Experimental Eye Research 103 (2012) 22–32

Contents lists available at SciVerse ScienceDirect

Experimental Eye Research

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

Expression profiles of aquaporins in rat conjunctiva, cornea, lacrimal gland and Meibomian gland

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ARTICLE INFO

Article history:
Received 10 April 2012
Accepted in revised form 12 July 2012
Available online 22 July 2012

Keywords:
ion transport
tear fluid
conjunctiva
cornea
lacrimal gland
Meibomian gland

ABSTRACT

The aim of the study was to elucidate aquaporin (AQP) family member mRNA expression and protein expression/localization in the rat lacrimal functional unit. The mRNA expression of all rat AQPs (AQP0–9, 11–12) in palpebral, fornical, and bulbar conjunctiva, cornea, lacrimal gland, and Meibomian gland was measured by Reverse Transcriptase-Polymerase Chain Reaction (RT-PCR) and real time RT-PCR. Antibodies against AQP1, 3, 4, 5, 9, and 11 were used in Western blotting and immunohistochemistry to determine protein expression and distribution. Our study demonstrated characteristic AQP expression profiles in rat ocular tissues. AQP1, 3, 4, 5, 8, 9, 11, and 12 mRNA were detected in conjunctiva. AQP0, 1, 2, 3, 4, 5, 6, 11, and 12 mRNA were expressed in cornea. AQP0, 1, 2, 3, 4, 5, 7, 8, and 11 mRNA were detected in lacrimal gland. AQP1, 3, 4, 5, 7, 8, 9, 11, and 12 mRNA were identified in Meibomian gland. By Western blot, AQP1, 3, 5, and 11 were detected in conjunctiva; AQP1, 3, 5, and 11 were identified in cornea; AQP1, 3, 4, 5, and 11 were detected in lacrimal gland; and AQP1, 3, 4, 5, 9, and 11 were present in Meibomian gland. Immunohistochemistry localized AQPs to distinct sites in the various tissues. This study rigorously analyzed AQPs expression and localization in rat conjunctiva, cornea, lacrimal gland, and Meibomian gland tissues. Our findings provide a comprehensive platform for further investigation into the physiological or pathophysiological relevance of AQPs in ocular surface.

1. Introduction

The lacrimal functional unit is an integrated system consisting of the lacrimal gland, the ocular surface (cornea, conjunctiva, and Meibomian gland), and the interconnecting sensory and motor innervation (McKown et al., 2009; Stern et al., 2004). These components function together to produce a normal tear film that provides protection against infection from environmental contacts, lubrication of the optical surface for light refraction, and a proper microenvironment for maintenance of ocular surface health (Gipson, 2007; Klenkler et al., 2007; Montes-Mico, 2007; Rolando and Zierhut, 2001; Schoenwald et al., 1998). The tear film has an inner aqueous-mucin layer derived mainly from the lacrimal gland, corneal, and conjunctival secretions that is rich in proteins, electrolytes, and mucins, and an outer lipid layer from the Meibomian gland that is rich in lipid and other proteins (Dartt et al., 1981; Greiner et al., 1996; Jumblatt et al., 1999; McCulley and Shine, 2003; Watanabe, 2002; Yu et al., 2008). Tear film homeostasis is continuously maintained by fluid transport across all these tissues.

Water transport through plasma membranes is mediated mainly through aquaporin (AQP) water channels in a variety of tissues. AQPs are a family of small transmembrane proteins that facilitate transport of water and other small solutes across cell membranes. A total of 13 mammalian AQPs, designated as AQP0–12, have been reported to date. Based on their transport properties, AQPs are divided into classical water selective aquaporins (AQP0, 1, 2, 4, and 5), aquaglyceroporins (AQP3, 7, 9, and 10) and “unorthodox” aquaporins (AQP6, 8, 11, and 12) (Rojek et al., 2008). Aquaglyceroporins mediate water as well as other small molecules, e.g., glycerol and urea, permeation in response to hydrostatic and osmotic gradients. Unorthodox aquaporins do not exhibit conventional water transport properties. AQP6 and 8 were reported to be able to mediate ion transport (Rojek et al., 2008). AQP11 and AQP12 have unique pore forming NPA (asparagine–proline–alanine) boxes distinct from other AQPs (Gorelick et al., 2006; Itoh et al., 2005). Besides the unconventional structure of the unorthodox AQPs, oocytes injected with rat AQP11 did not exhibit conventional water or other small solutes transport properties (Gorelick et al., 2006), suggesting that they may have alternative functions. Compelling evidence for the involvement of AQPs in cell migration and proliferation (Papadopoulos et al., 2008),

