The Pharmacologic Assessment of A Novel Lymphocyte Function-Associated Antigen-1 Antagonist (SAR 1118) for the Treatment of Keratoconjunctivitis Sicca in Dogs

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Purpose. Keratoconjunctivitis sicca (KCS) is characterized by inflammation and decreased production of tears containing increased levels of cytokines. The release occurs in the setting of conjunctival and lacrimal gland inflammation, potentially mediated by the interaction between lymphocyte function–associated antigen (LFA)-1, a cell surface protein found on lymphocytes, and its cognate ligand intercellular adhesion molecule (ICAM)-1. SAR 1118 is a novel LFA-1 antagonist and may be an effective therapeutic agent for the treatment of KCS. The following studies were performed to assess the in vitro activity of SAR 1118 and to evaluate the clinical efficacy of topical SAR 1118 for the treatment of idiopathic canine KCS.

Method. Pharmacodynamics were assessed by measuring the ability of SAR 1118 to inhibit Jurkat T-cell binding with recombinant human ICAM-1 and to inhibit cytokine release from human peripheral blood mononuclear cells (PBMCs) stimulated by staphylococcal enterotoxin B. For the assessment of clinical efficacy, 10 dogs diagnosed with idiopathic KCS were treated with SAR 1118 1% topical ophthalmic solution three times daily for 12 weeks. Schirmer's tear test (STT) was used to measure tear production.

Results. SAR 1118 demonstrated concentration-dependent inhibition of Jurkat T-cell attachment, inhibition of lymphocyte activation, and release of inflammatory cytokines, particularly the Th1, Th2, and Th17 T-cell cytokines IFN-γ, IL-2, and IL-17F, respectively. Mean STT values increased from 3.4 mm during week 1 to 5.8 mm at week 12 ($P < 0.025$). No SAR 1118-related adverse events were observed.

Conclusions. SAR 1118 appears to be an effective anti-inflammatory treatment for KCS. Additional studies are warranted to establish the efficacy of SAR 1118 for the treatment of KCS in humans. (*Invest Ophthalmol Vis Sci.* 2011;52:3174–3180) DOI: 10.1167/iovs.09-5078

Keratoconjunctivitis sicca (KCS) is an inflammatory condition characterized by decreased tear production. It occurs in humans and some animal species including dogs. The etiologies of KCS include autoimmune disease, drug toxicities, and autonomic denervation.[1–3] In humans, KCS is a common manifestation of Sjögren's syndrome.[4] Certain dog breeds are prone to a condition similar to human Sjögren's syndrome that causes spontaneous KCS.[1,5,6] In these dogs, KCS occurs secondary to an immune-mediated destruction of lacrimal tissues.[7,8]

Histologic examination of conjunctival epithelia of dogs and humans with KCS has revealed an increased number of lymphocytes that can form foci surrounded by areas of active inflammation. This infiltration may be responsible for the decreased density of conjunctival goblet cells and disrupted architecture of the affected lacrimal glands.[9] Together, this phenomenon leads to the mucopurulent ocular discharge, reduced tear volume, and hyperosmotic tear production that are commonly observed in dogs with KCS.[1,5,6]

Lymphocyte function–associated antigen (LFA)-1, a cell surface protein found on lymphocytes, mediates the cell-to-cell interactions essential for immune responses and inflammation[10] via an engagement with its cognate ligand, intercellular adhesion molecule (ICAM)-1, in diseased tissue. Inflammation involving specific $CD4^+$ lymphocytes (T-cells) appears to play an important role in the etiology of KCS.[11] It is also characterized by the upregulation of ICAM-1 expression in periocular tissues including the conjunctiva and lacrimal glands and the infiltration and proliferation of $CD3^+$ T-cells in affected tissue.[9,12,13] The infiltrating T-cells have been predominantly characterized as Th1 and Th17 cells, whose presence may reflect a pathologic suppression of Treg cells.[14,15]

Cyclosporine, a calcineurin antagonist and a well-recognized T-cell-modulating agent, has been successfully used for the treatment of canine KCS for many years.[16–18] Supporting the concept of a common pathogenic mechanism for canine and human KCS, cyclosporine has also been shown to increase tear production in human patients.[19,20] More recently, the T-cell inhibitors tacrolimus[21] and pimecrolimus[22] have been evaluated for the treatment of canine KCS.

Immune stimulation via LFA-1 has been shown to mediate many T-cell-dependent immune functions, including the adhe-

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sion of T-cells to endothelial and epithelial cells, T-cell migration into inflamed tissue, T-cell activation, T-cell cytokine release, T-cell-dependent B-cell proliferation and target cell lysis.[10] This interaction is specific to leukocyte infiltration and activation, which leads to the normal immune responses and inflammation; however, inappropriate T-cell infiltration and chronic inflammation in ocular tissues may contribute to the clinical signs and symptoms of KCS. SAR 1118 is a novel small molecule capable of inhibiting the association of LFA-1 with ICAM-1 and may therefore be an effective therapeutic agent for the treatment of KCS.

