoscopy was used to induce the occlusion by creating a patch of laser burns around an inferior branch vein to create a narrowing of the vein followed by laser application directly on the vein until stagnation of the venous blood flow was observed. Stagnation of the venous blood flow was generally observed after 30–40 laser applications with a power of 400 mW and a duration of 550 ms. Peripheral flame-shaped hemorrhages developed within 10 min after application of the laser burns.

After 24 h the animals were given an intervention with ranibizumab (Fig. 1B). The animals were anesthetized as described above followed by topical anesthesia with Oxybuprocaine Hydro 0.4%