Available online at www.sciencedirect.com
SCIENCE DIRECT
Experimental Eye Research 82 (2006) 505–511
EXPERIMENTAL EYE RESEARCH
www.elsevier.com/locate/yexer

Secreted leukocyte protease inhibitor is present in aqueous humours from cataracts and other eye pathologies

Sabina Janciauskiene[a,*], Lars Brandt[b], Anders Wallmark[a], Ulla Westin[c], Torsten Krakau[b]

[a]*Department of Clinical Sciences, Wallenberg Laboratory, Ing.46, Plan 2, University Hospital Malmö, S-20502 Malmö, Sweden*
[b]*Department of Ophthalmology, University Hospital Malmö, S-20502 Malmö, Sweden*
[c]*Department of Otolaryngology and Head and Neck Surgery, University Hospital Malmö, S-20502 Malmö, Sweden*

Received 14 March 2005; accepted in revised form 11 August 2005
Available online 3 October 2005

Abstract

Previous studies identified serine, cysteine and metalloproteases in normal aqueous humours (AH) and suggested that a balance between proteases and their inhibitors may play a role in the modulation of the AH outflow. We aimed to determine whether secretory leukocyte protease inhibitor (SLPI), a serine protease inhibitor, is present in AH of patients with cataract and other eye pathologies. AH was collected from 117 cataract patients of which 55 were diagnosed with more when one eye disease: cataract only ($n=62$), pseudoexfoliation (PEX) ($n=26$), glaucoma ($n=6$), diabetes retinopathy ($n=4$), iritis–uveitis ($n=4$) and macular degeneration ($n=28$). The total protein in AH was determined by a Bradford assay and SLPI was analyzed by Western blot and ELISA methods. The average concentration of total protein and SLPI in AH samples was 160 ± 15 μg/ml ($n=117$, $\pm$SEM) and 500 ± 94 pg/ml ($n=105$), respectively. The cataract patients with additional eye disease(s) showed higher protein levels (201 ± 35 μg/ml) than cataract (controls) (128 ± 31 μg/ml), $P<0.01$. It is noteworthy that no correlation was found between SLPI and the total protein concentrations in AH, but SLPI was positively correlated with age ($r=0.2$, $P<0.05$). No statistical difference in SLPI levels was found between controls (cataract) and other pathologies, while patients with iritis/uveitis had higher SLPI levels compared to those with diabetes ($P<0.05$). We show here for the first time that SLPI is present in AH and may play a role as well as serve as a marker in pathological states.

Keywords: secretory leukocyte protease inhibitor; aqueous humours; eye; protease inhibitors

1. Introduction

Aqueous humour (AH) is an important intraocular fluid made continuously by the ciliary epithelium and is responsible for the supply of nutrients to and removal of metabolic wastes from the avascular tissues of the eye (To et al., 2002). It is also indispensable for the maintenance of the optical properties of the eye. A number of studies of the ion, amino acid, and protein composition of AH (To et al., 2002; Dernouchamps, 1982; Russell and Epstein, 1992) have been done. Normal human AH protein concentrations range from 5 to 30 mg/100 mL (Sawa et al., 1988) and has been shown to increase during ageing (Oshika et al., 1989). It has been suggested that most of the proteins enter the AH through the iris (Kolodny et al., 1996), although they can also be released from tissues in the eye (Barsotti et al., 1992). For example, the lens is an epithelial structure derived from primitive ectoderm, and the protein concentration within the lens is higher than that of any other tissue in the body. Lens protein α-crystallin is one candidate protein which may be released into the AH (Sandberg and Closs, 1979; Horwitz, 2003).

Earlier studies by Ringvold and co-workers showed that AH from cataract eyes with and without exfoliation syndrome (PEX) contain high concentrations of proteins with a broad range of sizes (Ringvold et al., 1989), some of which were protein fragments with estimated molecular weight of 14.4 and 16.3 kDa (Ringvold et al., 1989; Koliakos et al., 2000). Recently, it has been demonstrated that AH of PEX patients contains protein amyloid (Berlau et al., 2001). Similarly, in AH from eyes of young

* Corresponding author.
E-mail address: sabina.janciauskiene@medforsk.mas.lu.se (S. Janciauskiene).

0014-4835/$ - see front matter © 2005 Elsevier Ltd. All rights reserved.
doi:10.1016/j.exer.2005.08.010

monkeys (*Macaca mulatta*), the major protein components were found to range between 14 and 170 kDa (Russell and Epstein, 1992). The composition of AH proteins varies dramatically among different ocular conditions, such as inflammation, glaucoma, and diabetic retinopathy (Peretz and Tomasi, 1961; Sung and Barton, 2004; Funatsu et al., 2005). Despite extensive research on AH protein composition, the profile of proteins in AH fluid is still incomplete.