The following pharmacologic studies were performed to assess the activity of SAR 1118 with respect to the inhibition of LFA-1/ICAM-1 binding, T-cell adhesion to ICAM-1 and T-lymphocyte activation in vitro, and the safety and ocular penetration of SAR 1118 in healthy dogs in vivo. In a preliminary evaluation of the clinical efficacy of topical SAR 1118 drops on tear production, signs of corneal surface disease, and inflammatory cell infiltration into conjunctival biopsies were studied in a series of dogs with idiopathic KCS.

Methods

In Vitro Pharmacodynamics

Inhibition of Jurkat T-Cell Attachment. To evaluate the ability of SAR 1118 to inhibit the attachment of Jurkat cells to intercellular adhesion molecule (ICAM)-1 in vitro, we prepared 100-mM stock solutions of SAR 1118, and a positive control was prepared in an aqueous solution of dimethylsulfoxide (1:1) and then diluted by adding assay medium to achieve and maintain the desired concentration throughout the assay. The reported LFA-1 antagonist compound 4[23,24] was used as the positive control. The Jurkat cells were labeled with an 8-μM solution of 2′,7′-bis-(2-carboxyethyl)-5-(and-6)-carboxyfluorescein in growth medium at room temperature for 15 minutes. Labeled cells were incubated in 70 μL of assay medium in each well of a 96-well plate at 500,000 cells per well with 70 μL of SAR 1118 or the positive control in assay medium at 37°C for 30 minutes. A 100-μL aliquot of this fluorescence-labeled Jurkat cell suspension was allowed to settle in the presence of SAR 1118 or the positive control in wells of a 96-well plate coated with recombinant human ICAM-1 expressed as an Fc chimera (R&D Systems, Minneapolis, MN) at 37°C for 1 hour. A dose titration of each test compound was run (10^{-11} –10^{-6} M) in two separate lanes on a single plate, and the assay was replicated on a second, separate plate (Fig. 1). Nonadherent cells were removed by washing and centrifugation at 100 g for 1 minute. Adherent cells were quantitated as the intensity of adherent fluorescence, and the IC_{50} values were calculated by using a standard four-parameter logistic nonlinear regression analysis of the data (GraphPad Software Inc., San Diego, CA). The reported IC_{50} is the average of data for two plates.

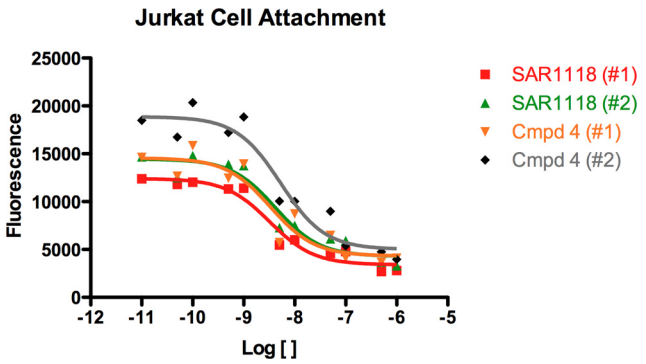

Figure 1. SAR 1118 demonstrated concentration-related inhibition of Jurkat cell attachment to ICAM-1 coating a microtiter plate. The concentration response curve and IC_{50} of SAR 1118 in this assay was comparable to that of the known direct competitive LFA-1 antagonist, compound 4.[20]

Inhibition of Cytokine Release by Activated Lymphocytes. To evaluate the ability of SAR 1118 to inhibit the release of cytokines from stimulated human mononuclear cells (PBMCs), we prepared stock solutions of SAR 1118, cyclosporine, and the superantigen staphylococcal enterotoxin B (SEB) in culture medium. SEB stimulation with vehicle (0.25% DMSO/medium) served as the positive control, and cells without SEB stimulation served as the negative control. Human PBMCs frozen in a cryopreservative were thawed, washed with RPMI culture medium containing 10% fetal bovine serum in growth medium, and seeded onto a 96-well plate at 20,000 cells/well containing 180 μL of culture medium. The cells were incubated in the presence of SAR 1118 or cyclosporine at 37°C for 1 hour before and after stimulation with SEB 1 ng/mL (IL-2, IFNγ, MIP-1α, and TNFα) or 10 ng/mL. Supernatants from SEB-stimulated cells were harvested at 6 (IL-2, IFNγ, MIP-1α, and TNFα), 16 (IL-4 and IL-10) and 48 (IL-1 and IL-17) hours. Cytokine levels in the assay supernatants were determined using a multiplex assay (Luminex; Invitrogen Corporation, Carlsbad, CA), and IC_{50} values were calculated with a standard four-parameter logistic nonlinear regression analysis of the data (GraphPad Software Inc.).

In Vivo Ocular Penetration of Topical SAR 1118

To assess ocular penetration, a solution of radiolabeled SAR 1118 was prepared in a sodium bicarbonate and preservative solution composed of methylparaben, propylparaben, edetate disodium, sodium chloride, mono- and dibasic phosphates, and sterile water. The pH was adjusted to 6.32 to 6.83 with dilute solutions of sodium hydroxide and hydrochloric acid and passed through a 0.22-μm filter before administration. The radiolabeled test article used was ^{14}C-SAR 1118 (PerkinElmer Life and Analytical Services, Waltham, MA) with a mean specific activity of 19.4 μCi/mg, once formulated.

