Tolerability of Topical Tocilizumab Eyedrops in Dogs: A Pilot Study

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Abstract

Purpose: The aim of this study was to examine the tolerability of topical tocilizumab eyedrops in normal dogs and to assess whether this preparation alters tear film cytokine levels or conjunctival cytokine mRNA expression.

Methods: Two percent tocilizumab eyedrops were administered to the right eyes and artificial tears to the left eyes of 10 dogs with no anterior segment pathology 3 times daily for 4 weeks. Portable slit-lamp examinations and Schirmer tear testing were performed at baseline and day 1 week 4, as well as 2 weeks after treatment (week 6). Schirmer strips were also used to collect tears for analyses of cytokine levels using multiplex bead array. Median levels of 8 cytokines in the tear film [interferon gamma (IFN- γ), tumor necrosis factor (TNF)- α , interleukin (IL)-1 α , IL-1 β , IL-2, IL-6, IL-8, and IL-10] were compared among tocilizumab-treated and control eyes. Conjunctival biopsies from both eyes were collected at week 4, and mRNA levels of cytokines were also evaluated. Blood samples were collected at baseline and at the end of treatment to monitor for changes in complete blood count, basic metabolic panel, or liver function tests.

Results: At week 4, conjunctival biopsies and tear samples showed no significant differences in either tear cytokine or mRNA levels for IFN- γ , TNF- α , IL-2, IL-6, IL-8, and IL-10. There was no evidence of local irritation or changes in bloodwork results from the topical tocilizumab formulation.

Conclusions: Topical application of tocilizumab eyedrops was well tolerated when used on healthy dog eyes in this pilot study.

Keywords: dry eye, tocilizumab, topical, eyedrops, canine, dog, interleukin-6

Introduction

DRY EYE OR KERATOCONJUNCTIVITIS SICCA (KCS) is highly prevalent, especially among women, and has been shown to have an adverse impact on vision-related quality of life.^{1–4} In addition, severe KCS can lead to corneal breakdown, infection, and permanent scarring.⁵ Although the etiology of dry eye is multifactorial,¹ inflammation of the ocular surface plays a key role in the pathogenesis of the disease and involves the complex interaction of various cytokines and cells on the ocular surface.

Inflammation of the ocular surface is a complex process that results in secretion of various cytokines and downstream effects.⁶ Animal studies indicate that inflammatory cytokines play a key role in the pathogenesis of KCS. For example, experimental dryness significantly increases the expression of interleukin (IL)-2, IL-6, and tumor necrosis factor (TNF)

transcripts in the corneal epithelium and conjunctiva of C57BL/6 mice.^{7,8} Similarly, several studies in dry eye patients have detected increased concentrations of numerous proinflammatory cytokines and chemokines in the tear fluid, such as IL-1, IL-6, and TNF- α .^{9–13} Significantly increased levels of IL-1, IL-6, IL-8, TNF, and transforming growth factor (TGF) RNA transcripts were found in the conjunctival epithelium of Sjogren's syndrome patients compared with controls, pointing to the conjunctiva as one possible source of inflammatory cytokines in the tear film.¹⁴

Selectively blocking key inflammatory cytokines on the ocular surface using an immunobiologic approach is a potential strategy for the development of new therapies for KCS.¹⁵ Tocilizumab, a monoclonal antibody that inhibits the IL-6 receptor,^{16,17} is an FDA-approved systemic medication for the treatment of rheumatoid arthritis and juvenile idiopathic arthritis.¹⁸ Tocilizumab binds both the soluble (sIL-

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6R) and transmembrane IL-6 receptors (IL-6R), thereby blocking IL-6-mediated signal transduction.¹⁹ Blockade of IL-6 on the ocular surface theoretically could result in decreased inflammation, thereby providing a novel strategy for the treatment of KCS. Adverse events that have previously been reported with intravenous systemic tocilizumab have included infections, infusion reactions, and gastrointestinal perforation.²⁰ However, the tolerability of topical tocilizumab eyedrops has not previously been described. Therefore, the purpose of this study was to evaluate the tolerability of topical tocilizumab in dogs and to gain a better understanding of the effect of tocilizumab eyedrops on cytokine levels in the tear film.

Methods

This study was approved by the University of Pennsylvania (Penn) Institutional Animal Care and Use Committee (IACUC). This study was also conducted in accordance with the Association for Research in Vision and Ophthalmology (ARVO) Statement for the Use of Animals in Ophthalmic and Visual Research and was approved by the Local Animal Care and Ethical Committee.