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0014-4835/$ – see front matter © 2012 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.exer.2012.07.005

further implicates this family of proteins important in a variety of physiological and/or pathological processes.

Several aquaporins transcripts and proteins have been previously reported to be expressed and play important roles in the ocular surface. Immunohistochemical analysis localized AQP0 in mouse lens fiber cells (Chepelinsky, 2003). Mutations in AQP0 are associated with autosomal dominant cataracts in mice and humans (Berry et al., 2000; Shiels and Bassnett, 1996). AQP1 is expressed in corneal endothelium and stromal keratocytes of mouse, rat, bovine, and humans (Hamann et al., 1998; Macnamara et al., 2004; Ruiz-Ederra and Verkman, 2006; Wen et al., 2001). An AQP1 deletion did not alter lens transparency but accelerated cataractgenesis in mice (Ruiz-Ederra and Verkman, 2006). AQP1 protein is also dramatically reduced in patients with endothelial corneal disease (Fuchs' dystrophy, bullous keratopathy, and graft endothelial failure) and mouse models of corneal endothelial injury, but remains unchanged in patients of non-endothelial corneal disease (keratoconus and corneal scarring) (Kenney et al., 2004; Macnamara et al., 2004).

AQP3 has been localized to mouse corneal epithelial cells (Levin and Verkman, 2006), rat conjunctival epithelium (Funaki et al., 1998; Hamann et al., 1998), and mouse lacrimal gland acinar cells (Ishida et al., 1997; Moore et al., 2000). There was increased AQP3 expression in corneal epithelium and AQP4 expression in corneal endothelium of Fuchs' dystrophy and bullous keratopathy patients compared with normal subjects (Kenney et al., 2004). AQP4 was not found in rat cornea (Hamann et al., 1998), but was present in mouse lacrimal gland acinar cells (Ishida et al., 1997; Moore et al., 2000).

AQP5 protein has been found in human and rabbit conjunctiva (Oen et al., 2006), mouse, rat, and human corneal epithelium (Funaki et al., 1998; Garfias et al., 2008; Levin and Verkman, 2004), and lacrimal gland acinar cells (Funaki et al., 1998; Hamann et al., 1998; Hirai et al., 2000; Tsubota et al., 2001). The AQP5 transcript expression was shown to be much lower in keratoconus etiopathogeny than normal subjects (Rabinowitz et al., 2005), while in another study no differences were detected in AQP5 mRNA or protein levels between those groups (Garfias et al., 2008). Although AQP5 protein levels were similar in lacrimal gland of Sjögren's syndrome (SS) patients and normal subjects, defective AQP5 trafficking was observed in lacrimal gland of SS patients (Ma et al., 1999). Quantification of AQP5 protein showed significantly higher level in tears of SS patients, which suggests that the damaged lacrimal gland and corneal epithelium may shed AQP5 into tears (Ohashi et al., 2003). Consistently, lacrimal gland tissue destruction and leakage of AQP5 into tears were observed in dacryoadenitis mice model (Hirai et al., 2000).

