Experimental Eye Research 142 (2016) 13–18

Contents lists available at ScienceDirect

Experimental Eye Research

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

Research article

Proinflammatory status in the aqueous humor of high myopic cataract eyes

Xiangjia Zhu [a, b, c], Keke Zhang [a, b, c], Wenwen He [a, b, c], Jin Yang [a, b, c], Xinghuai Sun [a, b, c], Chunhui Jiang [a, b, c], Jinhui Dai [a, b, c], Yi Lu [a, b, c, *]

[a] Department of Ophthalmology, Eye and Ear, Nose, and Throat Hospital, Fudan University, 83 Fenyang Road, Shanghai 200031, China
[b] Key Laboratory of Myopia, Ministry of Health, Shanghai 200031, China
[c] Shanghai Key Laboratory of Visual Impairment and Restoration, Fudan University, Shanghai 200031, China

ARTICLE INFO

Article history:
Received 25 September 2014
Received in revised form
19 February 2015
Accepted in revised form 20 March 2015
Available online 22 March 2015

Keywords:
High myopia
Aqueous humor
Inflammation
Cytokine
Interleukin-1 receptor antagonist (IL-1ra)
Monocyte chemoattractant protein-1 (MCP-1)
Capsular contraction syndrome
Fibrosis

ABSTRACT

High myopia has long been recognized as an inflammation-related disease, and high myopic eyes are thought to have a proinflammatory internal microenvironment, which might predispose to the occurrence of certain inflammation-related complications such as fibrotic capsular contraction syndrome after cataract surgery. Therefore, the purpose of this study was to detect inflammatory cytokines expressed in the aqueous humor (AH) of high myopic cataract (HMC) patients. The cytokines were screened using a RayBio Human Cytokine Antibody Array in AH samples from 15 age-related cataract (ARC) patients and 15 HMC patients. Those detected by the screening assays were verified using a Bio-Plex Suspension Array System in AH samples from 35 ARC patients and 45 HMC patients. The cytokine antibody array showed that the expression level of interleukin-1 receptor antagonist (IL-1ra) in the AH was higher in ARC than in HMC, whereas opposite trends were found for monocyte chemoattractant protein-1 (MCP-1), regulated on activation, normal T-cell expressed and presumably secreted (RANTES), IL-8, platelet-derived growth factor-BB, and IL-6 (all $P < 0.05$). In the verification assay using the suspension cytokine array, only the expression levels of IL-1ra and MCP-1 were significantly different between the ARC and HMC groups ($P = 0.014$ and 0.038, respectively); these results were confirmed by western blot assays. Our results demonstrated that the expression of IL-1ra was significantly lower and the expression of MCP-1 was significantly higher in the AH of HMC than in ARC, suggestive of a proinflammatory status in the anterior chamber of HMC eyes.

High myopia, defined as eyes with axis length ≥26 mm, is a disorder that affects almost the entire human eye. It constitutes an important cause of visual impairment in Asian populations, as its prevalence is 4–5 times greater in Asian adults than in American or European adults (Sawada et al., 2008; Tano, 2002; Zhu et al., 2013).

High myopia is thought to be an inflammation-related disease (Herbort et al., 2011). A typical example is capsular contraction syndrome, a rare fibrotic complication occurs after cataract surgery, is more common in eyes with high myopic cataract (HMC) (Chomanska et al., 2010; Jin-Poi et al., 2013), retinitis pigmentosa (Hayashi et al., 1998), pseudoexfoliation (Schlotzer-Schrehardt and Naumann, 2006), uveitis (Gimbel et al., 2005), or diabetic retinopathy (Takamura et al., 2013), which all indicate a potential role of inflammation in dysfunction of the blood–aqueous barrier (BAB). High myopia is also closely associated with other inflammatory disorders, such as choroidal neovascularisation (CNV) (Neelam et al., 2012), multiple evanescent white dot syndrome, and multifocal choroiditis (Herbort et al., 2011).

Therefore, high myopic eyes may have a special internal microenvironment. This microenvironment includes elevated transforming growth factor (TGF)-β2 expression in the sclera (McBrien et al., 2009), retina, retinal pigment epithelium, and choroid, and elevated vascular endothelial growth factor (VEGF) expression in the AH of eyes with active myopic CNV compared with healthy eyes (Chan et al., 2008). As a result, the expression profiles of inflammatory cytokines in the AH of HMC patients may change as well, generating conditions that might predispose to the onset of certain inflammation-related complications, such as fibrotic capsular contraction syndrome after cataract surgery.

* Corresponding author. Department of Ophthalmology, Eye and Ear, Nose, and Throat Hospital, Fudan University, 83 Fenyang Road, Shanghai 200031, China.
E-mail address: luyieent@163.com (Y. Lu).

http://dx.doi.org/10.1016/j.exer.2015.03.017
0014-4835/

However, very few studies have evaluated the inflammatory cytokines expressed in the AH of HMC eyes to date either because of the lack of proper instrumentation (el-Maghraby et al., 1993; Ladas et al., 2005) or the low expression of proteins in the AH. Recently, the advantages of the cytokine antibody array technique have been demonstrated by the simultaneous detection of a large number of selected biomarkers on one membrane for samples containing very low protein concentrations. Therefore, we used this method to determine the inflammatory cytokine expression profile in the AH of HMC patients to elucidate the inflammatory status in the anterior chamber of HMC eyes.

