Experimental Eye Research 103 (2012) 55–62

Contents lists available at SciVerse ScienceDirect

Experimental Eye Research

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

Aqueous humor oxidative stress proteomic levels in primary open angle glaucoma

A. Bagnis [a,*], A. Izzotti [b], M. Centofanti [c], S.C. Saccà [d]

[a] University of Genova, Eye Clinic, Department of Neurosciences, Ophthalmology and Genetics, University of Genoa, Genoa, Italy
[b] Department of Health Sciences, University of Genoa, Genoa, Italy
[c] University of Rome "Tor Vergata" and Glaucoma Research Bietti Foundation, Rome, Italy
[d] Ophthalmology Unit, Department of Head/Neck Pathologies, San Martino Hospital, Genoa, Italy

ARTICLE INFO

Article history:
Received 16 February 2012
Accepted in revised form 31 July 2012
Available online 21 August 2012

Keywords:
aqueous humor
primary open angle glaucoma
proteomic analysis
oxidative damage

ABSTRACT

The purpose of this work was to investigate the expression of glutamine synthase (GS), nitric oxide synthase (NOS) superoxide dismutase (SOD) and glutathione transferase (GST) in the aqueous humor of patients with primary open angle glaucoma and controls. Aqueous humor proteome was analyzed by antibody microarray. The expression of tested proteins was detected by protein Cy3/Cy5 labeling, column purification and hybridization on antibody-spotted glass microarray. Fluorescent signals were detected by fluorescence laser scanning. Aqueous humor levels of SOD as well as of GST were significantly lower (2.0- and 2.2-fold, $p < 0.01$) among patients than controls; both NOS and GS expression were significantly higher (2.2- and 2.6 fold, $p < 0.01$) among patients than controls. Our data showed substantial differences of GS, NOS2, SOD and GST aqueous humor levels between glaucomatous patients and controls as measured by antibody microarray technology. The overproduction of NO through inducible NOS can form toxic products and change the metabolic conditions of the TM. The GS over-expression might be related to neuronal injury or to the potential role of glutamate as a modulator in the ciliary body signaling. The reduced expression of the antioxidant enzymes SOD and GST could aggravate the unbalance between both oxygen- and nitrogen-derived free radicals production and detoxification. Based on our results, GS, NOS2, SOD and GST as measured by antibody microarray technology may be useful oxidative markers in aqueous humor of glaucomatous patients.

1. Introduction

Aqueous humor (AH) plays a pivotal role in the physiopathology of the eye. It has both optical and metabolic properties and supplies with nutrients the tissues of the anterior chamber (AC) of the eye (To et al., 2002; Fuchshofer, 2009). The volume of the AC is about 0.25 ml and the rate of AH production is roughly 2–2.5 μl/min. Approximately 1% of the AC and 3% of the posterior chamber AH volume is replaced each minute (Gerometta et al., 2005). The rate of production and elimination of AH contained in the AC is responsible for the intraocular pressure (IOP) levels and the accumulation of excessive amounts leads to increased IOP, the most important risk factor for primary open angle glaucoma (POAG) development. A decline in trabecular meshwork (TM) cellularity has been advocated as the main cause for IOP increasing during POAG (Alvarado et al., 1984). Oxidative free radicals and reacting oxygen species (ROS) are able to affect the cellularity of the TM and specifically its endothelial cells (Saccà et al., 2007). Visible and ultraviolet light continuously induce the formation of ROS targeting the TM and other parts of the eye, the AH representing a barrier against such kind of damage. The AH has well demonstrated antioxidant properties and any condition altering the oxidant–antioxidant balance at this level could contribute to the pathogenesis of POAG (Wood et al., 2007). A decrease of the AH antioxidant capacity has been demonstrated in patients with POAG together with dramatic changes in its protein content (Ferreira et al., 2004; Izzotti et al., 2010a). Moreover, mitochondria, the main endogenous source of reactive oxygen species has been shown to be targeted by the pathogenic processes in case of both POAG and pseudoexfoliative glaucoma (Izzotti et al., 2011, 2010b; Pamplona, 2011). For this reason, subjects bearing specific adverse gene pools are more susceptible to TM endothelial cell oxidative damage, regardless the exposure to environmental risk factors such as light exposure (Saccà et al., 2009).

The aim of the present study was to investigate whether the antioxidant capacity decrease detectable during POAG involves the

* Corresponding author. Clinica Oculistica, Università degli Studi di Genova, Viale Benedetto XV 5, 16132 Genova, Italy. Tel.: +39 010 353 8469; fax: +39 010 353 8494.
E-mail address: alebagnis@libero.it (A. Bagnis).

0014-4835/$ – see front matter © 2012 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.exer.2012.07.011

following enzymes showing oxidative production/detoxification activities (Table 1):

- Glutamate–ammonia ligase (glutamine synthase) (GS)
- Nitric oxide synthase 2 (NOS 2)
- Superoxide dismutase (SOD1/2)
- Microsomal glutathione S-transferase 1 (GST1)

To this purpose we used an antibody microarray technique that we selected for its clinical applicability. Antibody microarray has been already extensively validated in molecular medicine within cancer tissue (Sanchez-Carbayo, 2006; Madoz-Gúrpide et al., 2007; Bartling et al., 2005) and blood plasma (Carlsson et al., 2008; Orchekowski et al., 2005; Shafer et al., 2007; Szodoray et al., 2007, 2004). This innovative method is based on the use of specific antibodies and allows for the simultaneous detection of a great number of proteins (1200) from a single sample. The required sample size is lower than that used in other techniques. By this way it is possible to quantify at nanogram scale the amount of a given protein and to compare it to the others at the same time. Starting from 100 to 200 μl samples it is therefore possible to study the whole AH proteomic expression for each selected patient and to compare results from POAG patients and controls using standard computer algorithms such as Principal Component of Variance Analysis. Due to its accuracy, this method allows to obtain reliable results from experimental groups of only 10–15 patients.

