Experimental Eye Research 127 (2014) 153–160

Contents lists available at ScienceDirect

Experimental Eye Research

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

Does estrogen deficiency cause lacrimal gland inflammation and aqueous-deficient dry eye in mice?☆

Raheleh Rahimi Darabad [a, b], Tomo Suzuki [a, b], Stephen M. Richards [a, b], Frederick A. Jakobiec [b, c], Fouad R. Zakka [b, c], Stefano Barabino [a, b], David A. Sullivan [a, b, c, *]

[a] *Schepens Eye Research Institute, Boston, MA, USA*
[b] *Department of Ophthalmology, Harvard Medical School, Boston, MA, USA*
[c] *Massachusetts Eye and Ear Infirmary, Boston, MA, USA*

ARTICLE INFO

Article history:
Received 28 March 2014
Accepted in revised form 21 July 2014
Available online 30 July 2014

Keywords:
lacrimal gland
mice
estrogen
male
female
sex difference
inflammation
tear volume
gene expression

ABSTRACT

Researchers have proposed that estrogen deficiency will lead to a Sjögren's syndrome (SjS)-like lacrimal gland inflammation, aqueous tear deficiency and dry eye. The purpose of this study was to determine whether this proposal is correct. Lacrimal glands were obtained from adult, age-matched wild type (WT) and aromatase knockout (ArKO) mice, in which estrogen synthesis is completely eliminated. Tissues were also obtained from autoimmune MRL/Mp-lpr/lpr (MRL/lpr) mice as inflammation controls. Tear volumes in WT and ArKO mice were measured and glands were processed for molecular biological and histological evaluation. Our results demonstrate that estrogen absence does not lead to a SjS-like inflammation in lacrimal tissue or to an aqueous-deficient dry eye. There was no upregulation of genes associated with inflammatory pathways in lacrimal glands of male or female ArKO mice. Such inflammatory activity was prominent in autoimmune MRL/lpr tissues. We also found no evidence of inflammation in lacrimal gland tissue sections of estrogen-deficient mice, and tear volumes of ArKO males were actually increased as compared to those WT controls. Interestingly, our study did show that estrogen absence influences the expression of thousands of lacrimal gland genes, and that this impact is sex- and genotype-specific. Our findings demonstrate that estrogen absence is not a risk factor for the development of SjS-like lacrimal gland inflammation or for aqueous-deficient dry eye in mice.
© 2014 Elsevier Ltd. All rights reserved.

1. Introduction

The lacrimal gland is the primary source of the tear film's aqueous layer. This gland secretes proteins, electrolytes and water that collectively play a prominent role in maintaining ocular surface integrity, protecting against microbial challenge and preserving visual acuity (Sullivan, 2004). Malfunction of the lacrimal gland, in turn, leads to aqueous tear deficiency. An example is Sjögren's syndrome (SjS), an autoimmune disease characterized by extensive glandular inflammation, immune-mediated dysfunction and/or destruction of acinar and ductal epithelial cells, a precipitous decline in aqueous tear output, and consequent dry eye disease (Sullivan, 2004).

It is important to note that dry eye disease occurs predominantly in women (The epidemiology of dry eye disease: report of the Epidemiology Subcommittee of the International Dry Eye WorkShop, 2007). This prevalence has been linked to estrogen status. Investigators have proposed that estrogens positively modulate the structure, function and secretion of the lacrimal gland and that estrogen deficiency leads to numerous lacrimal gland sequelae, including acinar cell disruption, apoptosis and necrosis, cellular vacuolization, DNA degradation, inflammation, glandular regression and aqueous-deficient dry eye (Azzarolo et al., 2003; Konttinen et al., 2012; Mostafa et al., 2012; Song et al., 2014; Sullivan, 2004[1]). Researchers have also posited that estrogen insufficiency promotes a SjS-like autoimmune exocrinopathy (Arakaki et al., 2010; Hayashi et al., 2004; Ishimaru et al., 2003, 1999; Shim et al., 2004; Sullivan, 2004; Takahashi et al., 1997). Conversely, estrogen treatment purportedly corrects such defects in lacrimal gland anatomy and physiology, and promotes aqueous

☆ This research was supported by NIH grant R01EY05612 and the Margaret S. Sinon Scholar in Ocular Surface Research Fund.

