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Cornea & External Disease

Corticosteroid–Antibiotic Interactions in Bacteria that Cause Corneal Infection

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Received: April 1, 2022
Accepted: April 22, 2023
Published: May 15, 2023

Keywords: antibiotics; corticosteroids; cornea; drug interaction

Citation: Lee H, Kim SM, Rahaman MI, Kang DJ, Kim C, Kim T, Kim SW. Corticosteroid–Antibiotic interactions in bacteria that cause corneal infection. Transl Vis Sci Technol. 2023;12(5):16, https://doi.org/10.1167/tvst.12.5.16

Purpose: Although a comprehensive knowledge of antibiotic/corticosteroid combinations is essential for the appropriate treatment of eye infections, the impact of their co-administration has not been well studied to date. A systematic pharmacodynamic/pharmacokinetic study to determine the effects of cotreatment with various antibiotics and corticosteroids was conducted.

Methods: Four bacterial strains, seven antibiotics, and four corticosteroids were used in the analyses. Drug interactions were evaluated by considering antibacterial effects with a checkerboard assay and intracellular concentrations in human corneal epithelial cells.

Results: The drug combinations that showed the most stable effects against *Pseudomonas aeruginosa* was levofloxacin-prednisolone. Stable combinations against the three types of Gram-positive bacteria were neomycin-prednisolone, ofloxacin-dexamethasone, ofloxacin-prednisolone, and polymyxin-dexamethasone. The cellular concentrations were changed for the gatifloxacin-fluorometholone, moxifloxacin-fluorometholone, tobramycin-dexamethasone, and tobramycin-prednisolone combinations.

Conclusions: Loteprednol and fluorometholone reduced the antibacterial effects of all of the tested antibiotics in this study. Dexamethasone and prednisolone showed various effects in this regard, depending on the co-administered antibiotic. Prior knowledge of specific antibiotic/corticosteroid interactions provides valuable information to clinical practitioners by combining data on the antibacterial and intracellular uptake effects of their co-administration.

Translational Relevance: When using antibiotics and corticosteroids, drug combinations can be selected by referring to the results of this study.

Introduction

Interactions between systemic medications are an important issue for clinicians and their patients and numerous studies have focused on this topic, ranging from in vitro/in silico to in vivo analyses. Recently, such information has been integrated in the process of developing new drugs, leading to more sophisticated treatment approaches.[1,2]

However, drug interactions tend to receive little attention in the field of ophthalmology, despite the routine of administration of multiple topical ophthalmic medications to patients simultaneously or sequentially.[3] One common scenario with respect to drug interactions is the concurrent use of two types of eye drops, typically involving an antibiotic preparation to prevent bacterial infection and a corticosteroid to control intraocular inflammation. Antibiotics are routinely used to prevent serious postsurgical complications, such as endophthalmitis, which can cause significant vision loss and, in extreme cases, loss of the eye.[4] Along with antibiotics, the use of corticosteroids is essential to control intraocular inflammatory response after intraocular surgery, such as cataract surgery.[5] Steroids can be effective in preventing or reducing the severity of cystoid macular edema after cataract surgery.[6] In addition to intraocular surgery, topical antibiotics and corticosteroids are simultaneously applied to the ocular surface for the treatment of bacterial keratitis.[7–9] Bacteria account for most cases of infectious keratitis. The onset of bacterial keratitis involves penetration of the corneal epithelium and superficial stromal layer by these pathogens, and their subsequent replication in the host tissue.[10–13] A key component of the effective treatment of bacterial keratitis is the administration of antimicrobial agents to eliminate the causative bacteria at an early stage. Corticosteroids are also recommended to reduce corneal tissue damage and restore vision by inhibiting the inflammatory response.[14,15] Corticosteroids have been known to inhibit collagen degradation by corneal fibroblasts and to attenuate the infiltration of mononuclear cells, consequently inhibiting the interaction between polymorphonuclear neutrophils and corneal fibroblasts.[16,17] Corticosteroids can be applied as an adjunctive therapy in patients with bacterial keratitis.[14] Thus, in this situation, it is necessary to ascertain which corticosteroids increase or at least do not impede the antibacterial effect of antibiotics when administered in combination.