The study drug was administered in 30-μL aliquots containing 3 mg of SAR 1118, which was instilled into the cul-de-sac of both eyes of 6- to 7 month-old male (n = 5) and female (n = 5) beagles (Covance Research Products, Inc., Kalamazoo, MI). The upper and lower eyelids were then gently held together for approximately 45 to 60 seconds to limit the loss of material and distribute the dose across the eye. Each animal was restrained for approximately 1 to 2 minutes to prevent it from rubbing its eyes and an Elizabethan collar was placed on each animal for 2 hours.

Tissues were collected from one animal of each gender at 0.5, 2, 8, 12, and 24 hours after the drug was administered. The animals were euthanized via exsanguination under pentobarbital sodium anesthesia. Both eyes were enucleated from each animal, and samples of the following tissues were weighed and stored on dry ice: aqueous humor, choroid (including retinal pigmented epithelium), ciliary body, bulbar and palpebral conjunctiva, cornea, extraocular muscle, iris, lens, optic nerve, retina, sclera, and vitreous humor.

All samples were analyzed for radioactivity in liquid scintillation counters (Model 2900TR; Packard Instrument Company, Meriden, CT) for at least 5 minutes or 100,000 counts. All samples were analyzed in duplicate if sample size allowed. If results from sample duplicates (calculated as ^{14}C dpm/g sample) differed by >10% from the mean value, the sample was rehomogenized and reanalyzed. This 10% specification was met for all sample aliquots that had radioactivity greater than 100 dpm.

Four-Week Tolerability Assessment in Normal Beagles

Study Drug. SAR 1118 1%, 3%, and 10% ophthalmic drops were prepared as the sodium salt in individual 2.5-mL dropper bottles. The solution was buffered with sodium phosphate at pH 7.0, and sodium chloride was used to adjust the concentration to 290 mOsM/L. The filled dropper bottles were protected from light and refrigerated at 2°C to 8°C (36–46°F) before use.

Procedure. SAR 1118 ophthalmic solution was administered to three beagles (Covance Research Products, Inc.) in a dose-escalating study design. Initially, the study drug was administered as a 1% solution three times daily (TID) in an 8-hour period for 14 days followed by a 3-day washout. The washout was followed by 7 days of 3% solution TID with a 3-day washout and finally a 4-day dose regimen with 10% SAR 1118 drops administered TID. Dogs were examined daily at the first dose of the day by a board certified veterinary ophthalmologist using a slit lamp biomicroscope to look for signs of discomfort and effects on the ocular surface. Tear samples were taken on preweighed Schirmer strips at 1, 2, 4, 8, and 24 hours after the first dose of the day on days 1, 7, 14, 17, 24, and 31. SAR 1118 was eluted from the Schirmer strips, and tear levels were quantified by LC/MS/MS.

Thirteen-Week Tolerability Assessment in Normal Beagles

Normal beagles were treated with vehicle ($n = 10$), 0.3% ($n = 6$), 1% ($n = 6$), or 3% ($n = 10$) SAR 1118 ophthalmic drops TID for 13 weeks, to establish the safety, tolerability, and pharmacokinetics of SAR 1118 ophthalmic drops. The animals were evaluated in the predose phase; on days 1, 8, 15, and 29 of the dosing phase; on weeks 9 and 13 of the drug administration phase; and on day 28 of the recovery phase, for changes in ocular irritation by slit lamp biomicroscopy (model SL-14; Kowa Company Ltd., Tokyo, Japan), corneal fluorescein staining (Flu-Glo Fluorescein Sodium Ophthalmic Strip, USP; 1.0 mg; Akorn Inc., Fair Lawn, NJ), conjunctival staining, applanation tonometry (Tono-Pen Vet; Reichert Ophthalmic Instruments, Depew, NY), indirect ophthalmoscopy (model 12500; Welch Allyn Skaneateles Falls, NY, with a 28-D condensing lens Nikon, Tokyo, Japan), or STT. The presence and duration of ocular squinting was also noted, beginning with the first daily dose on day 1, continuing through the third daily dose on day 7, and immediately after each of three doses on 1 day weekly thereafter during the dosing phase.

Clinical Efficacy

Study Drug. SAR 1118 was prepared and dispensed as a 1% solution in multiple individual dropper bottles containing 2.5 mL of SAR 1118 as a sodium salt. The solution was buffered with sodium phosphate at pH 7.0, and sodium chloride was used to adjust the concentration to 290 mOsM/L. The filled dropper bottles were protected from light and refrigerated at 2°C to 8°C (36°F–46°F) before use.

