Title

Impact of topical corticosteroid pretreatment on susceptibility of the injured murine cornea to Pseudomonas aeruginosa colonization and infection

Authors

Wu, Yvonne T
Truong, Tan N
Tam, Connie
et al.

Publication Date

2019-02-01

DOI

10.1016/j.exer.2018.10.010

Peer reviewed

HHS Public Access

Author manuscript

Exp Eye Res. Author manuscript; available in PMC 2020 February 01.

Published in final edited form as:

Exp Eye Res. 2019 February ; 179: 1–7. doi:10.1016/j.exer.2018.10.010.

Impact of topical corticosteroid pretreatment on susceptibility of the injured murine cornea to *Pseudomonas aeruginosa* colonization and infection

Yvonne T. Wu[a], **Tan N. Truong**[a,b], **Connie Tam**[a,1], **Myra N. Mendoza**[a], **Lucia Zhu**[a], **David J. Evans**[a,c], and **Suzanne M. J. Fleiszig**[a,b,d,*]

[a]School of Optometry, University of California, Berkeley, CA, USA

[b]Vision Science Program, University of California, Berkeley, CA, USA

[c]College of Pharmacy, Touro University California, Vallejo, CA, USA

[d]Graduate Groups in Microbiology, and Infectious Diseases & Immunity, University of California, Berkeley, CA, USA

Abstract

Research with animal models of *Pseudomonas aeruginosa* keratitis has shown that use of a topical corticosteroid alone against an established infection can significantly increase the number of colonizing bacteria or worsen clinical disease. Moreover, retrospective analysis has suggested that corticosteroid use in humans is associated with an increased risk of keratitis in eyes with pre-existing disease. Thus, while corticosteroids are often used to reduce ocular inflammation in the absence of infection, the risk of opportunistic infection remains a concern. However, the effect of corticosteroids on the intrinsic barrier function of uninfected corneas is unknown. Here, we tested if short-term topical corticosteroid treatment of an uninfected murine cornea would increase susceptibility to *P. aeruginosa* colonization or infection after epithelial injury. Topical prednisolone acetate (1 %) was administered to one eye of C57BL/6 mice three times a day for 3 days; control eyes were treated with sterile PBS. Prior to inoculation with a cytotoxic *P. aeruginosa* corneal isolate strain 6206, corneas were subject to superficial-injury by tissue paper blotting, or scratch-injured followed by 12 h of healing. Previously we have shown that blotting renders mouse corneas susceptible to *P. aeruginosa* adhesion, but not infection, while 12 h healing reduces susceptibility to infection after scratching. Corneas were evaluated at 48 h for bacterial colonization and microbial keratitis (MK). To monitor impact on wound healing, corneal integrity was examined by fluorescein staining immediately after scarification and after 12 h healing. For both the tissue paper blotting and scratch-injury models, there was no significant difference in *P. aeruginosa* colonization at 48 h between corticosteroid-pretreated eyes and controls. With the

*Corresponding author. Dr. Suzanne M. J. Fleiszig. School of Optometry, University of California, Berkeley, CA 94720, USA. Tel. 1 (510) 643-0990, Fax. 1 (510) 643-5109, fleiszig@berkeley.edu. Authors have no commercial or proprietary interest in any concept or product described herein.

1.Present address. Cole Eye Institute. Cleveland Clinic. Cleveland, OH 44195, USA

blotting model, one case of MK was observed in a control (PBS-pretreated) cornea; none in corticosteroid-pretreated corneas. With the 12 h healing model, MK occurred in 6 of 17 corticosteroid-pretreated eyes versus 2 of 17 controls, a difference not statistically significant. Corticosteroid-pretreated eyes showed greater fluorescein staining 12 h after scarification injury, but this did not coincide with increased colonization or MK. Together, these data show that short-term topical corticosteroid therapy on an uninfected murine cornea does not necessarily enhance its susceptibility to *P. aeruginosa* colonization or infection after injury, even when it induces fluorescein staining.

Keywords

Corticosteroid pretreatment; murine cornea; *Pseudomonas aeruginosa*; bacterial colonization; microbial keratitis; superficial injury; epithelial healing

1. Introduction

Microbial keratitis is a vision-threatening corneal disease most often associated with contact lens wear or ocular injury. Once initiated, it can continue to progress rapidly despite appropriate antimicrobial therapy. *Pseudomonas aeruginosa* is among the most common causes of microbial keratitis associated with contact lens wear and other predisposing circumstances (Chen et al., 2017; Lim et al., 2016; Noureddin et al., 2016). While *P. aeruginosa* expresses an array of virulence mechanisms that allow corneal infection after epithelial injury (Lee et al., 2003a; Zolfaghar et al., 2006, 2003), healthy corneas show a remarkable ability to resist *P. aeruginosa* colonization and infection (Evans and Fleiszig, 2013). Indeed, in murine models, even large inocula of bacterial pathogens such as *Pseudomonas aeruginosa* are rapidly cleared from a healthy cornea (Augustin et al., 2011; Mun et al., 2009). Many factors contribute to defending the corneal epithelium against *P. aeruginosa in vivo* including; secretory IgA (Masinick et al., 1997), surfactant proteins (Alarcon et al., 2011), antimicrobial peptides (Augustin et al., 2011), and mucins (Fleiszig et al., 1994). MyD88, a key adaptor protein of innate defense signaling, also provides an important component of constitutive defense of the corneal epithelium, and its barrier function against *P. aeruginosa* (Metruccio et al., 2017; Reins et al., 2017; Sullivan et al., 2015; Tam et al., 2011).