It is notable that few studies to date have systematically presented information on the interactions between antibiotics and corticosteroids in the eyes. The present study evaluated the interactions between seven antibiotics and four corticosteroids that are commonly used in the ophthalmic field for the four bacteria that most commonly cause corneal infection.[18] An in vitro system was used to focus on changes in the antibacterial effect of antibiotics and to reveal the mutual effects of various combinations of antibiotics and corticosteroids, without considering the immunologic reactions that can be seen in corneal infections.

Materials and Methods

Cell Line Management

Human corneal epithelial (HCE) cells were maintained in Dulbecco's modified eagle's medium (LM001-05, Welgene, Korea) supplemented with 10% fetal bovine serum (16000044, Gibco, USA) without penicillin/streptomycin. Cells were incubated at 37°C in a 5% CO_2 humid incubator.

Bacteria Management

The 4 species of bacteria accounting for the highest frequency of ocular infections were selected by accumulating 10 years' worth of identified bacterial information in bacterial keratitis patients.[18] *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Staphylococcus epidermidis*, and *Streptococcus pneumoniae* were provided by professor Jeon Han Park and Kyungwon Lee at Yonsei University College of Medicine. *P. aeruginosa* and *S. aureus* were grown on Luria-Bertani broth (L3022, Sigma-Aldrich, USA). *S. epidermidis* and *S. pneumoniae* were grown on brain heart infusion broth (MB-B1008, MBcell, Korea). All bacterial strains were cultured in media for 18 hours at 37°C, 150 rpm in a shaking incubator. All strains were stored in cryogenic vials at −70°C with media containing 25% glycerol.

Colony Forming Unit Measurement

Colony forming unit (CFU) measurement was performed according to a previous paper.[19] CFU measurement was performed with Mueller Hinton agar (MHA; MB-M1033, MBcell). After culturing each bacterium by incubation in media, 10 bacteria samples were diluted and seeded on MHA plates. After 16 hours of incubation at 37°C, colony numbers were counted to determine the CFUs of bacteria. One $\times 10^6$ CFU/mL of bacterial solutions were prepared for experiments to a final concentration of 5×10^5 CFU/ mL.

Minimum Inhibitory Concentration Values

The minimum inhibitory concentration (MIC) value was measured as previously described.[19] The MIC value of the antibiotic was determined as the average of the values determined from three independent tests. Various concentrations of antibiotics were added to a 96-well plate inoculated with 5×10^5 CFU/ mL of each bacteria separately. After 24 hours, optical density was measured at 600 nm (OD 600) using a

microplate spectrophotometer (Epoch; Biotek, USA) to determine microbial growth. The minimum concentration of each drug for which no bacterial growth could be detected was designated the MIC (MIC_{100}).

Antibiotics and Corticosteroids Interaction Analysis

We selected seven antibiotics widely used in the ophthalmic field (gatifloxacin, levofloxacin, moxifloxacin, neomycin, ofloxacin, polymyxin, and tobramycin) and four corticosteroids (dexamethasone, fluorometholone, loteprednol, and prednisolone). Gatifloxacin (1288408), levofloxacin (28266), moxifloxacin (SML1581), neomycin (N6386), ofloxacin (O8757), polymyxin (P4932), tobramycin (T4014), dexamethasone (D4902), fluorometholone (F9381), loteprednol (SML0547), and prednisolone (P6004) were all purchased from Sigma-Aldrich. Full-scale antibiotic/corticosteroid interaction analysis was conducted with the MIC values obtained for these seven different antibiotics against the four bacterial strains that were tested (Supplementary Fig. S1), using a previously described checkerboard assay.[19,20] Then, 50 µL of media was added to each well of a 96-well plate and 50 µL of serially diluted antibiotic solution added from row A to row H of the 96-well plate. The final concentrations of antibiotic were 8 MICs (row A), 4 MICs (row B), 2 MICs (row C), MIC (row D), 1/2 MICs (row E), 1/4 MICs (row F), 1/8 MICs (row G), and 0 (row H). Subsequently, 50 µL of corticosteroid was added in different concentrations (0 to 1000 µM) to each column. Finally, 100 µL of bacteria was added to a final concentration of 5×10^6 CFU/ mL to each well. After 24 hours of incubation at 37°C, OD 600 was measured using a microplate spectrophotometer.