* Corresponding author. Schepens Eye Research Institute, 20 Staniford Street, Boston, MA 02114, USA. Tel.: +1 617 912 0287.

E-mail address: david.sullivan@schepens.harvard.edu (D.A. Sullivan).

[1] The "Sullivan, 2004" review contains over 40 additional references about estrogen's possible influence on the lacrimal gland. These papers were published between 1930 and 2002.

http://dx.doi.org/10.1016/j.exer.2014.07.017
0014-4835/© 2014 Elsevier Ltd. All rights reserved.

secretion (Affinito et al., 2003; Guaschino et al., 2003; Mostafa et al., 2012; Sullivan, 2004).

If these investigators' proposals are correct, we would predict that complete estrogen absence would induce an upregulation of inflammatory pathways in the lacrimal gland, a SjS-like inflammation in lacrimal tissue, and aqueous tear deficiency. To test this prediction, we examined molecular biological and histological attributes of the lacrimal glands, as well as tear volumes, of male and female aromatase knockout (ArKO) mice. These animals were generated by the targeted disruption of exon IX in the cyp19 gene and have no aromatase, the cytochrome P450 enzyme that controls the formation of estrogens. Consequently estrogen synthesis is completely eliminated in ArKO mice (Fisher et al., 1998).

2. Materials & methods

2.1. Animals and tissue collections

Breeding pairs of C57BL/6J – aromatase knockout (ArKO) heterozygous mice were acquired from Dr. Orhan Oz (University of Texas Southwestern Medical Center, Dallas, TX). Animals were sent to Charles River Breeding Laboratories (Wimington, MA) for initial quarantine, health monitoring and serology, and then shipped to the Animal Facilities of the Schepens Eye Research Institute (Boston, MA). Mice were maintained and bred in constant temperature rooms with fixed light/dark intervals of 12 h length. Offspring were genotyped by following a published protocol (Fisher et al., 1998). In brief, genomic DNA was isolated from tails by utilizing a GenEluteTM Mammalian Genomic DNA Miniprep Kit (Sigma–Aldrich, Saint Louis, MO). The DNA was amplified by PCR with a Hybaid OMN-E thermocycler (Thermo Electron Corp, Burlington, Ontario, Canada) by employing exon 9 gene primers (forward: GTGACAGAGACATAAAGATCG; reverse: GTAAATTCATTGGGCTTAGGG) and *neo* gene primers (forward: ATCAGGATGATCTGGACGAAGA; reverse: CCACAGTCGATGAATCCAGAA). The PCR conditions were 1 cycle (3 min at 94 °C), 35 cycles (40 s at 94 °C, 30 s at 55 °C, 45 s at 72 °C) and 1 cycle (5 min at 72 °C) and amplicons were examined on 2.5% agarose gels. Band sizes equaled 220 bp from wild type (WT) mice, 170 bp from ArKO mice, and both fragments from heterozygotes. In addition to these animals, adult female and male MRL/Mp-lpr/lpr (MRL/lpr) mice were obtained from Jackson Laboratories (Bar Harbor, ME) to serve as controls.

When indicated, mice were sacrificed by CO_2 inhalation and lacrimal glands were removed for either molecular biological procedures or histology. Glandular samples were prepared by combining tissues from 5 to 6 mice/sex/group. Three different sample preparations were made for each tissue/sex/group and then processed for RNA analysis.

For histological evaluation, tissues were fixed in 10% buffered formalin, dehydrated, embedded in methacrylate, cut into 3 μm sections and stained with hematoxylin and eosin or with Periodic-acid Schiff, according to previously described techniques (Sato and Sullivan, 1994). Sections were taken from different tissue areas, all separated by minimal distances of at least 30 μm. Slides were observed with an Olympus BH-2 light microscope and micrographs were obtained with a Nikon Eclipse E800 and SPOT camera (MicroVideo Instruments Inc., Avon, MA).