1. Methods

The Institutional Review Board of the Eye and ENT Hospital of Fudan University, Shanghai, China, approved this case–control study. The study was registered at www.clinicaltrials.gov (clinical trial accession number: NCT01824927). Written informed consent was obtained from each patient before participation. All procedures adhered to the Declaration of Helsinki and were conducted in accordance with the approved research protocol.

1.1. Subjects

We recruited 50 consecutive age-related cataract (ARC) patients (axial length $\leq$ 24.00 mm) and 60 consecutive HMC patients (axial length $\geq$ 26.00 mm) who underwent uneventful cataract surgery at our center between September 2013 and March 2014. The axial length of these patients was measured using an IOLMaster device (Carl Zeiss AG, Oberkochen, Germany), which was operated by one experienced technician. Just before measurement, the patients were asked to blink completely to spread an optically smooth tear film over the cornea. Then, with the assistance of the instrument monitor and an internal fixation target, the eyes were aligned. During measurement, the in-focus condition of the eyes was maintained when the instrument head was approximately 5.5 cm away from the subject's eyes.

All cataract surgeries were performed by one surgeon (Prof. Y.L.). No anti-inflammation therapy including topical NSAIDS were used in these cases before cataract surgery. Eyes with uveititis, glaucoma, previous trauma, zonular weakness, myopic choroidal neovascularization, or diabetic retinopathy were excluded from this study. The design of the study is presented in Supplementary Fig. 1.

1.2. AH collection

After swabbing the eyelids and the surrounding skin with disinfectant, we created a corneal paracentesis and gently inserted a 26 G needle through the paracentesis to aspirate the AH (100–200 μl) before commencing cataract surgery. The samples were immediately stored at −80 °C until further analysis.

1.3. Bradford protein assay

Before the array assays, the total protein in each AH sample was estimated using a Bradford protein assay (#500-0202; Bio-Rad Labs, Richmond, CA, USA). Bovine serum albumin standards (1 μl) with concentrations ranging from 0.125 to 2 μg/μl or AH samples (2 μl) were added to each well of a 24-well plate, along with distilled H_2O, to a volume of 100 μl. The samples were mixed on a microplate mixer after adding 1 × 1 ml of dye reagent to each well. After incubation for 5 min at room temperature, the absorbance values of the standards and AH samples were measured at 595 nm.

1.4. Cytokine antibody arrays

The cytokine antibody arrays were used to detect inflammatory cytokines in AH samples obtained from 15 ARC patients and 15 HMC patients. The Human Inflammation Array 3 (QAH-INF-3, RayBio; RayBiotech; Norcross, GA, USA) was used for simultaneous analysis of 40 selected cytokines. The assay was performed according to the manufacturer's instructions. The assay is based on the sandwich immunoassay principle. Seventy microliters of sample was applied to each block. Antibodies targeting the selected cytokines are immobilized in specific locations on the surface of the array membrane. Cytokines present in the samples are captured by the corresponding antibodies and a cocktail of biotinylated antibodies is added to detect the bound cytokines. The signals were visualized using fluorescent dye conjugated with streptavidin (cy3 equivalent) and were detected with a GenePix 4000B system (Axon Instruments, Foster City, CA, USA). GenePix Pro 6.0 software (Axon Instruments) was used for densitometric analysis of the spots.

1.5. Suspension cytokine array

To verify the concentrations of cytokines identified in the cytokine antibody arrays, the AH samples obtained from 35 ARC patients and 45 HMC patients were tested using a human 6-plex assay (Bio-Plex Suspension Array System; Bio-Rad, Hercules, CA, USA) according to the manufacturer's instructions (Steuerwald et al., 2013). The selected cytokines included interleukin-1 receptor antagonist (IL-1ra), monocyte chemoattractant protein-1 (MCP-1; monocyte chemotactic and activating factor, MCAF), regulated on activation, normal T-cell expressed and presumably secreted (RANTES), IL-8, platelet-derived growth factor (PDGF)-BB, IL-6. Fifty microliters of sample was added to each well, and incubated with capture antibody-coupled magnetic beads. The samples were washed three times in a Bio-Plex Pro wash station, and were then incubated with the biotinylated detection antibody for 30 min in the dark. The captured cytokines were visualized with streptavidin-conjugated phycoerythrin and the expression levels were quantified using a BioPlex array reader. Bio-Plex Manager software (Bio-Rad) was used to calculate the concentrations of each cytokine.

1.6. Western blot assay

After determining the protein concentration, each AH sample (containing 4 μg of total AH protein) was added to 5 × sodium dodecyl sulfate–polyacrylamide gel electrophoresis loading buffer and denatured at 100 °C for 5 min. Then, the samples were separated by 15% gradient acrylamide sodium dodecyl sulfate-polyacrylamide gel electrophoresis. Protein bands were transferred onto a polyvinylidene difluoride blotting membrane (Millipore, Bedford, MA, USA), and subjected to immunolabeling using primary rabbit anti-human antibodies for IL-1ra (1:1000 dilution; ab2573; Abcam, Cambridge, MA, USA), MCP-1 (1:1000 dilution; ab151538; Abcam), and β-actin (1:1000 dilution; ab8226; Abcam). The membranes were then incubated with goat anti-rabbit IgG horseradish peroxidase (HRP)-conjugated secondary antibody (1:1000 dilution; ab6721; Abcam) for 30 min at room temperature. The immunoblotted bands were revealed by enhanced chemiluminescence reagent (Pierce ECL Western Blotting Substrate; Thermo Fisher Scientific, Waltham, MA, USA).

1.7. Statistical analysis

All data are expressed as the mean ± standard deviation. Student's *t* test was used to examine the differences between the two groups. The χ^2 test was used to examine differences between