The purpose of the current study was to characterize expression of the entire rat AQP family in rat conjunctiva, cornea, lacrimal gland, and Meibomian gland. We explored the expression of AQPs in Meibomian gland to search for evidence of the involvement of AQPs in secretion of this gland. Twelve reported rat AQPs (AQP0–9, and AQP11–12) were screened at the gene expression level, and a subset of AQPs were examined for protein expression and localization based upon antibody availability. We dissected palpebral, fornical, and bulbar conjunctiva separately to evaluate regional differences in AQPs expression. This systematic AQPs expression profiles identification serves as a basis for exploring the functional roles of AQPs in the lacrimal functional unit.

2. Materials and methods

2.1. Experimental animals

Male Sprague–Dawley rats (Charles River, Wilmington, MA) weighing 250–300 g were studied at age 10–12 weeks. Animals were treated according to the ARVO statement for the Use of Animals in Ophthalmic and Vision Research and were handled under protocols approved by the Institutional Animal Care and Use Committee of the University of North Carolina at Chapel Hill.

2.2. Reverse Transcriptase-Polymerase Chain Reaction (RT-PCR) analysis

Rats were sacrificed by CO_2 asphyxiation. Under a dissecting microscope, Meibomian gland tissues were carefully dissected from the palpebral conjunctiva. Then palpebral, fornical, and bulbar conjunctiva were dissected individually according to their anatomic distributions, with the width of each section being 1.5–2 mm. Finally, cornea and lacrimal gland were removed for analysis. Total RNA was isolated with RNeasy Plus Mini kit (Qiagen, Valencia, CA) according to the manufacturer's instructions. Complementary DNA (cDNA) was prepared from of 1 μg of total RNA with SuperScript III reverse transcriptase (Invitrogen, Carlsbad, CA). PCR was performed with PCR master mix (Fermentas, Glen Burnie, MA) under the following conditions: 95 °C 10 min, 40 cycles of 95 °C 45 s, 55 °C 45 s, and 72 °C 1 min, and 72 °C 7 min. The amplified products were analyzed on ethidium bromide stained agarose gels. Rat AQPs family genes (AQP0–9, and AQP11–12) were characterized with the primer sets listed in Table 1. Primers for the internal standard, 18s RNA, were obtained from Ambion (Austin, TX). Reactions substituting reverse transcriptase with water were used as negative controls.

2.3. Real time RT-PCR

Complimentary DNA was prepared as described above and further analyzed with Fast SYBR Green Master Mix by ABI 7900HT Sequence Detection System (Applied Biosystems Inc., Foster, CA). The PCR reaction was performed in a 96-well optical plate. The thermal cycler profile started with 2 min at 95 °C, followed by 40

Table 1
Oligonucleotide primers for regular RT-PCR.

Gene	Sequence (forward)	Sequence (reverse)	Accession number	Product size
AQP0	acggctcaagagtgtttctga	gttgacacctctttccccttc	NM_001105719.1	409bp
AQP1	cttacctccaggacccttcc	agctcatccacacgtgctc	NM_012778.1	232bp
AQP2	gttcccagtgcagagtagctg	gtcccccagaaggagtctatg	NM_012909.2	237bp
AQP3	cttccactgaggcagagaatg	ggttcccttgagctgctagtt	NM_031703.1	461bp
AQP4	catgctcatctttgttctgctc	cagtaacatcagtccgtttgga	NM_012825.3	428bp
AQP5	atccattggcttgtctgtcac	attatgggcttctgctcctgt	NM_012779.1	465bp
AQP6	gtcaacgtggtccacaacag	tgcaaacttcccaacaatga	NM_022181.1	240bp
AQP7	tcagttcctgggttccttctta	gcatatcctgtgttcatgccta	NM_019157.2	318bp
AQP8	gaccatgctgctaattccctac	cacccatacacacagccaatac	NM_019158.2	209bp
AQP9	acttctggtggatacctgtcgt	taggcacacaggtgacatcttc	NM_022960.2	234bp
AQP11	ctggacgctgacactgatctac	tgtcgaaatggtacttggtcag	NM_173105.1	221bp
AQP12	acgtctgccttcttcaatcct	tagggttcggtatttgcttt	NM_001109009.1	202bp