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Research article

Sjögren's syndrome associated dry eye in a mouse model is ameliorated by topical application of integrin α4 antagonist GW559090☆

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ABSTRACT

Sjögren's syndrome is an autoimmune disease associated with inflammation of exocrine glands with clinical manifestations of dry eye and dry mouth. Dry eye in this disease involves inflammation of the ocular surface tissues – cornea and conjunctiva. While systemic blockade of adhesion molecules has been used to treat autoimmune diseases, the purpose of this study was to determine the therapeutic efficacy of topical application of an integrin α4 adhesion molecule antagonist in a mouse model of dry eye associated with Sjögren's syndrome. To assess this spontaneously developed ocular surface inflammation related to Sjögren's syndrome in TSP-1null mice (12 wks) was evaluated. Mice were treated with topical formulations containing 0.1% dexamethasone or 30 mg/ml GW559090 or vehicle control. Corneal fluorescein staining and conjunctival goblet cell density were assessed. Real-time PCR analysis was performed to assess expression of the inflammatory marker IL-1β in the cornea and Tbet and RORγt in the draining lymph nodes. Ocular surface inflammation was detectable in TSP-1null mice (≥12 wk old), which resulted in increased corneal fluorescein staining indicative of corneal barrier disruption and reduced conjunctival goblet cell density. These changes were accompanied by increased corneal expression of IL-1β as compared to WT controls and an altered balance of Th1 (Tbet) and Th17 (RORγt) markers in the draining lymph nodes. Topically applied dexamethasone and GW559090 significantly reduced corneal fluorescein staining compared to vehicle treatment ($p = 0.023$ and $p < 0.001$, respectively). This improved corneal barrier integrity upon adhesion molecule blockade was consistent with significantly reduced corneal expression of pro-inflammatory IL-1β compared to vehicle treated groups ($p < 0.05$ for both treatments). Significant improvement in goblet cell density was also noted in mice treated with 0.1% dexamethasone and GW559090 ($p < 0.05$ for both). We conclude that similar to topical dexamethasone, topically administered GW559090 successfully improved corneal barrier integrity and inflammation in an established ocular surface disease associated with Sjögren's syndrome.

1. Introduction

Autoimmune Sjögren's syndrome is the second most common rheumatic disease next to arthritis with as many as 1 to 2 million individuals affected in the United States. The prevalence of Sjögren's syndrome is reported to range between 0.05 and 4.8% of the population (Pillemer et al., 2001). Clinical presentation of this disease typically includes oral and ocular manifestations as both salivary and lacrimal glands are targeted by the autoimmune responses. The ocular symptoms, referred to as Keratoconjunctivitis sicca (KCS), include an inflammation of the cornea (keratitis) and conjunctiva (conjunctivitis). The glandular inflammatory infiltrates interfere with the secretory function resulting in compromised tear quality, which leads to ocular surface inflammation or KCS. As a chronic condition Sjögren's syndrome typically affects middle-age

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patients with an average onset age of 40 years.

Sjögren's syndrome is a complex disease with a multifactorial etiology that includes environmental stimuli as well as genetic factors. The immunopathogenesis of this disease involves activation of both T and B cells. While glandular histology is marked with lymphocytic infiltration, the presence of autoantibodies in the serum represents another hallmark of Sjögren's syndrome. Both of these features were reported as detectable in thrombospondin-1 deficient (TSP-1null) mice (Turpie et al., 2009). The progressive and chronic nature of the disease in humans is also reproduced in TSP-1null mice. A loss of lacrimal gland secretory function is concurrently detected with increased apoptosis as early as 8 weeks of age in TSP-1null mice. These changes are accompanied by compromised tear quality as indicated by reduced tear mucin levels (Contreras-Ruiz et al., 2013). The earliest sign of ocular surface damage in TSP-1null mice is detected by 12 wks of age when significant disruption of corneal barrier integrity is noted with increased corneal fluorescein score. The pathological changes in the corneal epithelium during ocular surface inflammation are attributed to IL-1β based on *in vitro* and *in vivo* evidence (Chen et al., 2010; Li et al., 2010). Furthermore, the ocular surface damage in Sjögren's related KCS is reported as associated with increased corneal expression of inflammatory markers including IL-1β (Solomon et al., 2001). More recently inflammatory changes in the TSP-1 deficient conjunctiva were also detected (Contreras-Ruiz et al., 2013) similar to those reported in Sjögren's patients that are believed to result in a loss of mucin secreting conjunctival goblet cells (Kunert et al., 2002; Pflugfelder et al., 1999). Thus ocular surface pathology in TSP-1null mice bears a striking resemblance to that seen in KCS and is clearly associated with inflammatory immune responses.