Corticosteroids are a valuable therapeutic agent for treatment of ocular inflammation (Gan et al., 2005; Holmberg, 1953; Noble and Goa, 1998; O'Gallagher et al., 2017). However, their use is not without potential complications, e.g. risk of opportunistic infection, or elevated intraocular pressure, making judicious prescribing critical. For example, in animal models of *P. aeruginosa* keratitis, corticosteroid treatment alone significantly favored bacterial survival (Badenoch et al., 1985), and was associated with worse clinical disease (Gritz et al., 1990). Retrospective analysis of corticosteroid use in humans has also shown an association with infectious keratitis in eyes with pre-existing disease (Luchs et al., 1997). However, combining corticosteroid treatment with antibiotic therapy for established *P. aeruginosa* keratitis in a murine scarification model did not adversely impact disease resolution (Lee et al., 2003b). Indeed, clinical studies have shown that combined use of a corticosteroid with

antibiotic therapy for active bacterial keratitis may be beneficial for long-term clinical outcomes (Srinivasan et al., 2014; Tallab and Stone, 2016). However, other studies have not shown a benefit for corticosteroids (Blair et al., 2011; Bohigian and Foster, 1977). Indeed, for *P. aeruginosa* keratitis, the benefit of combining a corticosteroid and antibiotic was strain-dependent: corticosteroid inclusion with antibiotic was associated with more improvement in best-corrected visual acuity in patients with ulcers caused by invasive (*exoS* genotype) strains, and less improvement if cytotoxic (*exoU* genotype) strains were involved (Borkar et al., 2013).

It is not known, however, if corticosteroid use affects the intrinsic barrier function of the corneal epithelium against potentially pathogenic bacteria in the absence of prior infection. Since ethical considerations prevent deliberate bacterial inoculation of human subjects, this knowledge gap was explored using mice. Thus, healthy murine corneas were pretreated with topical prednisolone acetate (1 %) 3 times a day for 3 days, then subject to different forms of epithelial injury that partially compromise resistance to *P. aeruginosa*; 1) superficial injury by tissue-paper blotting that allows *P. aeruginosa* to adhere to the cornea without penetrating beyond the epithelial surface (Alarcon et al., 2011), and 2) corneal scarification followed by 12 h of healing time, which allows the cornea time to re-establish resistance to infection (Lee et al., 2003c). Corticosteroid-pretreated eyes were compared to PBS-pretreated controls for fluorescein staining at 12 h, and bacterial colonization and susceptibility to *P. aeruginosa* keratitis 48 h after exposure to a cytotoxic clinical isolate. Results showed that short-term corticosteroid pre-treatment had minimal impact on the ability of the injured murine cornea to resist *P. aeruginosa* colonization and infection under all conditions.

2 Materials and methods

2.1 Bacteria

P. aeruginosa strain 6206 was used (Lee et al., 2003a). Bacterial inocula were prepared by growing bacteria on a trypticase soy agar (TSA) plate overnight for ~16 h at 37 °C, followed by suspension in Dulbecco's Modified Eagle's Medium (DMEM; Sigma-Aldrich, St. Louis, MO) to a concentration of ~10^{11} colony forming units (CFU)/mL or ~10^{8} CFU/mL An absorbance of 0.1 at 650 nm corresponds to ~10^{8} CFU/mL. To confirm higher concentrations, inocula were diluted 1000-fold to achieve an absorbance of 0.1 at 650 nm. Inocula were confirmed by viable counts.

2.2 Mice

Six-week-old female C57BL/6 mice (Jackson Laboratory, Bar Harbor, ME) were used. Mice were anesthetized using a mixture of ketamine, xylazine, and acepromazine (50 mg, 10 mg, and 1 mg/Kg body weight, respectively) before each procedure. Experiments involved between 4 to 17 animals per group and were repeated at least twice. All procedures involving animals were conducted in accordance with the Association for Research in Vision and Ophthalmology Resolution on the Use of Animals in Research and contained within a protocol approved by the Animal Care and Use Committee of the University of California, Berkeley.

2.3 Topical corticosteroid pretreatment

Mice were treated with 5 μL of prednisolone acetate suspension (1 %) (Alcon Laboratories, Fort Worth, TX) or sterile PBS, administered topically onto the surface of the right eye three times a day for three days.

2.4 Tissue paper blotting murine model

Prior to each experiment, murine corneas were checked for health and integrity. Mice were then randomly assigned to one of two study groups; topical prednisolone acetate pre-treatment followed by tissue paper blotting of the cornea, or topical PBS pre-treatment followed by tissue paper blotting. Immediately after pre-treatments concluded, mice were anesthetized, corticosteroid treated eyes were rinsed once with PBS, and each cornea blotted 3 times with 1-ply tissue paper (Kimwipe; Kimberly-Clark, Irving, TX) as previously described (Alarcon et al., 2011). Pretreated eyes were then challenged with 5 μL of *P. aeruginosa* suspension containing ~10^{11} CFU/mL (~5×10^8 CFU/eye). Mice were evaluated with a slitlamp after 12, 24, and 48 h for evidence of visible pathology, with the identity of groups masked from the observer. A diagnosis of microbial keratitis was made by a clinically-trained observer, with the aid of a slit-lamp microscope, and was based on the presence of corneal opacity (Beisel et al., 1983; Lee et al., 2003b). At 48 h, eyes were enucleated, transferred to a glass homogenizer using sterile tweezers, and homogenized in 1 mL PBS containing Triton X-100 (0.25% vol./vol.) to prevent bacterial clumping. The number of viable bacteria per eye was determined by viable counts of the homogenate using TSA plates.