2. Material and methods

This was a case-control study. All the enrolled subjects furnished an informed written consent and were treated in accordance with the Declaration of Helsinki. The study has been approved by the Ethical Committee of the San Martino Hospital on October 12th 2010.

Both patients and controls underwent ocular surgery for therapeutic purposes. AH samples were obtained from 10 clinically uncontrolled POAG patients (cases) and 10 senile cataract patients (controls) immediately before trabeculectomy and cataract surgery, respectively.

Cases samples were collected from 10 POAG patients (5 males, 5 females) with no tonometric compensation. Main elements for POAG diagnosis were IOP values, characteristic optic nerve head (ONH) and visual field defects. Exclusion criteria were the presence of any other ocular, systemic, or neurological diseases other than POAG-related optic-nerve damage and the diagnosis of other types of glaucoma. All glaucomatous patients were under treatment with maximal therapy that included topical beta-blockers, prostaglandin analogs, topical carbonic anhydrase inhibitors, and systemic carbonic anhydrase inhibitors. POAG patient mean age was 74.9 ± 3.10 years (mean ± SD).

All patients underwent a Humphrey 30-2 computerized visual field examination (750 Humphrey Field Analyzer II; Humphrey Ind., San Leandro, California) 2–4 weeks before surgery. The Glaucoma Staging System (GSS 2) (Brusini and Filacorda, 2006) was used in order to stage the visual field damage. All patients underwent daily tonometry curve (every 2 h between 8:00 AM and 8:00 PM) the week before surgery by the same physician using Goldmann tonometry (Table 1).

The inclusion criteria for 10 age (mean: 72.9 ± 3.54 years) and gender (5 males, 5 females) matched controls were: an open anterior chamber angle; no history of previous filtration surgery; pupil size >5 mm after dilatation; absence of pseudoexfoliation syndrome, diabetes, uveitis, systemic collagenopathy, and objective neurological signs, no history of use of systemic antihypertensive drugs, and no administration of corticosteroids during the five weeks before surgery (Saccà et al., 2001).

Sample collection from controls was performed before surgical interventions for cataract. Mean IOP among controls as measured by Goldmann tonometry was 14.1 ± 2.0 mmHg (mean ± SD).

Clinical characteristics of glaucomatous patients and controls are summarized in Table 1.

Mean AH sample volume was 115 μl, ranging from 100 μl (min) to 160 μl (max). AH samples were immediately stored in a deep

Table 1
Clinical characteristics of glaucomatous patients and results of AH protein analysis by antibody microarray. The difference of protein amounts between cases and controls was statistically significant ($p < 0.01$) for each tested enzyme. na, not available. POAG therapy: TBB = topical beta-blockers; PGA = prostaglandin analogs; CAI = carbonic anhydrase inhibitors.

Patient code	Age (years)	Gender	Mean 12 h IOP (mmHg)	Min IOP (mmHg)	Max IOP (mmHg)	VF defect stage	POAG therapy	Glutamate–ammonia ligase (glutamine synthase)	Nitric oxide synthase 2	Superoxide dismutase (SOD1/2)	Microsomal glutathione S-transferase 1
Controls ($n = 10$)											
C1	70	F	na	na	na	na	None	0.4	0.7	0.8	1.60
C2	65	F	na	na	na	na	None	0.5	0.9	1.8	1.10
C3	75	F	na	na	na	na	None	0.7	0.5	1.4	1.00
C4	73	F	na	na	na	na	None	0.8	0.9	0.6	2.00
C5	71	F	na	na	na	na	None	0.4	0.6	1.0	1.20
C6	72	M	na	na	na	na	None	0.6	0.3	0.9	1.70
C7	76	M	na	na	na	na	None	0.6	0.7	0.5	1.90
C8	70	M	na	na	na	na	None	0.5	0.8	11.7	1.50
C9	77	M	na	na	na	na	None	0.4	0.4	0.8	1.10
C10	74	M	na	na	na	na	None	0.6	0.8	0.7	1.60
Mean ± SD	72.9 ± 3.54	–	–	–	–	–	–	0.6 ± 0.1	0.7 ± 0.2	1.0 ± 0.5	1.4 ± 0.4
POAG ($n = 10$)											
P1	72	F	21	15	29	4	TBB, PGA, CAI	1.9	1.1	0.3	0.5
P2	71	F	20	19	23	2	TBB, PGA, CAI	1.0	2.5	0.4	0.9
P3	77	F	21	18	24	2	TBB, PGA, CAI	2.0	1.0	0.5	0.7
P4	73	F	20	16	28	3	TBB, PGA, CAI	2.1	1.5	0.6	0.8
P5	72	F	19	13	22	3	TBB, PGA, CAI	1.2	3.4	0.5	0.9
P6	75	M	22	18	25	4	TBB, PGA, CAI	1.1	1.2	0.3	0.6
P7	78	M	21	13	26	4	TBB, PGA, CAI	1.9	1.1	0.5	0.5
P8	74	M	20	19	28	4	TBB, PGA, CAI	1.5	1.0	0.4	0.3
P9	81	M	21	17	24	2	TBB, PGA, CAI	2.0	1.3	0.7	0.6
P10	76	M	19	15	27	3	TBB, PGA, CAI	1.0	3.2	0.5	0.3
Mean ± SD	74.9 ± 3.10		20.2 ± 3.5	16.3 ± 2.3	25.6 ± 2.4	3.1 ± 0.9		1.6 ± 0.5	1.73 ± 0.94	0.47 ± 0.11	0.6 ± 0.2