Drug Concentration Measurement in Cells Using Liquid Chromatography With Tandem Mass Spectrometry

HCE cells were seeded on 60 mm dish at a density of 200,000 cell/dish and maintained at 37°C in a 5% CO_2 humid incubator. After 24 hours, cells were treated with 100 µM antibiotic and corticosteroid for 48 hours. Cells were harvested using 0.25% trypsin-ethylene-diamine-tetraacetic acid solution (LS015-10, Welgene) and washed with phosphate buffered saline (IBS-BP007, Intron Biotechnology, Korea). Then, 400,000 cells were moved into Eppendorf tubes and centrifuged at 3000 rpm for 3 minutes. Subsequently, the supernatants were removed and cells were stored at −70°C.

Further, 200 µL of distilled water was added to the cell pellet and mixed gently, then ultrasonicated for 1 to 2 minutes. Thereafter, lysis buffer (50 µL) and internal standard solution (methyltestosterone 100 ng/ mL in methanol, 10 µL) were added and mixed for 3 seconds. Methanol (50 µL) was then added and the solution was mixed again for 30 seconds. Samples were centrifuged at 12,000 rpm at 4°C for 2 minutes. Formic acid (0.1%, 80 µL) was added to the supernatant, giving a final volume of 320 µL. After mixing the samples well, 20 µL was used in the experiment.

Liquid chromatography with tandem mass spectrometry (LC-MS/MS) analysis was performed using AB SCIEX 4000 Q Trap LC-MS/MS and Shimadzu LC 20A systems. The intracellular drugs were expressed as amount per cell. The amount of intracellular drugs was converted into logscales and analyzed as a *t*-test.

Statistical Analysis

A simple linear regression approach was used to correlate the co-administered corticosteroid concentrations from the checkerboard assay results with the antibacterial effects of the antibiotics. A linear function using the corticosteroid concentration as an independent variable and the antibacterial effect as a dependent variable was generated, and correlations were determined using the slope and *P* value of the straight line. An unpaired *t*-test was used to compare changes in the antibacterial effects of the antibiotics in the presence or absence of corticosteroids. Because two types of statistical analysis were performed based on one experimental result, a Bonferroni correction was applied with significance set at a *P* value of 0.025.

A ratio paired *t*-test was used to compare changes in the intracellular drug concentrations. In this case, statistical differences were defined as significant when the *P* value was less than 0.05.

Results

The schema for this present study, which aimed to explore the interactions between co-administered antibiotics and corticosteroids for the treatment of bacterial infections of the cornea, is presented in Figure 1. After it was confirmed whether a particular corticosteroid had an impact on the effectiveness of an antibiotic in a combination treatment, changes in the concentrations of these co-administered drugs in human corneal cells were assessed.