2.5 Scarification and healing murine model

Murine corneas were checked for health and assigned to one of two treatment groups; topical prednisolone acetate treatment or PBS control. Immediately after pre-treatments concluded, mice were anesthetized, corticosteroid treated eyes were rinsed once with PBS then 3 parallel 1 mm wounds incised on the central cornea with a sterile 25-gauge needle (Lee et al., 2003a). Corneas were allowed to heal for 12 h, then challenged with 5 μL of *P. aeruginosa* suspension containing ~10^8 CFU/mL (~5×10^5 CFU/eye). Mice were monitored at 12, 24, and 48 h after bacterial challenge for evidence of disease pathology. The diagnosis of microbial keratitis was made based on detection of corneal opacity (as described above). At 48 h, eyes were harvested and homogenized for viable counting of bacteria on TSA plates. Using the same experimental protocol, but prior to bacterial inoculation, corneal epithelial integrity was examined using 1 μL sodium fluorescein (fluorescein sodium ophthalmic strips, HUB Pharmaceuticals LLC). Fluorescein staining was evaluated using a slit-lamp with a cobalt blue light and a yellow filter, immediately after scarification and after 12 h of healing.

2.6 Statistical analysis

The number of viable bacteria recovered per eye was determined and expressed as a median for each group. The significance of difference between groups was determined using Mann-Whitney U test. The Chi-square test was used to determine if the incidence of *P. aeruginosa*

keratitis was significantly different between groups. $P < 0.05$ was considered significant, and Prism software (GraphPad, La Jolla, CA) was used for analysis.

3. Results

3.1 Effects of corticosteroid pretreatment on corneal susceptibility to P. aeruginosa colonization and keratitis in a superficial injury model

Corneas were treated with topical prednisolone acetate (1 %) three times per day for 3 days then superficially-injured (tissue paper blotted) before bacterial inoculation. Those corneas showed a median *P. aeruginosa* colonization at 48 h similar to PBS-pretreated, blotted controls (Fig. 1) ($P > 0.05$, Mann-Whitney U test). Indeed, an absence of bacterial colonization was observed for some mice in both the control and corticosteroid treated groups (overall bacterial clearance > 99.99 % of the original inoculum). Only one cornea developed an infection, and it was a control that was not corticosteroid-pretreated (shown in Fig. 1). All other corneas in both the control and corticosteroid-pretreated groups remained clear for the entire 48 h follow-up period despite having been superficially-injured by blotting and subsequently challenged with *P. aeruginosa*. Thus, the incidence of microbial keratitis was not significantly different between the groups ($P = 1.0$, Chi-square test).

3.2 Effects of corticosteroid pretreatment on corneal susceptibility to P. aeruginosa colonization and keratitis in the scarification-12 h epithelial healing injury model

In the next experiments, murine corneas were corticosteroid-pretreated as before, but rather than being blotted they were instead scarified and allowed to heal for 12 h before bacterial inoculation. Previously we showed that corneas allowed to heal for ~ 12 h after scarification regained their resistance to MK despite residual fluorescein staining (Lee et al., 2003c). The results showed a trend towards corticosteroid-pretreatment enhancing susceptibility to MK compared to controls (6 of 17 versus 2 of 17, respectively by 48 h) (Fig. 2), This difference was not statistically significant ($P = 0.22$, Chi-square test). Interestingly, bacterial colonization was significantly higher in the corticosteroid-pretreated eyes ($P = 0.02$, Mann-Whitney U test), correlating with the MK rates. For eyes remaining healthy, bacterial colonization levels were similar for the two treatment groups, with bacterial clearance being > 99 % of the original inoculum in both instances.

3.3 Corticosteroid-pretreatment increases corneal fluorescein staining in the 12 h healing model.

Fluorescein staining was used to evaluate the impact of corticosteroid-pretreatment on corneal integrity of some of the healing scratch-injured corneas. After 12 h of healing, only 2 of 10 control group (PBS-pretreated) eyes showed fluorescein staining, compared to 7 of 11 eyes in the corticosteroid-pretreated group ($P = 0.04$, Chi-square test) showing corticosteroid-pretreatment could reduce epithelial healing after scarification injury. Fig. 3 shows the typical appearance of scarification and fluorescein staining at the time of injury (Figs 3A and 3B), and examples of absence (Fig. 3C) and presence (Fig. 3D) of staining 48 h after corticosteroid pretreatment.

In the same experiment, eyes were inoculated with *P. aeruginosa* (same method as Fig. 2). After 48 h, a single case of MK occurred in each group (Fig. 4); both were eyes that had displayed residual fluorescein staining after the 12 h healing period. Mirroring this lack of difference in MK susceptibility, there was also no significant difference in bacterial colonization rates between corticosteroid-pretreated eyes and controls (P = 0.41, Mann-Whitney U test).

4. Discussion

Topical corticosteroids are often used in the treatment of ocular disorders, their powerful anti-inflammatory and immunosuppressive effects being of considerable benefit in facilitating disease resolution. However, a continuing concern with corticosteroid use is the risk of opportunistic infection. In this study, two in vivo murine models were used to examine the impact of short-term corticosteroid pretreatment on corneal susceptibility to P. aeruginosa colonization and infection after injury. Results from both models indicated that 3 days pretreatment of the murine cornea with topical prednisolone (1 %) three times a day, did not significantly impact the susceptibility of injured corneas to P. aeruginosa colonization or microbial keratitis. While corticosteroid pretreatment increased the incidence of corneal epithelial fluorescein staining at 12 h after scarification injury, that did not correlate with susceptibility to P. aeruginosa infection, consistent with our previous work showing that fluorescein staining does not necessarily predict infection risk (Lee et al., 2003c).