Study Animals. Twelve dogs of various breeds (five male, seven female) were voluntarily entered into the study by their respective owners after reviewing and signing an informed consent. To be included in the study, the dogs were required to be more than 1 year of age and were diagnosed with unilateral or bilateral spontaneous keratoconjunctivitis sicca (KCS). Specifically, study eyes were required to have an STT score of <10 mm wetting/min and demonstrate at least one of the following clinical signs: blepharospasm, conjunctival hyperemia, exposure keratopathy (irregular surface), corneal pigmentation, corneal neovascularization, or mucopurulent ocular discharge. The subjects were excluded from the study if they demonstrated systemic disease, facial nerve paralysis, any other form of keratoconjunctivitis, such as congenital, neurogenic, traumatic or toxic keratoconjunctivitis, or had been treated with topical cyclosporine or tacrolimus during the previous 6 months.

Study Procedures

One drop of SAR 1118 1% solution was instilled into the KCS-affected eye TID, with 3 to 5 hours allowed between doses for 12 weeks. Animal owners were shown how to topically administer SAR 1118 during the initial visit with emphasis on delivering drops in a manner that prevented microbial contamination of the solution. Owners were instructed to record the administration of each dose in a diary. Compliance was determined by weighing the dropper bottles(s) to monitor usage at each clinical visit. Owners were also given artificial tears (Genteal GelDrops; Novartis Pharmaceuticals Corporation, East Hanover, NJ) for twice-daily administration. On clinic visit days, both eye drops were withheld until ophthalmic examinations were completed.

Ophthalmic Examination. The animals were examined by a board-certified veterinary ophthalmologist during the initial visit and again during four follow-up clinic visits after 2, 4, 8, and 12 weeks of treatment. The adnexa and anterior portion of the both eyes were examined with a slit lamp biomicroscope and the ocular fundus of both eyes was examined using an indirect ophthalmoscope. The eyes were dilated with a mydriatic when applicable to allow evaluation of the lens and fundus including the retina. Rose bengal and fluorescein dyes were used to assess the eyes for epithelial abnormalities and corneal erosions or ulcers, respectively. A modified McDonald-Shadduck scoring system[25] was used in conjunction with the slit lamp ophthalmic examinations to grade clinical findings during each visit. Intraocular pressure was measured using applanation tonometry (Tono-Pen Vet; Reichert Ophthalmic Instruments, Depew, NY).

Schirmer Tear Test. Tear production of the study eye was the primary study outcome measure and was evaluated using an STT in nonanesthetized dogs during the initial clinic visit and at each of the five follow-up evaluations. This testing was performed before the instillation of any topical agents. At each visit, one strip of STT paper was used for each study eye. The STT paper was placed in the inferior cul-de-sac at the junction between the medial two thirds and lateral one third of the lower lid margin for 60 seconds and the length (in millimeters) of wetting below the notch of the strip was recorded.

Conjunctival Biopsies. Conjunctival biopsies were collected at baseline and again after 12 weeks of treatment from the ventral medial fornix of each eye after application of topical proparacaine and 10% phenylephrine. The repeat biopsy was obtained from a location approximately 1 mm lateral to the initial biopsy. These tissue samples were shipped overnight in formalin fixative at 4°C. They were rinsed, dehydrated, and then embedded in butyl-methylmethacrylate (BMMA) resin and polymerized at 4°C. Semithin 0.5-μm sections were stained with 1% toluidine blue and evaluated for degree of inflammatory cellular infiltration using a 0 to 4 severity scale (0, no infiltration; 4, very severe infiltration).

Statistical Analysis. Statistical analyses were limited to simple expressions of variation, such as mean and SD. Dose tables were compiled with mean and SD values calculated in a spreadsheet program (Excel, ver. 11.01; Microsoft Corporation, Redmond, WA). Radioanalysis data tables were generated by an automated and validated data capture and management system for data collection in absorption, distribution, and excretion studies using a radiolabeled test article (Debra, ver. 5.6.2.60; LabLogic Systems Ltd., Sheffield, UK).

Ethics. The studies described here were approved by the Institutional Animal Care and Use Committees at Covance Laboratories, Inc. and the University of Wisconsin at Madison as an open-label study. Denial of care in a placebo arm with pets recruited from the community was considered unwarranted for this pilot study. The dogs used in this veterinary clinical study were handled in compliance with institutional Animal Care and Use Committee guidelines and with the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research.

Results

In Vitro Pharmacodynamics

Inhibition of Jurkat T-Cell Attachment. SAR 1118 demonstrated concentration-dependent inhibition of Jurkat cell attachment (Fig. 1). The concentration-response curve and 2.98 nM IC_{50} of SAR 1118 in this assay was comparable to that of the known LFA-1 antagonist, compound 4 (IC_{50} 2.39 nM),[23] demonstrating SAR 1118 to be a potent antagonist of LFA-1/ICAM-1–mediated T-cell binding.

Inhibition of Cytokine Release from Activated Lymphocytes. SAR 1118 demonstrated potent concentration-dependent inhibition of superantigen-induced inflammatory cy-

TABLE 1. Inhibition of Human PBMC Stimulation with SEB

	IFNγ*	TNFα*	MIP-1α*	IL-1α	IL-1β	IL-2*	IL-4	IL-6	IL-10	IL-17A	IL-17F
SAR 1118	0.0016	0.076	0.020	0.24	0.36	0.002	0.14	3.9	0.15	200	3
Cyclosporine	0.0005	0.0005	0.001	0.002	0.003	0.001	0.006	0.021	0.029	0.055	0.033

Data are the EC_{50} needed for cytokine release (μM) and were obtained after stimulation with SEB.