Tocilizumab eyedrops

Two percent tocilizumab eyedrops were compounded from sterile intravenous solution (Actemra; Genentech, San

TABLE 1. CYTOKINE LEVELS, mRNA LEVELS, AND SCHIRMER TEAR TEST IN TREATED (RIGHT) EYES (N=10) COMPARED WITH UNTREATED (LEFT) EYES (N=10) AT 4 WEEKS

Marker	Mean (SD) ^a Median (25%, 75%) ^a % Detectable (cytokines only)		Treated eye (compared with untreated eye)			P ^b
	Control eye	Treated eye	Lower	Same	Higher	
Cytokine level in tear film (pg/mL)						
IFN- γ	57.6 (84.1) 32.4 (17.4, 42.5) 80%	104.7 (214.6) 20.3 (8.1, 30.3) 60%	7 (70%)	2 (20%)	1 (10%)	0.07
TNF- α	22.1 (48.9) 4.6 (3.2, 12.8) 100%	31.7 (66.5) 9.0 (8.8, 17.1) 90%	3 (30%)	0 (0%)	7 (70%)	0.34
IL-2	128.4 (295.8) 27.5 (13.4, 64.2) 100%	83.1 (165.2) 29.8 (17.5, 35.1) 100%	3 (30%)	0 (0%)	7 (70%)	0.34
IL-6	125.8 (326.6) 11.7 (8.0, 48.1) 100%	129.7 (351.2) 15.8 (5.5, 33.8) 100%	5 (50%)	1 (10%)	4 (40%)	1.00
IL-8	1,972 (906) 1,757 (1,651, 2,668) 100%	2,830 (1,494) 2,869 (1,672, 3,724) 100%	1 (10%)	0 (0%)	9 (90%)	0.02
IL-10	4.4 (3.1) 4.3 (2.4, 4.7) 50%	19.3 (29.4) 3.5 (1.3, 53.2) 30%	3 (30%)	6 (60%)	1 (10%)	0.63
mRNA from conjunctival biopsy (normalized to GAPDH) ^c						
IFN- γ	0.004 (0.003) 0.004 (0.002, 0.007)	0.006 (0.004) 0.008 (0.002, 0.009)	2 (22%)	0 (0%)	7 (78%)	0.18
TNF- α	0.007 (0.005) 0.007 (0.005, 0.008)	0.009 (0.009) 0.009 (0.004, 0.009)	3 (33%)	0 (0%)	6 (67%)	0.51
IL-1 α	0.028 (0.018) 0.025 (0.018, 0.027)	0.034 (0.015) 0.035 (0.030, 0.038)	2 (22%)	0 (0%)	7 (78%)	0.18
IL-1 β	0.004 (0.003) 0.003 (0.002, 0.005)	0.005 (0.004) 0.006 (0.002, 0.008)	3 (33%)	0 (0%)	6 (67%)	0.51
IL-2	0.007 (0.006) 0.006 (0.001, 0.011)	0.009 (0.008) 0.010 (0.001, 0.015)	5 (56%)	0 (0%)	4 (44%)	1.00
IL-6	0.020 (0.011) 0.021 (0.015, 0.024)	0.023 (0.015) 0.018 (0.012, 0.034)	3 (33%)	0 (0%)	6 (67%)	0.51
IL-8	0.027 (0.026) 0.018 (0.014, 0.025)	0.044 (0.027) 0.037 (0.020, 0.074)	1 (11%)	0 (0%)	8 (89%)	0.04
IL-10	0.005 (0.003) 0.005 (0.002, 0.008)	0.007 (0.004) 0.007 (0.002, 0.010)	2 (22%)	0 (0%)	7 (78%)	0.18
Other						
Schirmer tear test (mm/5 min)	18.6 (3.3) 19.0 (17.0, 20.0)	18.7 (4.7) 19.5 (17.0, 23.0)	5 (50%)	2 (20%)	3 (30%)	0.73

^aAmong those in detectable range.

^bSign test.

^cOne dog had no detectable mRNA in either eye on assay.