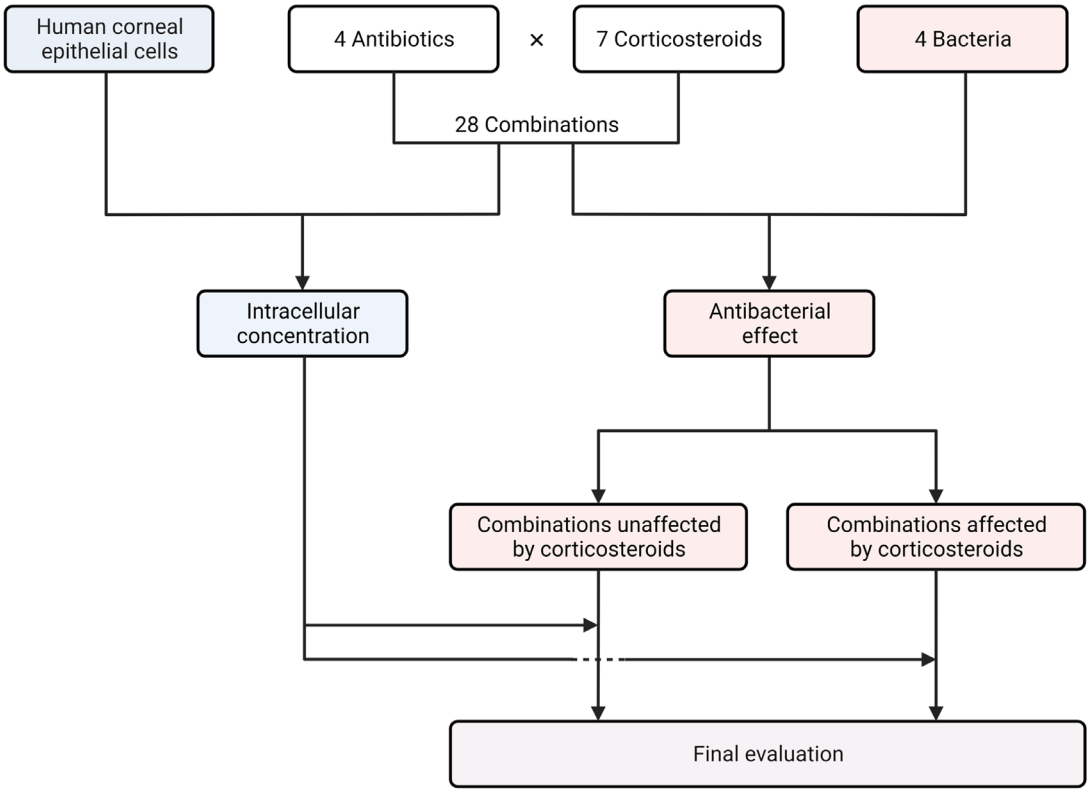

Figure 1. Study flow chart. The antibacterial impact of co-administering antibiotics with corticosteroids was confirmed for four types of bacteria. Corticosteroids that either lowered or had no effect on the effect of the combined antibiotic were identified. Drug interactions were also evaluated through an analysis of the concentration changes of these agents in human corneal cells.

Pharmacodynamic Interactions

Prior to the interaction experiment, the MIC values of seven antibiotics for four types of bacteria were measured. Supplementary Figure S1 shows bacterial survival rates according to antibiotic concentration, and the obtained MIC values are briefly summarized in Supplementary Table S1. Overall, the MIC value of six antibiotics was highest in *S. pneumoniae*.

Checkerboard assays were conducted to evaluate the interaction between antibiotics and corticosteroids in terms of antibacterial effects using MIC value information. The antibacterial effects were expressed as the survival rate of the bacteria after drug treatment (Supplementary Fig. S2). The survival rates of the bacteria exposed to corticosteroids over a 0 to 1000 µM range and the MIC of the antibiotics are shown as an x-shaped point. Linear regression analysis was used to determine whether the antibacterial effect was affected by an increased concentration of corticosteroids. As shown in Supplementary Figure S2, this is expressed as a straight line and the detailed formula is provided in Supplementary Table S2. The data indicated that as the concentration of a corticosteroid increased, the effectiveness of the co-administered antibiotic tended to decrease (upward direction of the regression lines and P values < 0.025).