Both of the in vivo models we used offer some degree of corneal vulnerability to P. aeruginosa: 1) The tissue paper blotting model induces superficial epithelial injury and fluorescein staining, allowing increased bacterial adhesion, but without enabling bacteria to traverse the epithelium or subsequent infection (Alarcon et al., 2011). The scarification-healing model involves deep corneal injury using a needle followed by healing time (12 h) to allow re-epithelialization and almost full recovery of resistance to fluorescein staining and infection (see Lee et al., 2003c for histological, and other validation of this model). While epithelial defenses mediating corneal resistance to P. aeruginosa infection in these models remain to be fully determined, our previous work with uninjured and superficially-injured murine corneas has shown defenses against infection involve surfactant proteins (Mun et al., 2009), antimicrobial peptides (Augustin et al., 2011; Tam et al., 2012), the innate defense signaling adaptor protein, MyD88 (Tam et al., 2011), and MyD88-dependent receptors (TLR4, TLR5, IL-1R) from corneal epithelial and dendritic cells (Metruccio et al., 2017). MyD88-dependent factors likely include antimicrobials and cytokine signaling (Metruccio et al., 2017; Redfern et al., 2011; Reins et al., 2017; Sullivan et al., 2015). Indeed, a recent study showed a role for keratin-derived antimicrobial peptides, whose upregulation is TLR4-dependent, in defending the murine cornea against bacterial colonization and helping to mediate ocular clearance of bacteria (Chan et al., 2018). Blotting also removes surface epithelial cells, and permeabilizes the remaining surface layer (Jolly et al., 2017). EGTA treatment of blotted corneal epithelia allows subsequent P. aeruginosa traversal (Alarcon et al., 2011; Sullivan et al., 2015) suggesting the involvement of Ca2+-dependent defenses (e.g. epithelial tight junctions, surfactant proteins). Additional factors are also likely to be involved in the re-establishment of corneal epithelial defense against P. aeruginosa at 12 h

after scarification (Lee et al., 2003c), including cell types with defensive and woundhealing functions, e.g. neutrophils, macrophages and myofibroblasts (Ljubimov and Saghizadeh, 2015).

Considering the scope of known constitutive and upregulated innate defenses protecting the injured murine cornea, and breadth of actions of topical corticosteroids (inhibition of inflammatory mediator synthesis, suppression of phagocyte degranulation, the inhibition of immune cell proliferation etc.), it was surprising that corticosteroid pretreatment had very little impact on bacterial colonization and susceptibility to P. aeruginosa infection in the injury models used in this study. This may reflect independence of one or more constitutive or upregulated innate defenses from corticosteroid inhibition, and if might relate to the parameters of treatment used. Indeed, given the observed trend towards increased MK in corticosteroid-pretreated eyes in the 12 h healing model in some of our experiments, it would be of value to extend the duration of treatment in future studies, and if an impact is found, evaluate factors involved, e.g. cytokines, antimicrobial peptides, resident or infiltrating cells.

The number of bacteria remaining in the eye 48 h post-inoculation was greatly reduced from initial inocula in both injury models, suggesting bacterial clearance of 99 % or greater. These results were consistent with our previous studies using “null-infection” murine models with healthy corneas (Augustin et al., 2011; Mun et al., 2009). The lack of a difference between the two groups suggested that corticosteroid-pretreatment did not impact tear fluid-mediated clearance. Factors that impact bacterial clearance in healthy corneas include; soluble mucins (Fleiszig et al., 1994), surfactant proteins (Mun et al., 2009; Ni et al., 2005), secretory IgA (Masinick et al., 1997), tear-derived antimicrobial peptides (McDermott, 2013), and tear effects on corneal epithelial defenses, e.g. expression of antimicrobials (Mun et al., 2011), alterations in microRNA expression (Mun et al., 2013). Since the present study involved injured corneas rather than healthy eyes, different factors might be at play in successful clearance of bacteria and our results suggest that key players are insensitive to inhibition by corticosteroids.

Scratched corneas showed more residual fluorescein staining at 12 h when pretreated with corticosteroids, consistent with the well known effects of topical corticosteroids in delaying wound healing in various animal models corneal epithelia (Barba et al., 2000; Kadmiel et al., 2016; Petroutsos et al., 1982). Multiple mechanisms are likely to be involved, including reduced cell migration. Why these corticosteroid-mediated effects on wound healing did not significantly increase susceptibility to P. aeruginosa colonization or keratitis is unclear. However, it suggests that the lack of barrier function corresponding with the increased residual fluorescein staining were counterbalanced by defenses against bacteria that are insensitive to corticosteroid inhibition, at least with the treatment regimen used in this study.

Interestingly, inclusion of the fluorescein staining step prior to bacterial inoculation (Fig. 4) correlated with lower bacterial recovery in both the control and corticosteroid-pretreatment groups compared to experiments in which fluorescein was not used (Fig. 2), despite the otherwise identical experimental protocol. It also removed the trend for increased

susceptibility to MK with corticosteroid pretreatment. Mechanisms for this observation would be of interest to explore in future studies.

In conclusion, the data presented in this report show that short-term corticosteroid use in healthy murine corneas does not necessarily affect subsequent susceptibility to P. aeruginosa colonization or keratitis under conditions of superficial injury or during healing after scarification - even though it can hinder the resolution of fluorescein staining. Thus, short-term corticosteroid use does not necessarily present a risk of opportunistic bacterial infection for a murine cornea not already infected. Whether this applies to other corticosteroids, different treatment regimens, other microbes, in the context of underlying diseases, and is also true for human corneas, remains to be explored.

Acknowledgements.

This work was supported by the National Institutes of Health EY011221 (SMJF), EY023000 (CT), EY003176 (Vision Science Training Grant), EY017269 (Berkeley Clinical Scientist Development Program), the Australian National Health and Medical Research Council CJ Martin Fellowship (YW), an American Optometric Foundation Ezell Fellowship (TNT), and Vistakon Research Grant (SMJF and YW).