Francisco). All topical tocilizumab and control eyedrop preparations were prepared by the Investigational Drug Service (IDS) at the University of Pennsylvania at the start of the study and were stored at 4°C for the duration of the study. Artificial tears (vehicle; Rugby/Major 1.7% polyvinyl alcohol, dibasic sodium phosphate, edetate disodium, monobasic sodium phosphate, purified water, sodium chloride, phosphoric acid, and benzalkonium chloride 0.01%) were used for the control group. The vehicle for tocilizumab was not used for the control group as most of the components are not readily available in sterile and liquid forms and the exact formula is not available. The formulation of topical tocilizumab eyedrops used for these studies is an off-label use and is investigational.

Treatment of dogs

The right eyes of 10 dogs with no anterior segment disease and normal baseline Schirmer tear test (STT) values²¹ were treated with 1% tocilizumab eyedrops 3 times daily for 4 weeks (7:00 AM, 11:00 AM, and 3:00 PM). The left eyes of the same dogs received treatment at the same frequency with artificial tears and served as controls. The dogs represent an outbred population with a common genetic background, and all received the same commercial dog food diet and were handled in the same manner by caretakers. The dogs were maintained in a kennel that runs under a cyclic light environment (7 AM on–7 PM off) with light intensities that vary between 175 and 350 lux.

Safety

Ocular surface examinations were performed using a Finoff transilluminator, a hand-held slit lamp (Kowas SL14) and the STT evaluation (Tear Flo™, HUB Pharmaceuticals, CA). Examinations were performed at baseline, day 1, week 4, and week 6 after the initiation of the treatment, with the examiner masked to the treatment.

Pain assessments were performed daily for the first week and then weekly thereafter in the morning when drops were applied by the caretakers and during scheduled eye examinations by a veterinarian. If any sign of discomfort or inflammation was noted, the dog was immediately brought to the attention of the veterinarian for a full ocular evaluation. Serum complete blood counts, basic metabolic panels, and liver function tests were checked before and immediately after the end of the treatment period to assess for any possible systemic side effects such as decreased blood counts, hyperlipidemia, and transaminemia.²²

Tear film samples

Schirmer tear testing without anesthesia was performed at baseline, week 1, week 2, week 3, week 4, and week 6. The Schirmer strips were also used for tear collection. Immediately after collection, the strips were frozen to –80°C until analyzed by the laboratory. All laboratory analyses for this study were performed by the Human Immunology Core Laboratory at the University of Pennsylvania. The strips were analyzed for levels of the following proinflammatory cytokines: interferon gamma (IFN-γ), tumor necrosis factor alpha (TNF-α), IL-1α, IL-1β, IL-2, IL-6, IL-8, and IL-10 using a previously described multiplex bead array,²³ using the Canine Cytokine Magnetic Bead Panel (Cat No. CCYTMG-90K-PX13; EMD Millipore, Temecula, CA) according to the manufacturer's instructions.

Briefly, Schirmer strips were removed from –80°C and thawed on ice. Elution was performed using sodium chloride, Tween, and assay buffer. Standards were prepared according to the manufacturer's protocol, with a 5 × serial dilution and PBS was used as background media.

Conjunctival biopsy samples

At the 4-week time point, conjunctival biopsy samples were taken from treated and untreated eyes of each dog. The biopsies were taken from the superior bulbar conjunctiva of awake dogs following topical instillation of proparacaine 0.5% (Akorn Incorporated, IL).

The Quantigene Plex 2.0 Assay Multiplex Gene Expression Analysis System (10 Plex, Cat No. QP1010, from Affymetrix, Santa Clara, CA) was used to detect RNA expression in the conjunctival biopsy samples by following the manufacturer's protocol. This system utilizes a hybridization-based assay using the xMAP Luminex magnetic beads and is performed on 96-well plates. The assay is based on direct quantification of RNA targets using xMAP Luminex beads for multiplexing of RNA targets (up to 80) and uses branched DNA (bDNA) signal amplification technology. The array plate was read using the Bioplex 200 Luminex instrument (Bio-Rad, Hercules, CA) and experiments were performed by staff from the Human Immunology Core Laboratory at the University of Pennsylvania. The mean fluorescence intensity was used to measure the gene expression level and the housekeeping gene GAPDH was used as a sample loading control. Messenger RNA (mRNA) levels of the following cytokines were assessed (normalized to GAPDH): IFN-γ, TNF-α, IL-1α, IL-1β, IL-2, IL-6, IL-8, and IL-10, utilizing the appropriate positive and negative controls. The conjunctival biopsy sample from 1 dog did not yield any detectable mRNA results and was therefore not included in the analysis.