The antibacterial outcomes when the highest concentration of a corticosteroid (1000 µM) was administered in combination were next compared with those from using the same antibiotic alone (Supplementary Fig. S3). The average value of the antibacterial effects of the antibiotics at concentrations above the MIC (1, 2, and 4 MIC; see Supplementary Fig. S3) was compared between the antibiotic administered alone and in combination with the corticosteroid (Fig. 2; detailed data and P values are provided in Supplementary Table S3).

For *Pseudomonas aeruginosa*, the antibacterial effects of gatifloxacin, ofloxacin and polymyxin were not weakened when combined with dexamethasone ($P = 0.038$, 0.035, and 0.032, respectively), or of gatifloxacin, levofloxacin, ofloxacin and polymyxin coadministered with prednisolone ($P = 0.044$, 0.35, 0.068, and 0.044, respectively).

For *S. aureus*, there was no change in the antibacterial effects of levofloxacin, neomycin, ofloxacin, and polymyxin when they were combined with

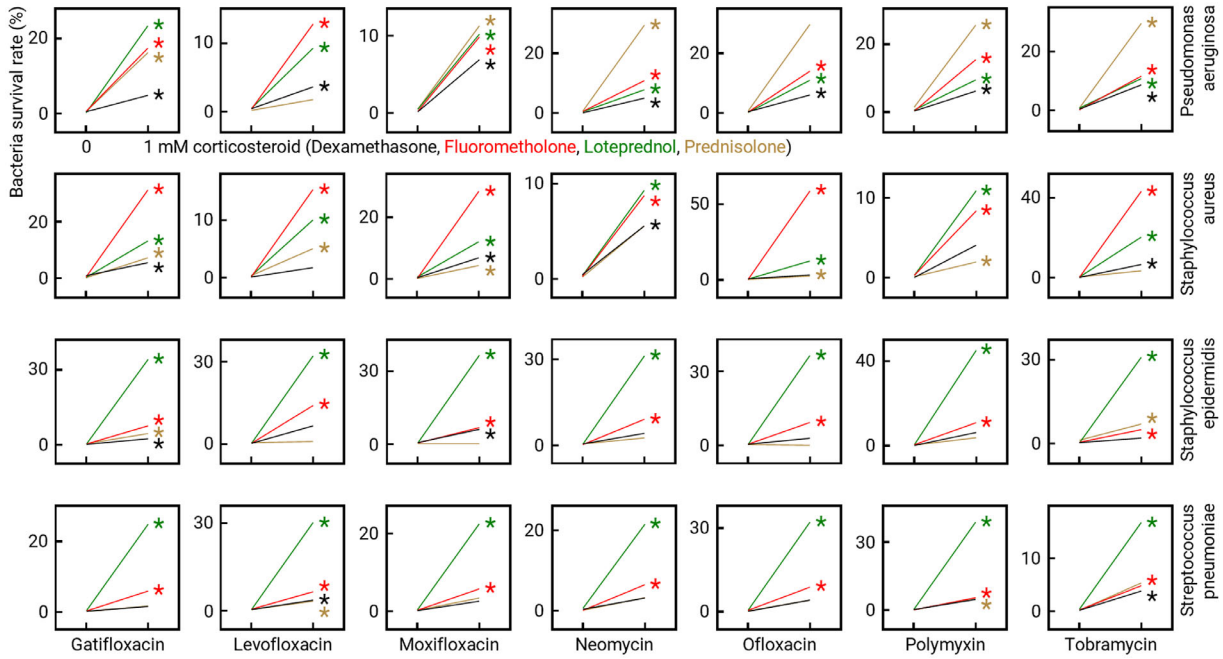

Figure 2. Changes in the effect of antibiotics in the presence or absence of corticosteroids. The antibacterial effects of seven different antibiotics were evaluated when administered alone or in combination with four types of corticosteroids (at a 1 mM concentration). These assessments were conducted using the four indicated bacterial strains. Two points are connected on the X-axis and represented by *lines*. The left and right points represent the bacterial survival rate when antibiotics are administered alone or in combination with corticosteroids, respectively. The average and standard deviation values for the antibacterial effects of each tested antibiotic at a concentration above the MIC (MIC, 2 MICs, and 4 MICs) were then calculated. The black line denotes treatment with or without dexamethasone, the red line with or without fluorometholone, the green line with or without loteprednol, and the gold line with or without prednisolone. Comparisons between the groups with or without the co-administration of corticosteroids were conducted using an unpaired *t*-test. This experiment was conducted three times independently. The asterisks indicate the results with a *P* value of less than 0.05. Detailed numerical values are listed in Supplementary Table S3.