Cell adhesion molecules play a key role in several inflammatory pathologies (Golias et al., 2007; Sfikakis and Mavrikakis, 1999) as extravasation of circulating inflammatory leukocytes into a peripheral tissue involves their interactions with adhesion molecules expressed on vascular endothelium. Targeting adhesion molecules to block tissue migration of inflammatory effectors is emerging as an effective approach to treat inflammatory conditions as noted in several experimental animal studies (Ecoiffier et al., 2008; Ghosh and Panaccione, 2010; Glasner et al., 2005; Gong et al., 2006). In humans blockade of adhesion molecules has proven to be an effective therapeutic approach in preventing progression and relapses of autoimmune disease such as multiple sclerosis (Miller et al., 2003; Polman et al., 2006). Increased expression of adhesion molecules is also reported in Sjögren's syndrome – in the conjunctiva as well as lacrimal glands (Saito et al., 1993; Stern et al., 2002). In this study the efficacy of a topically applied integrin α4 antagonist (GW559090) in ameliorating ocular surface inflammation associated with autoimmune Sjögren's syndrome was evaluated using a preclinical mouse model (TSP-1null). GW559090 had been selected because of its high affinity for integrin α4β1 (Kd = 0.19 nM) and potent blockade of cell adhesion of integrin α4β1 to VCAM-1 (vascular cell adhesion molecule 1) and fibronectin (IC_{50} = 7.72 nM and 8.04 nM, respectively) and integrin α4β7 to MAdCAM (mucosal addressin cell adhesion molecule 1; IC_{50} = 23.0 nM) *in vitro* (Krauss et al., 2015). The compound had previously undergone clinical testing by the inhalation route for the treatment of asthma (Ravensberg et al., 2006).

2. Materials and methods

2.1. Mice

C57BL/6 (*H-2b*) mice, 6–8 weeks old, were purchased from Charles River Laboratories (Wilmington, MA) and served as wild type (WT) control mice. TSP-1null mice (C57BL/6 background), originally received from Dr. J. Lawler (BIDMC, Harvard Medical School, Boston, MA) were bred in house in a pathogen-free facility at Schepens Eye Research Institute, Boston, MA. All animal experiments were conducted in accordance with institutional guidelines and the GSK Policy on the Care, Welfare and Treatment of Laboratory Animals after review by the GSK and Schepens Institutional Animal Care and Use Committees. The work conformed to the standards in the ARVO Statement for Use of Animals in Ophthalmic and Vision Research.

2.2. Treatment regimen

Early development of secretory deficit in TSP-1 deficient lacrimal glands and conjunctival goblet cells by 8 weeks of age progressively leads to disruption of corneal barrier integrity by 12 weeks of age. At this age inflammation is detectable locally in the conjunctiva as well as systemically in cervical lymph nodes indicating established dry eye disease. Therefore we chose to use 12 weeks old mice in this study. Thrombospondin-1 deficient mice were treated with 0.1% dexamethasone or GW559090 ((S)-3-(4-((4-carbamoylpiperidine-1-carbonyl)oxy)phenyl)-2-((S)-4-methyl-2-(2-(otolyloxy)acetamido)pentanamido)propanoic acid; synthesized by GSK) (30 mg/ml in phosphate buffered saline, pH 7) or control vehicle (phosphate buffered saline, pH 7) in both eyes. The dose was chosen based on a dose–response study in a different mouse model of dry eye (Krauss et al., 2015). An eyedrop (5 μL) was topically applied to the eyes of the mice without anesthesia twice a day for a period of 3 weeks. The untreated WT and TSP-1null mice, both 12 wk old at baseline, received no eyedrops. Ocular signs of dry eye disease (corneal barrier integrity as determined by fluorescein staining) were measured at baseline (prior to initiating treatment) and at the end of each week (i.e week 1, 2 and 3). Mice were then euthanized at the end of the study period for cellular and molecular studies.

2.3. Corneal fluorescein staining

Corneal fluorescein staining was performed as described previously (Turpie et al., 2009). Sodium fluorescein (1%), 1 μl, was applied to the cornea of mice under anesthesia. Three minutes later, eyes were flushed with PBS to remove excess fluorescein, and corneal staining was evaluated and photographed with a slit lamp biomicroscope (Humphrey-Zeiss, Dublin, CA) using a cobalt blue light. Punctate staining was recorded using a standardized National Eye Institute grading system of 0–3 for each of the five areas of the cornea (Lemp, 1995).

Following numbers of animals were included in different groups in this study - 12 WT and 9 TSP-1null mice to compare age related changes in untreated mice over 3 weeks of study period, 10 TSP-1null mice to determine effect of dexamethasone (5 each in vehicle and experimental group) and 19 TSP-1null mice to evaluate the efficacy of GW559090 (10 in vehicle and 9 in experimental group).

2.4. Real-time PCR

At the end of study period (3 weeks) corneas (one per mouse) and cervical lymph nodes were harvested from mice in each treatment groups. These are described in previous section with specific numbers per group. Total RNA was isolated from harvested tissues using RNA STAT-60 kit (Tel-Test, Inc., Friendswood, TX) according to the manufacturer's instructions. cDNA was synthesized by reverse transcribing RNA using oligo dT and M-MLV RT (Promega, Madison, WI). SYBR green real-time PCR assay was used