* 1 or 10 ng/mL. Shaded columns highlight those cytokines whose presence in tear correlates with the clinical severity of human KCS.[26] SAR 1118 remains at concentrations significantly higher than these EC_{50} values (e.g., >1 μM) for 24 hours after application to the normal dog eye as a 1% solution. Levels of cyclosporine at the cornea surface were measured at 254 ng/g of tissue (0.00021 μM) 12 hours after a twice-daily dose of 0.05% cyclosporine to the normal dog eye.[27]

tokine release from human PBMCs, particularly the Th1 and Th2 T-cell cytokines, IFN-γ and IL-4, respectively (Table 1). The inhibition of Th17 T-cell cytokine release was moderate for IL-17F and poor for IL-17A. The inhibition of lymphocyte activation and cytokine release by SAR 1118 was significant at 1 μM, particularly for IFN-γ, IL-1α, IL-1β, IL-10, and MIP-1α, whose levels in tears of KCS patients correlate with the severity of clinical signs of the disease.[26]

Ocular Penetration of Topical SAR 1118

Radioactivity was found in most ocular tissues examined after topical administration. Representative amounts of radioactivity recovered from the ocular tissue of male dogs are summarized in Table 2. The highest average concentrations of SAR 1118 (nanogram equivalents ^{14}C-SAR 1118/g) were determined to be in the anterior tissues: bulbar conjunctiva (4510), palpebral conjunctiva (3790), and cornea (2130) after 0.5 hour. The C_{max} in sclera occurred at 0.5 hour. Concentrations of radioactivity in posterior tissues were lower, declined more rapidly, and, with the exception of sclera, generally approached the limit of quantification after 12 hours. After 24 hours, the concentration of radiation had decreased further but was still detectable in most ocular tissues, notably the bulbar and palpebral conjunctiva. Exceptions to this were the choroid, ciliary body, retina, and vitreous humor which remained below the limit of quantification at all time points.

Four-Week Tolerability Assessment in Normal Healthy Beagles

SAR 1118 was well tolerated at concentrations as high as 10% with no apparent adverse effects on the cornea surface or overall ocular health. Drug concentrations in tears did not show any signs of accumulation across days 1, 7, and 14 (1%), days 17 and 24 (3%), or days 29 and 31 (10%) (Fig. 2), although the drug concentration in tears increased with increasing dose strength. SAR 1118 concentrations at the 24-hour time points (trough) were >1 μM for all dose strengths.

Thirteen-Week Tolerability Assessment in Normal Beagles

SAR 1118 was well tolerated by normal beagles at concentrations as high as 3% TID for 13-weeks with no apparent adverse effects on the cornea surface or overall ocular health. In particular, no alterations were noted on McDonald-Shadduck ocular irritation scoring, slit lamp biomicroscopy, indirect ophthalmoscopy, corneal pachymetry, applanation tonometry, or ocular surface fluorescein staining. The principle ocular finding associated with the administration of SAR 1118 was a variable, short-lived (often <5–10 seconds) period of blinking and squinting immediately after instillation, noted on approximately 2.8%, 4.5%, 11.4%, and 18.9% of the observation periods for eyes receiving vehicle control or 0.3%, 1%, or 3% SAR 1118, respectively. The mean duration of blinking and squinting also declined after the first 1 to 2 days of instillation.

Clinical Efficacy

Ophthalmic Evaluation. Of the 12 KCS-affected dogs enrolled in the study, 10 (5 female, 5 male) completed all visits. Two of these 10 dogs had unilateral KCS, and data for 18 study eyes were analyzed. Two of the 12 enrolled dogs were noncompliant and failed to appear for scheduled clinic visits at week 1 and week 12. They were lost to follow-up and subsequently were dropped from the study analysis due to incomplete data. With a modified McDonald-Shadduck scoring system, the eyes in this study exhibited clinical signs frequently associated with KCS at baseline, including conjunctival discharge, conjunctival congestion, and increased corneal opacity (Table 3). In contrast to published reports in of the human disease, these animals demonstrated a complete absence of

TABLE 2. Mean SAR 1118 Concentrations in Ocular Tissue after Topical Administration in Male Dogs

Ocular Tissue	0.5 h	2 h	8 h	12 h	24 h
Aqueous humor	15.2	26.0	21.2	11.7	4.20
Choroid-retinal pigmented epithelium	—	—	—	—	—
Ciliary body	—	—	—	—	—
Conjunctiva (bulbar)	4510	1280	884	1920	2170
Conjunctiva (palpebral)	3790	1670	1560	4530	1040
Cornea	2130	1510	1240	690	498
Extraocular muscle	111	73.1	19.3	734	171
Iris	—	250	178	189	—
Lens	1.33	2.38	2.23	4.01	2.86
Optic nerve	—	42.7	—	17.6	—
Retina	—	—	—	—	—
Sclera	295	175	106	82.3	108
Vitreous humor	—	—	—	—	—

Each value represents the mean of two eyes of one animal. Values expressed as nanogram equivalents of ^{14}C-SAR1118/g. No value indicates that the level of radiation was below the limit of quantification.

