( ) R1 ( ) R2 (X) R3 ( ) PG0 ( ) PG1 ( ) Estagiário ( ) Tecnólogo ( ) PIBIC

Last Name - Saito
First Name - Caio Vinicius
Middle - Regatieri

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New approaches to study experimental choroidal neovascularization: effects of intravitreal injection of anti-TNF alpha

Regatieri, CVS1; Dreyfuss, JL2; Melo, GB1; Hossaka, S1; Lavinsky, D1; Nader, HB2; Maia, M1; Farah, ME1

1-Departament of Ophthalmology; 2-Discipline of Molecular Biology

Purpose: To study a laser-induced choroidal neovascularization (CNV) model in rat eyes by fluorescein angiogram and immunohistochemistry as well as to evaluate the effects of an anti-TNF alpha (infliximab) on CNV inhibition.

Methods: Four photocoagulation marks were performed around the optic disc in 30 heterozygots Zucker rats by argon laser Eyelite Alcon (Dallas, USA) using the following parameters: 1.Power: 300 mW; 2.Spot: 100 micrometers; 3.Exposure time: 100 ms. At the end of laser session, 2 concentrations of anti-TNF alpha infliximab (5 and 10 µg) were injected intravitreously in order to cause CNV inhibition in 10 animals. The remaining animals were used as control eyes. After three weeks, fluorescein angiogram and autofluorescence exams were performed using the Heidelberg Retina Angiograph – 2 (HRA-2) (Heidelberg, Germany) to detect the area of laser-induced CNV both groups. treated X not treated by intravitreal infliximab injection. Data was analyzed by two-tailed Student t Test. After clinical exam the eyes were enucleated and immunofluorescence was tested by a technique using anti-Von Willebrand factor directly on the eye cup – flat mount. Using serial cryosections (10µm), histochemistry for HA and immunofluorescence (IF) antibodies against fibronectin, VEGF receptor (VEGFR), syndecan4 (Syn4) were performed and analyzed by confocal microscopy. mRNA encoding Syn4, perlecam, VEGF and b-actin were analyzed by quantitative real time PCR. The expression of sulfated glycosaminoglycans were evaluated in retina and choroid/sclera from animals metabolically labeled with [35S]-sulfate before and after laser-induced CNV, using agarose gel electrophoresis in PDA buffer. Similar analyses were performed for hyaluronan (HA), which was measured by fluorometric ELISA-like assay.

Results: The CNV complex was easily analysed by HRA-2. Increase of GAG expression was observed (mainly HA, heparan and dermatan sulfate) in the choroids and also high levels of chondroitin sulfate (HS) was detected in the

retina after 48 hours from laser-induced CNV. The IF analysis demonstrated Syn4 co-localizing with VEGFR on retinal cellular surface. Real time PCR analysis showed an important increase in expression of perlecan and a slight increase in Syn4 in CNV lesions. However, no differences were observed in the expression of VEGF due to negative feedback at the time of experiment (after 3 weeks of laser-induced CNV). It was also observed a reduction of CNV perimeter from treated groups with infliximab 5 µg (1414 µm ; $p<0.05$) and 10 µg (1085 µm ; $p<0.05$) compared with control eyes (no infliximab injection ; 3613 µm)

Conclusions: The novel imaging technique using the HRA 2 system was easily performed to analyze the laser induced CNV lesions in this rat model. Changes in proteoglycans and glycosaminoglycans expression were observed in laser-induced CNV lesions, which is helpful to understand the molecular mechanisms of angiogenesis and to develop new treatments for the inhibition of CNV. Intravitreal infliximab reduced the area of laser-induced CNV lesions in rats demonstrating that it may be an useful antiangiogenic drug for human eye.