dexamethasone ($P = 0.116$, 0.046, 0.061, and 0.068, respectively), or of gatifloxacin, levofloxacin, neomycin, ofloxacin, and tobramycin when co-administered with prednisolone ($P = 0.029$, 0.032, 0.113, 0.03, 0.034, and 0.052, respectively).

In the case *Staphylococcus epidermidis*, the antibacterial effects of levofloxacin, moxifloxacin, neomycin, ofloxacin, polymyxin, and tobramycin did not change when they were combined with dexamethasone ($P = 0.178$, 0.025, 0.066, 0.067, 0.167, and 0.108, respectively), or of neomycin and ofloxacin co-administered with fluorometholone ($P = 0.028$ and 0.026, respectively) or of levofloxacin, moxifloxacin, neomycin, and polymyxin co-administered with prednisolone ($P = 0.425$, 0.94, 0.077, and 0.198, respectively).

For *Streptococcus pneumoniae*, the antibacterial effects of gatifloxacin, levofloxacin, moxifloxacin, neomycin, ofloxacin, polymyxin, and tobramycin were not affected when these drugs were combined with dexamethasone ($P = 0.259$, 0.046, 0.054, 0.128, 0.091, 0.116, and 0.044, respectively), nor were those of gatifloxacin, moxifloxacin, neomycin, ofloxacin, polymyxin, and tobramycin in combination with prednisolone ($P = 0.055$, 0.054, 0.081, 0.088, 0.032, and 0.068, respectively). In all of the remaining combinations tested in these analyses, it was observed that the effect of the antibiotics was weakened by co-administering corticosteroids (all $P < 0.025$). The most stark example of this loss of effectiveness was with the combination of ofloxacin and fluorometholone, in which *S. aureus* showed a 58.66% survival rate ($P = 0.02$).

Pharmacokinetic Interactions

Even when information is available on the pharmacodynamic interactions between antibiotics and corticosteroids, the output predictions will become far more accurate when there is knowledge of the drug concentration changes in the cell. Hence, changes in the intracellular concentrations of antibiotics and corticosteroids were evaluated upon their co-administration in our present analyses. Figure 3 presents the results of a 48-hour cotreatment with antibiotics and