The presence of proteases and their inhibitors has been reported in human AH using zymographic and immunoblot techniques (Ando et al., 1993; Huang et al., 1996; Kee et al., 1999; Rohde et al., 1998). Aberrations in enzyme systems are thought to be involved in extracellular matrix alterations associated with glaucoma (Lutjen-Drecoll et al., 1989). Various metalloproteases and their tissue inhibitors (TIMPs) have been identified in AH samples from patients with cataract, uveitis, PEX and glaucoma (Vadillo-Ortega et al., 1989; El-Shabrawi et al., 2000; Schlotzer-Schrehardt et al., 2003; Maatta et al., 2005), and are implicated in the regulation of AH outflow (Rao et al., 2000). At least a few serine protease inhibitors, such as α1-antitrypsin, α1-antichymotrypsin and α2-macroglobulin, were also detected in human AH (Ando et al., 1993; Janciauskiene and Krakau, 2003). Antithrombin III, another serine protease inhibitor, was described as a major heparin binding protein in porcine AH (Rao et al., 2000). Cystatin C which is a mammalian cysteine protease inhibitor has been found in the rat and mouse AH, and localized in mouse, rat and human retinas (Wasselius et al., 2004; Wasselius et al., 2001; Paraoan et al., 2000). It has been suggested that the metalloproteases, papain-like cysteine proteases, serine proteases, and their inhibitors found in AH may participate in the remodelling of extracellular matrices in the trabecular meshwork and other tissues bordering the anterior chamber.

Secretory leukocyte protease inhibitor (SLPI) is a potent serine protease inhibitor found in mucosal secretions. SLPI was initially considered an epithelial cell product, but later was also found in human neutrophils and alveolar macrophages (Abe et al., 1991; Mihaila and Tremblay, 2001; Sallenave et al., 1997). The suggested function of SLPI is to protect mucosal tissue from proteolytic degradation and play a role in host defence against certain bacterial and fungal infections (Sallenave, 2002). Recent studies reveal that SLPI plays an important role in mending wounds. For example, studies with genetically engineered mice that lack the SLPI gene, have shown that these mice exhibit greatly compromised wound healing, increased inflammation and increased activity of the serine protease, elastase (Ashcroft et al., 2000). The knowledge that SLPI is mainly produced by the epithelial cells and exhibits both protease-inhibitory and antimicrobial activities prompted us to investigate whether SLPI is present in AH from patients with cataract (controls) and other ocular pathologies.

2. Materials and methods

2.1. Patients

AH from 117 non-selected patients, 87 females between the ages of 68–98 (average age 83) and 30 males, between the ages of 59 and 93 (average age 79.5), were taken by one experienced surgeon during cataract surgery. Informed consent was obtained from patients prior to collecting the aqueous samples. The study was approved by the Ethics Committee of Lund University (LU-575-00). A small quantity (0.15–0.2 ml) of AH from the interior chamber of each eye was aspirated using a 27 gauge needle on a tuberculin syringe as soon as the eye was opened. Immediately after collection, AH were transferred to sterile plastic tubes and stored at −80 °C before analysis. Samples contaminated with blood were excluded. Samples from cases [$n=62$, ages 46–92 years (average 73.3)] diagnosed only for cataract were used as controls. Twelve patients, in addition to those with cataracts, had at least two other diseases. Patients included in the study were not grouped according to cataract type and therefore the influence of various types of cataract on measured parameters was not evaluated. The number of cases and the distribution between females and males diagnosed for cataract alone or cataract plus other diseases are presented in Table 1.

The glaucoma cases were treated with pressure-reducing drugs such as timolol, pilocarpine and latanoprost, the latter being a prostaglandin analogue which may affect iris pigmentation. One hour before the cataract surgery all patients were treated with a sedative, oral Diazepam (benzodiazepine). Before the operation patients were also treated locally with: 0.5% Chloramphenicol once, 0.1% Diclophenac twice, 10% Phenylefrin solution four times and 1% Cyclopentolate four times. The possible effects of this medication require a separate study. Patients who had

Table 1
Diagnostic Characteristics of the patients

Diagnosis	Male	Female	Total
Cataract	16	46	62
Cataract + PEX[a]	2	15	17
Cataract + macular degeneration	5	13	18
Cataract + iritis/uveitis	–	3	3
Cataract + diabetes	1	1	2
Cataract + glaucoma	1	2	3
Cataract + PEX + macular degeneration	3	4	7
Cataract + PEX + macular degeneration + diabetes	1	–	1
Cataract + PEX + glaucoma	–	1	1
Cataract + macular degeneration + glaucoma	1	–	1
Cataract + macular degeneration + iritis/uveitis	–	1	1
Cataract + diabetes + glaucoma	–	1	1
Total	30	87	117

[a] Pseudoexfoliation syndrome.