Statistical analysis

All statistical computations were performed with SAS 9.4 (SAS Institute, Inc., Cary, NC). Due to highly skewed cytokine levels and to incorporate levels below detection (which were all represented as equal), nonparametric statistics were used. Changes from baseline to week 4 were summarized as higher, lower, or the same and *P* values were

FIG. 1. Tear film interleukin-8 levels (pg/mL) in treated versus untreated eyes. Median levels of interleukin-8 (error bars indicate 25th and 75th percentiles) showed no significant difference between levels in either eye over time.

calculated using a sign test. Differences across weeks 0 to 6 were summarized as median and interquartile ranges, with *P* values being calculated using the Friedman test to address repeated measures on each dog.

Results

Tolerability

The topical tocilizumab eyedrops were well tolerated without any evidence of irritation, pain, abnormal lacrimation, inflammation, or damage of the ocular surface, including conjunctival erythema, chemosis, anterior chamber cell or flare, or corneal ulceration or breakdown. No systemic side effects such as decreased blood counts were observed (data not shown).

Cytokine and mRNA Levels

At 4 weeks after initiation of treatment, conjunctival biopsies and tear samples showed no significant differences in either tear cytokine or mRNA levels for IFN- γ , TNF- α , IL-2, IL-6, and IL-10 (Table 1). By the end of treatment at week 4, IL-8 levels were significantly higher in treated versus untreated eyes as measured either by tear cytokines ($P=0.02$) or mRNA ($P=0.04$), but this difference decreased at week 6 to a level that was not statistically significant (Fig. 1).

Discussion

In the present study, we examined the tolerability and ocular surface effects of topical tocilizumab eyedrops in dogs. We

TABLE 2. TRENDS IN CYTOKINE LEVELS IN TEAR FILM SAMPLES AND SCHIRMER TEAR TEST

Marker	Week	Control		Treated eye	
		Median (25%, 75%)	Friedman's P value	Median (25%, 75%)	Friedman's P value
IFN- γ (pg/mL)	0	17 (<d, 30)	<0.001	5.3 (<d, 28)	0.21
	1	<d (<d, 12)		<d (<d, 1.8)	
	2	<d (<d, 3.9)		<d (<d, 11)	
	3	<d (<d, 8.7)		4.1 (<d, 16)	
	4	26 (13, 38)		7.5 (<d, 24)	
	6	2.4 (<d, 5.0)		3.3 (<d, 34)	
TNF- α (pg/mL)	0	6.7 (1.6, 9.0)	1.00	3.5 (<d, 9.5)	0.04
	1	6.2 (<d, 10)		3.3 (<d, 11)	
	2	8.0 (2.0, 14)		6.8 (3.1, 19)	
	3	5.6 (1.6, 11)		3.7 (<d, 8.8)	
	4	4.6 (3.2, 13)		8.9 (1.6, 17)	
	6	4.2 (1.6, 21)		12 (5.4, 93)	
IL-2 (pg/mL)	0	26 (15, 44)	0.66	18 (8.5, 41)	0.10
	1	19 (8.7, 26)		12 (3.9, 26)	
	2	21 (13, 30)		20 (12, 23)	
	3	14 (8.5, 24)		17 (11, 29)	
	4	28 (13, 64)		30 (17, 35)	
	6	13 (6.7, 84)		63 (19, 238)	
IL-6 (pg/mL)	0	12 (6.8, 16)	0.50	10 (4.3, 21)	0.07
	1	5.7 (<d, 14)		4.3 (<d, 16)	
	2	8.7 (4.3, 21)		8.8 (6.1, 26)	
	3	3.8 (<d, 7.5)		4.7 (2.4, 22)	
	4	12 (8.0, 48)		16 (5.5, 34)	
	6	7.3 (3.6, 35)		53 (14, 450)	
IL-8 (pg/mL)	0	1,780 (1,088, 3,632)	0.15	2,332 (1,078, 2,665)	0.43
	1	2,971 (1,778, 6,555)		2,819 (2,075, 5,032)	
	2	2,277 (1,766, 4,048)		2,935 (1,924, 3,655)	
	3	2,525 (1,998, 3,068)		2,111 (1,572, 3,435)	
	4	1,757 (1,651, 2,668)		2,869 (1,672, 3,724)	
	6	3,167 (1,737, 3,388)		3,215 (2,827, 4,536)	
IL-10 (pg/mL)	0	<d (<d, <d)	0.09	<d (<d, <d)	0.33
	1	<d (<d, <d)		<d (<d, <d)	
	2	<d (<d, 0.19)		<d (<d, <d)	
	3	<d (<d, 0.060)		<d (<d, <d)	
	4	0.65 (<d, 4.3)		<d (<d, 1.3)	
	6	<d (<d, 1.6)		0.32 (<d, 24)	
Schirmer tear test (mm/5 min)	0	18 (16, 23)	0.09	19 (15, 20)	0.34
	4	19 (17, 20)		20 (17, 23)	
	6	22 (19, 23)		20 (17, 23)	