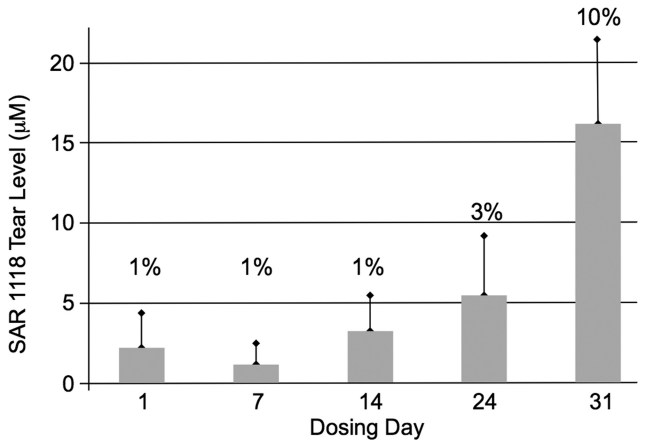

FIGURE 2. During a dose-escalation study, SAR 1118 concentrations in tear did not accumulate after administration of a 1% solution three times daily for 14 days followed by a 3-day washout period, a 3% solution three times daily for 7 days followed by a 3-day washout period, and a 10% solution three times daily for 4 days.

fluorescein corneal staining abnormalities at baseline. Rose bengal staining was also normal in all animals, except for transient stippling in two and persistent stippling in another. Consequently, it was not possible to observe improvements in corneal staining during the study. One corneal ulcer developed in one study eye during the course of treatment, which resulted in an increase in corneal fluorescein staining. This ulcer responded to treatment with triple antibiotic ointment and was judged to be unrelated to SAR 1118 treatment. Qualitative attenuation of ocular inflammation was observed in some animals (Fig. 3); however, only minor changes in McDonald-Shadduck scores were observed relative to baseline in conjunctival discharge, conjunctival congestion, and corneal opacity in the time frame of the study and were not statistically significant using a paired *t*-test (Table 3).

No clear and consistent pattern of IOP fluctuation was noted during treatment, nor did any dog develop any ocular changes suggestive of IOP abnormalities.

Schirmer Tear Test. At baseline, 9 of the 18 study eyes had STT values of 0 mm and 9 had STT wetting >0 mm. Mean STT values for all 18 KCS study eyes increased 2.4 mm (to 5.8 mm) at week 12 from a mean of 3.4 mm at baseline ($P < 0.025$; paired *t*-test; Table 4). SST values improved from baseline at week 12 in 11 of the study eyes, and STT values were unchanged or worsened for the remaining seven study eyes in this timeframe. An overall mean STT improvement of 4.8 mm was observed in the 11 improved eyes from six animals at week 12 (9.5 mm) versus baseline (4.7 mm). Five of the remaining seven STT nonresponding study eyes were from three dogs with baseline SST values of 0 mm. The conjunctival biopsies from two nonresponding dogs exhibited significantly stratified conjunctival epithelium, suggestive of a more advanced disease state or a different disease. In 4 of the 11 responding eyes, the STT at baseline was 0 mm and increased to 3, 4, 7, and 8 mm at week 12. In the parallel control group of normal dogs, 1% SAR 1118 did not increase STT values across 3 months of TID doses.

Conjunctival Biopsy. Conjunctival biopsies were stained with toluidine blue and analyzed with a semiquantitative histologic assessment of inflammation as defined by a degree of cellular infiltration into tissue on a 0 to 4 scale (Table 5). Using this method, an improvement was seen in the histologic scores for the 18 study eyes from 2.22 at baseline to 1.90 at week 12. This trend in anti-inflammatory improvement was most dramatic among the 11 responding study eyes with improved STT scores. The mean scores of these eyes improved from 2.54 at baseline to 2.0 at week 12 and approached statistical significance ($P = 0.07$) after 12 weeks of treatment. This improvement in the histologic assessment in this STT responding group could reach $P = 0.05$ with the addition of one more responding study eye ($n = 12$) exhibiting the observed ($n = 11$) mean histologic improvement from 2.5 to 2.0. There was no change in the histologic scores of any of the seven nonresponding study eyes that exhibited no change or worsening of STT values at week 12 relative to baseline. Attempts to more accurately quantitate specific leukocyte markers (e.g., CD3) on infiltrating inflammatory cells were unsuccessful because of an unacceptable level of nonspecific binding with commercially available reagents in tests of diseased and normal canine tissues.