References

Alarcon I, Tam C, Mun JJ, LeDue J, Evans DJ, Fleiszig SMJ 2011 Factors impacting corneal epithelial barrier function against *Pseudomonas aeruginosa* traversal. Invest. Ophthalmol. Vis. Sci 52, 1368–1377. [PubMed: 21051692]

Augustin DK, Heimer SR, Tam C, Li WY, Le Due JM, Evans DJ, Fleiszig SMJ 2011 Role of defensins in corneal epithelial barrier function against *Pseudomonas aeruginosa* traversal. Infect. Immun 79, 595–605. [PubMed: 21115716]

Badenoch PR, Hay GJ, McDonald PJ, Coster DJ 1985 A rat model of bacterial keratitis. Effect of antibiotics and corticosteroid. Arch Ophthalmol 103, 718–722. [PubMed: 3843063]

Barba KR, Samy A, Lai C, Perlman JI, Bouchard CS 2000 Effect of topical anti-inflammatory drugs on corneal and limbal wound healing. J. Cataract Refract. Surg 26, 893–897. [PubMed: 10889437]

Beisel KW, Hazlett LD, Berk RS 1983 Dominant susceptibility effect on the murine corneal response to *Pseudomonas aeruginosa*. Proc. Soc. Exp. Biol. Med 172, 488–491. [PubMed: 6844357]

Blair J, Hodge W, Al-Ghamdi S, Balabanian R, Lowcock B, Pan YI, Sherif H, AlMahmoud T, Fergusson D, Slomovic A 2011 Comparison of antibiotic-only and antibiotic-steroid combination treatment in corneal ulcer patients: double-blinded randomized clinical trial. Can. J. Ophthalmol 46, 40–45. [PubMed: 21283156]

Bohigian GM, Foster CS 1977 Treatment of *Pseudomonas* keratitis in the rabbit with antibiotic-steroid combinations. Invest. Ophthalmol. Vis. Sci 16, 553–556. [PubMed: 405346]

Borkar DS, Fleiszig SM, Leong C, Lalitha P, Srinivasan M, Ghanekar AA, Tam C, Li WY, Zegans ME, McLeod SD, Lietman TM, Acharya NR 2013 Association between cytotoxic and invasive *Pseudomonas aeruginosa* and clinical outcomes in bacterial keratitis. JAMA Ophthalmol. 131, 147–153. [PubMed: 23411878]

Chan JKL, Yuen D, Too PH-M, Sun Y, Willard B, Man D, Tam C, 2018 Keratin 6a reorganization for ubiquitin–proteasomal processing is a direct antimicrobial response. J. Cell Biol 217, 731–744. [PubMed: 29191848]

Chen HC, Lee CY, Lin HY, Ma DHK, Chen PYF, Hsiao CH, Lin HC, Yeh LK, Tan HY 2017 Shifting trends in microbial keratitis following penetrating keratoplasty in Taiwan. Med. (United States) 96, e5864.

Evans DJ, Fleiszig SMJ 2013 Why does the healthy cornea resist *Pseudomonas aeruginosa* infection? Am. J. Ophthalmol 155, 961–970 e2. [PubMed: 23601656]

Fleiszig SM, Zaidi TS, Ramphal R, Pier GB 1994 Modulation of *Pseudomonas aeruginosa* adherence to the corneal surface by mucus. Infect. Immun 62, 1799–1804. [PubMed: 8168942]

Gan IM, Ugahary LC, van Dissel JT, Feron E, Peperkamp E, Veckeneer M, Mulder PG, Platenkamp GJ, van Meurs JC 2005 Intravitreal dexamethasone as adjuvant in the treatment of postoperative endophthalmitis: a prospective randomized trial. Graefes Arch Clin. Exp. Ophthalmol 243, 1200–1205. [PubMed: 16235062]

Gritz DC, Lee TY, Kwitko S, McDonnell PJ 1990 Topical anti-inflammatory agents in an animal model of microbial keratitis. Arch. Ophthalmol 108, 1001. [PubMed: 2369336]

Holmberg A 1953 The local treatment of acute iridocyclitis with cortisone. Acta Ophthalmol. 31, 75–89. [PubMed: 13039983]

Jolly AL, Agarwal P, Metruccio MME, Spiciarich DR, Evans DJ, Bertozzi CR, Fleiszig SMJ 2017 Corneal surface glycosylation is modulated by IL-1R and *Pseudomonas aeruginosa* challenge but is insufficient for inhibiting bacterial binding. FASEB J. 31, 2393–2404. [PubMed: 28223334]

Kadmiel M, Janoshazi A, Xu X, Cidlowski JA 2016 Glucocorticoid action in human corneal epithelial cells establishes roles for corticosteroids in wound healing and barrier function of the eye. Exp. Eye Res 152, 10–33. [PubMed: 27600171]

Lee EJ, Cowell BA, Evans DJ, Fleiszig SMJ 2003a Contribution of ExsA-regulated factors to corneal infection by cytotoxic and invasive *Pseudomonas aeruginosa* in a murine scarification model. Invest. Ophthalmol. Vis. Sci 44, 3892–3898. [PubMed: 12939306]

Lee EJ, Truong TN, Mendoza MN, Fleiszig SMJ 2003b A comparison of invasive and cytotoxic *Pseudomonas aeruginosa* strain-induced corneal disease responses to therapeutics. Curr. Eye Res 27, 289–299. [PubMed: 14562165]

Lee EJ, Evans DJ, Fleiszig SMJ 2003c Role of *Pseudomonas aeruginosa* ExsA in penetration through corneal epithelium in a novel *in vivo* model. Invest. Ophthalmol. Vis. Sci 44, 5220–5227. [PubMed: 14638720]

Lim CHL, Carnt NA, Farook M, Lam J, Tan DT, Mehta JS, Stapleton F 2016 Risk factors for contact lens-related microbial keratitis in Singapore. Eye 30, 447–455. [PubMed: 26634710]

Ljubimov AV, Saghizadeh M 2015 Progress in corneal wound healing. Prog. Retin. Eye Res 49, 17–45. [PubMed: 26197361]

Luchs JI, Cohen EJ, Rapuano CJ, Laibson PR 1997 Ulcerative keratitis in bullous keratopathy. Ophthalmology 104, 816–22. [PubMed: 9160028]