All research studies with mice were approved by the Institutional Animal Care and Use Committee of The Schepens Eye Research Institute and adhered to the Association for Research in Vision and Ophthalmology Statement for the Use of Animals in Ophthalmic and Vision Research.

2.2. Measurement of aqueous tear production

Tear volumes were measured by using a modified phenol red thread test (Zone-Quick^{TM}, Lacrimedics, Eastsound, WA) (Barabino et al., 2005). Under direct visualization with a biomicroscope, meniscal tears were removed and then threads were placed in the lateral canthus of the right eye for 30 s. The tear migration distance in mm was determined by using a microscope (Zeiss S4, West Germany) and a hemocytometer. Tears measurements were repeated 3 times and the results were averaged. Statistical analyses of these data were performed with Student's *t*-test (two-tailed, unpaired).

2.3. Molecular biological procedures

Total RNA was extracted from lacrimal glands by using TRIzol reagent (Invitrogen Corp., Carlsbad, CA) and purified with RNAqueous spin columns (Ambion, Austin, Tx). The glandular RNA samples were treated with RNase-free DNase (Invitrogen), analyzed spectrophotometrically at 260 nm to determine concentration and assessed with a RNA 6000 Nano LabChip and an Agilent 2100 Bioanalyzer (Agilent Technologies, Palo Alto, CA) to verify RNA integrity. The RNA samples were then stored at −80 °C until processing.

Gene expression was determined by the use of two procedures. One involved the processing of RNA samples for hybridization to CodeLink UniSet Mouse 20K I Bioarrays (n ~ 20,000 genes/array; Amersham Biosciences/GE Healthcare, Piscataway, NJ), according to reported procedures (Richards et al., 2006). In brief, cDNA was synthesized from RNA (2 μg) with a CodeLink Expression Assay Reagent Kit (Amersham) and purified with a QIAquick purification kit (Qiagen, Valencia, CA). Samples were then dried, and cRNA was generated with a CodeLink Expression Assay Reagent Kit (Amersham), recovered with an RNeasy kit (Qiagen) and quantified with an UV spectrophotometer. Fragmented and biotin-labeled cRNA was incubated and shaken at 300 rpm on a CodeLink Bioarray at 37 °C for 18 h. After this time period, the Bioarray was washed, exposed to streptavidin-Alexa 647, and scanned by utilizing ScanArray Express software and a ScanArray Express HT scanner (Packard BioScience, Meriden, CT) with the laser set at 635 nm, laser power at 100%, and photomultiplier tube voltage at 60%. Scanned image files were examined by using CodeLink image and data analysis software (Amersham), which yielded both raw and normalized hybridization signal intensities for each array spot. The intensities of the ~20,000 spots on the Bioarray image were normalized to a median of 1. Standardized data, with signal intensities exceeding 0.50, were analyzed with sophisticated bioinformatic software (Geospiza, Seattle, WA). This comprehensive software also generated gene ontology, KEGG (i.e. Kyoto Encyclopedia of Genes and Genomes) pathway and z-score reports. The ontologies were organized according to the guidelines of the Gene Ontology Consortium (http://www.geneontology.org/GO.doc.html) (Ashburner et al., 2000).

The other procedure to analyze gene expression involved the hybridization of each cRNA (20 μg) sample to a GeneChip Mouse Genome 430A 2.0 Array (Affymetrix, Santa Clara, CA) according to the manufacturer's protocol. Hybridized GeneChips were scanned with an Affymetrix Model 700 Scanner and expression data files were generated from array images using Affymetrix Microarray Suite 4.0 software. GeneChip data were standardized by selecting the default scaling in Affymetrix GeneChip Operating Software, which yields a trimmed mean intensity of 500 for each GeneChip microarray. Normalized data with a quality value of 1.0 were then evaluated with Geospiza Genesifter software.

Download English Version:

https://daneshyari.com/en/article/6196799

Download Persian Version:

https://daneshyari.com/article/6196799

Daneshyari.com