DISCUSSION

KCS in humans is characterized by upregulation of ICAM expression and the infiltration and proliferation of $CD3^+$ T-cells in periocular tissues, including the conjunctiva and lacrimal gland.[9,12,13] This infiltration of T-cells results in chronic inflammation with reduced tear volume or hyperosmotic tear composition and ultimately damages the corneal surface of the eye. SAR 1118 is a potent inhibitor of LFA-1 binding to ICAM-1, human T-cell attachment to ICAM-1 and the release of inflammatory cytokines from activated human lymphocytes. As such, it may offer a therapeutic modality that regulates T-cell cytokines IFN-γ, IL-2, IL-4, IL-17, and other inflammatory mediators whose presence correlates with the severity of clinical signs in human KCS.[26] It should be noted that, in this study, SAR 1118

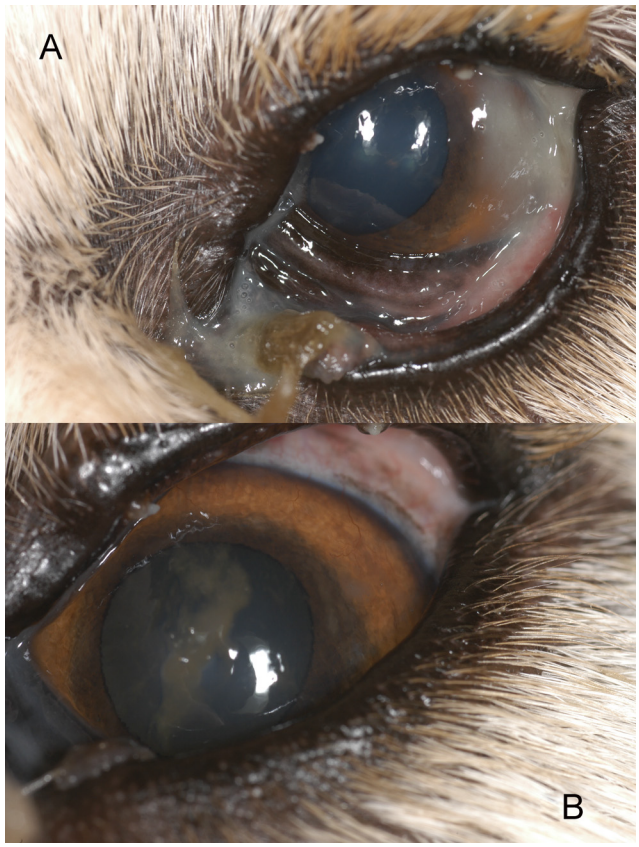

FIGURE 3. The attenuation of (**A**) baseline inflammation associated with the ocular application of SAR 1118 (**B**) after 1 week of treatment is evident in this study eye.

TABLE 3. Modified McDonald-Shadduck Test Results*

	Baseline	Wk 2	Wk 4	Wk 8	Wk 12
Conjunctival discharge	2.4/2.1 (0.7/1.1)	2.4/2.0 (0.7/1.0)	2.2/2.0 (0.7/1.0)	2.3/2.0 (1.7/1.2)	2.4/2.1 (1.0/1.1)
Conjunctival chemosis	0.2/0.1 (0.7/0.3)	0.3/0.2 (0.7/0.4)	0.1/0.1 (0.3/0.3)	0.1/0.1 (0.3/0.3)	0.1/0.1 (0.3/0.3)
Conjunctival congestion	1.4/1.1 (0.9/0.8)	1.3/1.0 (0.9/0.7)	1.3/1.2 (0.7/0.8)	1.2/1.1 (0.7/0.7)	1.1/0.9 (0.8/0.8)
% Area of corneal opacity	1.8/2.0 (1.6/1.6)	1.9/2.0 (1.5/1.6)	1.9/1.7 (1.5/1.6)	1.8/2.0 (1.3/1.6)	1.6/1.8 (1.2/1.6)
Corneal opacity	1.6/1.4 (1.3/1.2)	1.7/1.4 (1.2/1.2)	1.6/1.4 (1.2/1.3)	1.6/1.1 (1.2/1.3)	1.6/1.1 (1.2/1.)
Corneal stain (fluorescein)	0.0/0.0 (0.0/0.0)	0.0/0.0 (0.0/0.0)	0.2/0.02 (0.4/0.4)	0.2/0.2 (0.4/0.4)	0.2/0.2 (0.4/0.4)
% Area of corneal stain	0.0/0.0 (0.0/0.0)	0.0/0.0 (0.0/0.0)	0.2/0.2 (0.4/0.4)	0.2/0.2 (0.4/0.4)	0.2/0.2 (0.4/0.4)

* Data are the mean results OD/OS (SD) and do not include uninvolved eyes from two animals with unilateral KCS. $n = 18$.
† No significant change ($P < 0.05$, paired *t*-test) for any weekly OD/OS values vs. baseline value.

inhibited the superantigen SEB-stimulated secretion of these cytokines at concentrations achieved and maintained in tears after administration of SAR 1118 as an ophthalmic drop. Although IC_{50} values for cyclosporine may be lower than those for SAR 1118 in this in vitro assay, the levels of SAR 1118 in tears observed in this study 24 hours after administration of a 1% solution (i.e., 1 μM) offered equivalent or more significant inhibition of cytokine release than expected from the levels of cyclosporine 12 hours after administration of a 0.05% solution (i.e., 0.2 μM).[27] Furthermore, it should be noted that the level T-cell stimulation leading to cytokine secretion may be less in diseased tissue than is achieved in vitro with the co-incubation of the superantigen SEB. In this study, improvements in a histologic assessment of inflammation from biopsies of responding dogs supports a link between the inhibition of T-cell adhesion and cytokine release observed in vitro and an STT response to treatment in clinically symptomatic dogs with KCS.