Masinick SA, Montgomery CP, Montgomery PC, Hazlett LD 1997 Secretory IgA inhibits *Pseudomonas aeruginosa* binding to cornea and protects against keratitis. Investig. Ophthalmol. Vis. Sci 38, 910–918. [PubMed: 9112987]

McDermott AM 2013 Antimicrobial compounds in tears. Exp. Eye Res 117, 53–61. [PubMed: 23880529]

Metruccio MME, Tam C, Evans DJ, Xie AL, Stern ME, Fleiszig SMJ 2017 Contributions of MyD88-dependent receptors and CD11c-positive cells to corneal epithelial barrier function against Pseudomonas aeruginosa. Sci. Rep 7, 13829. [PubMed: 29062042]

Mun J, Tam C, Chan G, Kim JH, Evans D, Fleiszig S 2013 MicroRNA-762 is upregulated in human corneal epithelial cells in response to tear fluid and *Pseudomonas aeruginosa* antigens and negatively regulates the expression of host defense genes encoding RNase7 and ST2. PLoS One 8, e57850. [PubMed: 23469087]

Mun JJ, Tam C, Evans DJ, Fleiszig SMJ 2011 Modulation of epithelial immunity by mucosal fluid. Sci. Rep 1, 8. [PubMed: 22355527]

Mun JJ, Tam C, Kowbel D, Hawgood S, Barnett MJ, Evans DJ, Fleiszig SMJ 2009 Clearance of *Pseudomonas aeruginosa* from a healthy ocular surface involves surfactant protein D and is compromised by bacterial elastase in a murine null-infection model. Infect. Immun 77, 2392–2398. [PubMed: 19349424]

Ni M, Evans DJ, Hawgood S, Anders EM, Sack RA, Fleiszig SMJ, 2005 Surfactant protein D is present in human tear fluid and the cornea and inhibits epithelial cell invasion by *Pseudomonas aeruginosa*. Infect. Immun 73, 2147–2156. [PubMed: 15784557]

Noble S, Goa KL 1998 Loteprednol etabonate: clinical potential in the management of ocular inflammation. BioDrugs 10, 329–339. [PubMed: 18020605]

Noureddin GS, Sasaki S, Butler AL, Tilley P, Roscoe Di., Lyons CJ, Holland SP, Yeung SN 2016 Paediatric infectious keratitis at tertiary referral centres in Vancouver, Canada. Br. J. Ophthalmol 100, 1714. [PubMed: 27030278]

O'Gallagher M, Bunce C, Hingorani M, Larkin F, Tuft S, Dahlmann-Noor A 2017 Topical treatments for blepharokeratoconjunctivitis in children. Cochrane Database Syst. Rev 2, CD011965. [PubMed: 28170093]

Petroutsos G, Guimaraes R, Giraud J, Pouliquen Y 1982 Corticosteroids and corneal epithelial wound healing. Br. J. Ophthalmol 66, 705–708. [PubMed: 6896993]

Redfern RL, Reins RY, McDermott AM 2011 Toll-like receptor activation modulates antimicrobial peptide expression by ocular surface cells. Exp Eye Res 92, 209–220. [PubMed: 21195713]

Reins RY, Courson J, Lema C, Redfern RL 2017 MyD88 contribution to ocular surface homeostasis. PLoS One. e0182153 [PubMed: 28796783]

Srinivasan M, Mascarenhas J, Rajaraman R, Ravindran M, Lalitha P, O'Brien KS, Glidden DV, Ray KJ, Oldenburg CE, Zegans ME, Whitcher JP, McLeod SD, Porco TC, Lietman TM, Acharya NR 2014 The steroids for corneal ulcers trial (SCUT): Secondary 12-month clinical outcomes of a randomized controlled trial. Am. J. Ophthalmol 157, 327–333. [PubMed: 24315294]

Sullivan AB, Tam KPC, Metruccio MME, Evans DJ, Fleiszig SMJ 2015 The importance of the *Pseudomonas aeruginosa* type III secretion system in epithelium traversal depends upon conditions of host susceptibility. Infect. Immun 83, 1629–1640. [PubMed: 25667266]

Tallab RT, Stone DU 2016 Corticosteroids as a therapy for bacterial keratitis: An evidence-based review of "who, when and why." Br. J. Ophthalmol 100, 731–735. [PubMed: 26743622]

Tam C, LeDue J, Mun JJ, Herzmark P, Robey EA, Evans DJ, Fleiszig SMJ 2011 3D quantitative imaging of unprocessed live tissue reveals epithelial defense against bacterial adhesion and subsequent traversal requires MyD88. PLoS One 6, e24008. [PubMed: 21901151]

Tam C, Mun JJ, Evans DJ, Fleiszig SMJ 2012 Cytokeratins mediate epithelial innate defense through their antimicrobial properties. J. Clin. Invest 122, 3665–3677. [PubMed: 23006328]

Zolfaghar I, Evans DJ, Fleiszig SMJ 2003 Twitching motility contributes to the role of pili in corneal infection caused by *Pseudomonas aeruginosa*. Infect. Immun 71, 5389–5393. [PubMed: 12933890]

Zolfaghar I, Evans DJ, Ronaghi R, Fleiszig SMJ 2006 Type III secretion-dependent modulation of innate immunity as one of multiple factors regulated by *Pseudomonas aeruginosa* RetS. Infect. Immun 74, 3880–3889. [PubMed: 16790760]

Highlights

- Short-term pretreatment of a healthy murine cornea with a topical corticosteroid did not significantly increase susceptibility to *P. aeruginosa* colonization or infection after injury.
- Short-term pretreatment of healthy murine corneas with a topical corticosteroid was associated with increased incidence of corneal fluorescein staining 12 h after scarification injury, but this did not coincide with greater susceptibility to *P. aeruginosa* colonization or infection.