Median levels and interquartile levels of cytokine levels in tear film samples and Schirmer tear test results over the course of the study. <d indicates below level of detection.

found that our formulation of 1% tocilizumab eyedrops appeared to be well tolerated after 4 weeks of treatment without signs of local ocular toxicity or discomfort. In addition, the topical preparation did not cause any systemic changes in serum basic metabolic panels, liver function tests, or complete blood counts. To our knowledge, this is the first study examining the effects of topical tocilizumab eyedrops on the ocular surface in dogs.

After 4 weeks of treatment, there was no significant difference in STT values when comparing treated versus control eyes. This was an expected outcome as at baseline, the dogs had normal STT values, as opposed to dogs with KCS who have low STT values (Table 2). Because others have found evidence of increased production of proinflammatory cytokines such as IL-1, IL-6, IL-8, TGF- β 1, and TNF- α on the ocular surface of KCS patients,^{10–12,24} we hypothesized that topical tocilizumab treatment would decrease tear film and conjunctival mRNA levels of IL-6 and other inflammatory cytokines.^{16–19}

However, we found no significant differences between treated and control eyes in the majority of tear film cytokine levels or mRNA levels as determined from conjunctival biopsies (IFN- γ , TNF- α , IL-1 α , IL-1 β , IL-2, IL-6, and IL-10) at the end of the treatment period. Interestingly, an unexpected finding was that there was a statistically significant difference in the level of IL-8 in treated eyes compared with untreated eyes at 4 weeks, and this difference decreased after treatment was stopped. One possible explanation for this finding is that while tocilizumab binds to both the soluble and transmembrane IL-6 receptors, it does not impede signaling of other IL-6 family cytokines.¹⁹ It is possible that through partially blocking a subset of IL-6 receptors, tocilizumab triggered some reactive inflammation on the ocular surface, resulting in an increase in IL-8 in the tear film. Alternatively, as there was significant variability in cytokine levels over the course of the study, it is possible that this finding reflected this variability and is not an actual treatment effect. Additional larger studies will be needed to address this question.

There is evidence that IL-6, along with other cytokines, is elevated in dry eye patients, who therefore are potential targets for new therapies. For example, a recent study found elevated levels of IL-6, IL-8, and TNF- α in dry eye patients compared with controls.¹⁴ Similarly, another study found elevated cytokines in the tear film of patients with moderate evaporative dry eye disease.²⁵ The authors in that study also found that interleukin-1 receptor antagonist (IL-1ra), IL-6, IL-8, and epidermal growth factor levels correlated with pain and clinical parameters. There is also evidence from a mouse model that IL-6 may promote the development of T helper 17 (Th17) cells, resulting in consequent Th17 regulator T cell imbalance, which could play a role in the pathogenesis of autoimmunity.¹⁶ However, the exact role of each of these cytokines in the pathogenesis of KCS has not been fully elucidated and further studies are needed.

Our results should be interpreted in light of the limitations of our study, which include our small sample size, and the fact that the dogs used in this study did not have KCS. In addition, while it has previously been reported that anti-human IL-6 monoclonal antibodies show cross-reactivity with canine IL-6²⁶ and that there is a high degree of IL-6 gene sequence similarity across species, including dogs,²⁷ to our knowledge, no studies have directly shown that tocili-

zumab cross-reacts with the canine IL-6 receptor. Future larger studies that specifically examine the cross-reactivity of tocilizumab with canine IL-6 receptors and involve longer treatment periods in dogs with DED will be necessary to further examine the effect of topical tocilizumab eyedrop treatment on the ocular surface.

The identification and targeting of key cytokines involved in the pathogenesis of DED are new areas of research that may result in the discovery of new therapies. To our knowledge, we are the first to study the local and systemic tolerability of topical tocilizumab eyedrops in dogs without DED. Future larger studies in dry eye animal models and humans are needed to define the potential utility of blockade of IL-6 on the ocular surface for the treatment of DED.

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Author Disclosure Statement

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