The results of this preliminary clinical veterinary study suggest that SAR 1118 is safe and increased tear production when used for the treatment of spontaneous KCS in dogs when applied topically TID as a 1% solution. This treatment regimen produced sustained drug levels in tears above 1 μM. In this study, treatment with SAR 1118 improved the mean STT scores in 18 study eyes by 2.4 mm/eye/min at 12 weeks and reached statistical significance ($P < 0.05$) within the first 2 weeks of treatment. Eleven of the 18 study eyes exhibited an increase in STT values across the treatment period and the mean STT of this responding group increased by 4.8 mm/eye/min. This improvement in mean STT was steady across the treatment period and was statistically significant relative to baseline ($P < 0.05$) at weeks 2, 4 and 12 in a paired *t*-test. These results compare favorably with the improvement in STT observed after the treatment of canine KCS with topical cyclosporine 1%[17] and 2%[16] solutions. Improvement in the histologic assessment of conjunctival biopsies for inflammation was noted from 2.5 at baseline to 2.0 at week 12 ($P = 0.07$) in the STT responding population. Although this histologic improvement did not reach statistical significance, the data for this group did strongly trend toward improvement ($P = 0.07$). This result, coupled with the absence of any histologic improvement in the seven study eyes which did not improve in STT values, suggests a link between a reduction in conjunctival inflammatory cell infiltration and an improvement in STT.

The ocular application of a 10% dose of SAR 1118 resulted in relatively high and sustained concentrations of the drug in the cornea and bulbar and palpebral conjunctiva. These data indicate that the ocular application of SAR 1118 is likely to provide therapeutic drug exposure for several hours in humans. SAR 1118 was either undetectable or detected at very low concentrations in the vitreous fluid, indicating that it does not appear to accumulate unexpectedly in or remain long term in the vitreous fluid. Because of the limited absorption of the low ocular doses used, systemic plasma exposure is low in humans, even with multiple ocular doses.[28]

Contrary to the human condition, canine patients with KCS typically present to the ophthalmologist with significantly more advanced disease. Many of the ocular surface changes observed in dogs at initial examination are indicative of chronic illness and do not respond dramatically to therapeutic intervention over short time frames. In this and similar studies of cyclosporine and calcineurin antagonists in dogs, the only clinically and statistically significant improvement among multiple endpoints assessed was the increase in STT values.[16]

The ability of SAR 1118 to inhibit the components of inflammation and immune activation that contribute to the etiology of KCS in addition to improving tear production makes it an attractive candidate as a therapeutic agent for the treatment of KCS in humans.[28]

CONCLUSION

SAR 1118 inhibits LFA-1 attachment to ICAM-1, thereby decreasing the typical inflammatory response. After topical application, SAR 1118 reaches acceptable concentrations in the eye. Preliminary results after the treatment of 10 cases of spontaneous canine KCS suggest that the LFA-1 antagonist SAR 1118 increases tear production and decreases ocular inflammation when applied topically three times daily for 12 weeks. Addi-

TABLE 4. STT Results*

	Baseline	Wk 2	Wk 4	Wk 8	Wk 12
All KCS eyes, $n = 18$	3.4 (3.8)	5.1 (4.7) $P = 0.021$†	4.6 (5.7) $P = 0.089$	5.0 (6) $P = 0.11$	5.8 (6.2) $P = 0.025$
Responding eyes, $n = 11$	4.7 (3.9)	6.7 (5.0) $P = 0.001$	7.3 (5.8) $P = 0.08$	8.1 (5.3) $P = 0.011$	9.5 (4.5) $P = 0.004$
Nonresponding eyes, $n = 7$	1.4 (2.5)	2.6 (3.0)	0.30 (0.8)	0.1 (0.4)	0.0 (0.0)

* Data are the mean length of wetting in millimeters (SD). The mean does not include uninvolved eyes from two animals with unilateral KCS.
† Paired *t*-test.

TABLE 5. Conjunctival Biopsy Histology Score*

	Baseline	Week 12
All study eyes	2.22 (1.21)	1.90 (1.42); $P = 0.08$†
STT-responding study eyes, $n = 11$	2.54 (1.13)	2.0 (1.55); $P = 0.07$

* Data are the mean score (SD). Conjunctival biopsy histology was scored on 0–4 scale of inflammation (inflammatory cell infiltration): 0, no infiltration; 1, mild infiltration; 2, moderate infiltration; 3, severe infiltration; 4, very severe infiltration.

† Paired *t*-test versus baseline.

tional human studies of SAR 1118 for the treatment of KCS are warranted.

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