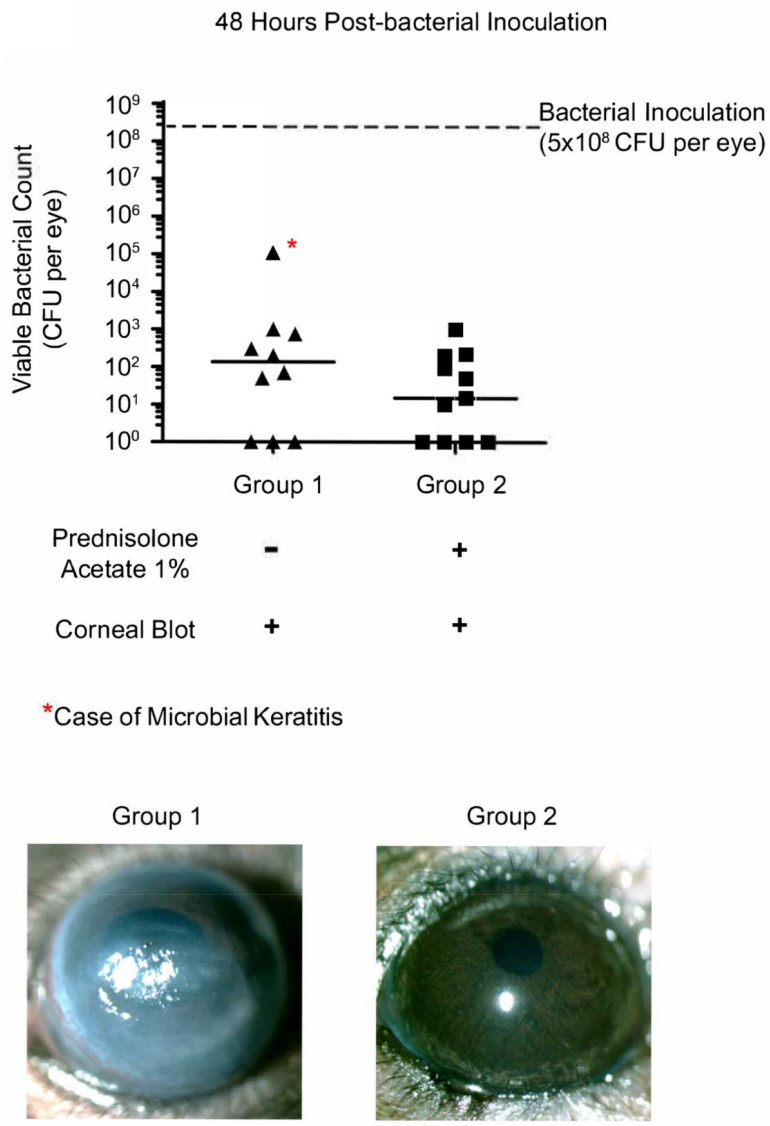

Figure 1.

Viable bacteria recovered from mouse eyes at 48 h post-challenge with *P. aeruginosa* strain 6206 (~5 × 10^8 CFU/eye, dashed line). Prior to inoculation, one eye from each mouse was pretreated with topical prednisolone acetate 1 % (or PBS control) for three times a day for 3 days, followed by blotting injury. The horizontal line represents the median number of *P. aeruginosa* recovered for each group. Differences between groups were not significant (P > 0.05, Mann-Whitney U test). The asterisk indicates a case of microbial keratitis in a PBS-pretreated, blotted control cornea (Group 1), slit-lamp photo-documentation of which is shown (left). A representative image of a corticosteroid-pretreated, blotted cornea without disease (Group 2) is also shown (right).

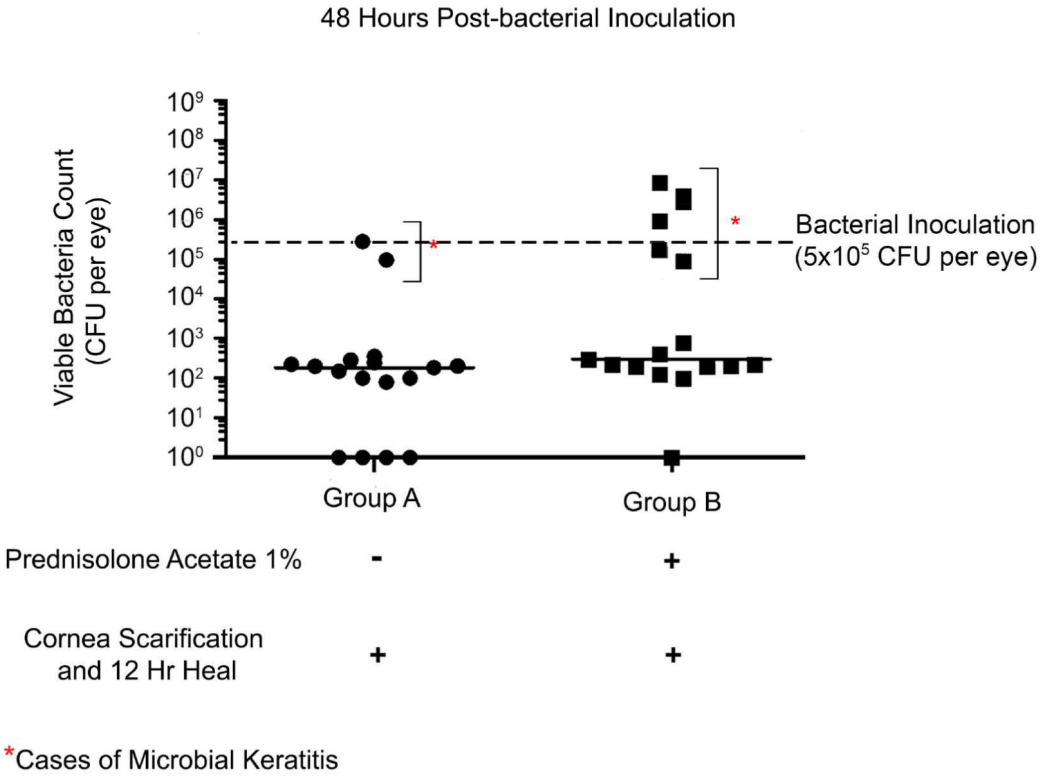

Figure 2.

Viable bacteria recovered from mouse eyes at 48 h post-challenge with *P. aeruginosa* strain 6206 (~5×10^5 CFU/eye, dashed line). Prior to inoculation, one eye from each mouse was pretreated with topical prednisolone acetate 1 % (or PBS control) for three times a day for 3 days, followed by scarification injury with 12 h of epithelial healing. The horizontal line represents the median number of *P. aeruginosa* recovered for each group. The difference between groups was significant (P = 0.02, Mann-Whitney U test). The asterisks indicate cases of microbial keratitis.

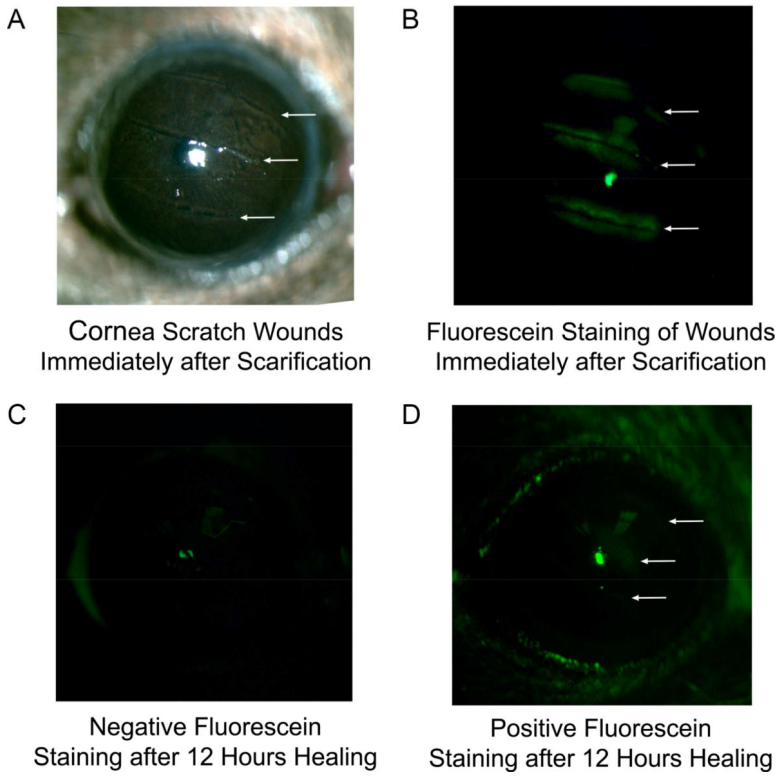

Figure 3.

Slit-lamp evaluation of the murine cornea immediately after scarification and after 12 h epithelial healing. Prior to scarification, the eye was treated with topical prednisolone acetate 1 % three times a day for 3 days. (A) Immediately after scarification, three wounds visible under white light, (B) the same wounds after addition of 1 μL sodium fluorescein, (C) a representative example of negative fluorescein staining 12 h after injury (an artifact of light reflection was present para-centrally), (D) a representative example of positive fluorescein staining 12 h after-injury (white arrows).

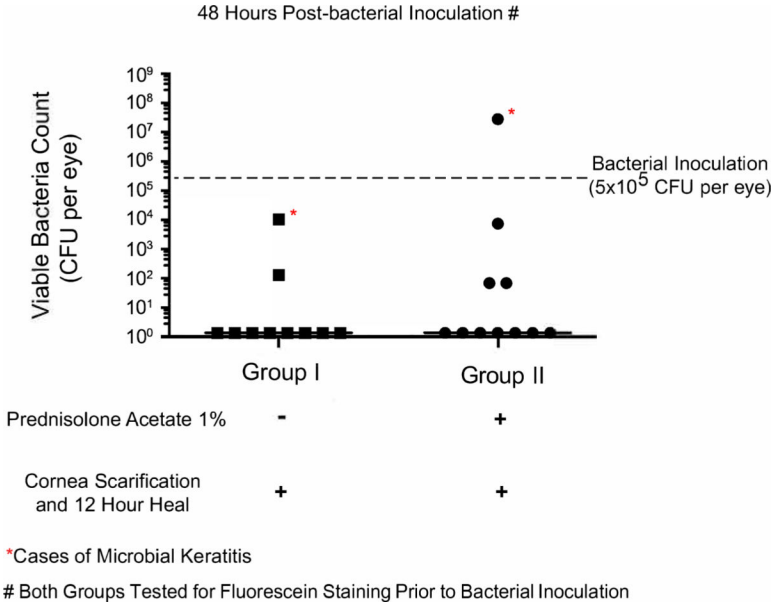

Figure 4.

Viable bacteria recovered from mouse eyes at 48 h post-challenge with *P. aeruginosa* strain 6206 (~5×10^5 CFU/eye, dashed line). Prior to inoculation, one eye from each mouse was pretreated with topical prednisolone acetate 1 % (or PBS control) for three times a day for 3 days, followed by scarification injury with 12 h of epithelial healing. In these experiments, both groups were also tested for fluorescein staining immediately after scarification and after 12 h of healing, and before bacterial inoculation. The horizontal line represents the median number of *P. aeruginosa* recovered for each group. The difference between groups was not significant (P = 0.41, Mann-Whitney U test). Asterisks indicate cases of microbial keratitis.