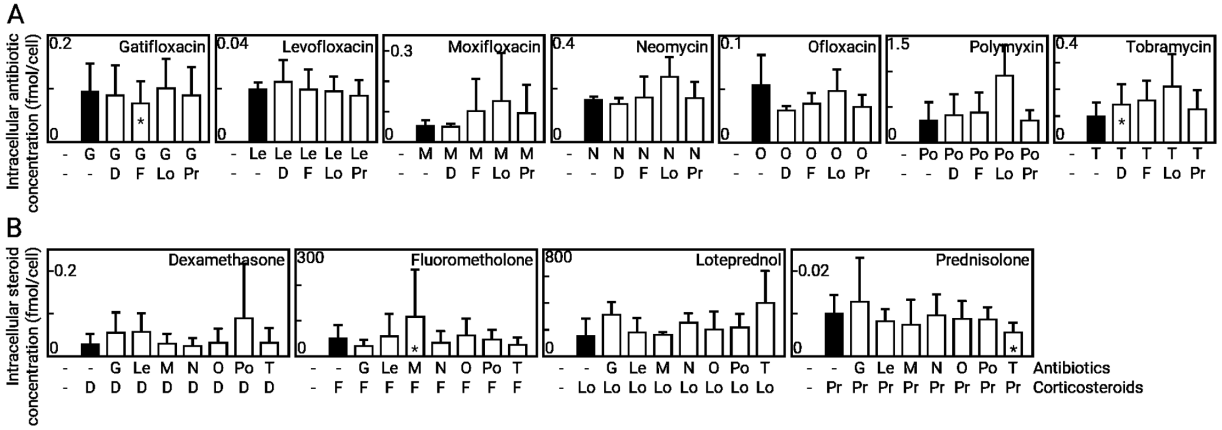

Figure 3. Pharmacokinetic interaction with antibiotics and corticosteroid combinations. Seven antibiotics and four corticosteroids were treated into human corneal epithelial cells in combination, and intracellular concentrations were measured. Antibiotic concentrations are indicated in (**A**) and corticosteroid concentrations in (**B**). The *asterisk* indicates that statistical significance was observed using a ratio paired *t*-test in the combination treatment group compared to the single treatment group. All experiments were performed independently for a minimum of three to a maximum of six times. Among the detailed numerical values presented in this graph, the intracellular antibiotic and corticosteroid concentrations are shown in Supplementary Tables S4 and S5, respectively. G, gatifloxacin; Le, levofloxacin; M, moxifloxacin; N, neomycin; O, ofloxacin; Po, polymyxin; T, tobramycin; D, dexamethasone; F, fluorometholone; Lo, loteprednol; Pr, prednisolone.

Table. Corticosteroids That Do Not Reduce the Potency of The Indicated Antibiotic When Used in Combination

	Gatifloxacin	Levofloxacin	Moxifloxacin	Neomycin	Ofloxacin	Polymyxin	Tobramycin
Pseudomonas aeruginosa	Gati-Dexa Gati-Pred	Levo-Pred			Oflo-Dexa Oflo-Pred	Poly-Dexa Poly-Pred	
Staphylococcus aureus	Gati-Pred	Levo-Dexa Levo-Pred		Neo-Dexa Neo-Pred	Oflo-Dexa Oflo-Pred	Poly-Dexa Poly-Pred	Tobra-Pred[b]
Staphylococcus epidermidis		Levo-Dexa Levo-Pred	Moxi-Dexa Moxi-Pred	Neo-Dexa Neo-Fluo Neo-Pred	Oflo-Dexa Oflo-Fluo	Poly-Dexa Poly-Pred	Tobra-Dexa[a]
Streptococcus pneumoniae	Gati-Dexa Gati-Pred	Levo-Dexa	Moxi-Dexa Moxi-Pred	Neo-Dexa Neo-Pred	Oflo-Dexa Oflo-Pred	Poly-Dexa Poly-Pred	Tobra-Dexa[a] Tobra-Pred[b]

[a]This combination is likely to increase the concentration of tobramycin inside the cell, that stabilizes the antibacterial effects of the drug.

[b]This combination is likely to reduce the concentration of prednisolone inside the cell, which can decrease the effect of corticosteroid.

corticosteroids (each 100 μM) in HCE cells. With most of the tested combinations, the intracellular concentration of the drugs did not fluctuate significantly. However, some combinations led to noticeable changes. Focusing on the antibiotic levels in the cells, statistically significant changes were observed for the following two combinations. The concentration of gatifloxacin decreased by 24.21% when combined with fluorometholone ($P = 0.04$) and that of tobramycin increased by 43.43% when coadministered with dexamethasone ($P = 0.02$). Significant changes in the internal cellular corticosteroid levels were also observed. The concentration of fluorometholone increased by 121.82% when combined with moxifloxacin ($P = 0.03$) and that of prednisolone decreased by 43.43% when cotreated with tobramycin ($P = 0.01$). The intracellular concentrations and P values analyzed using a ratio paired *t*-test are presented in Supplementary Tables S4 and S5.

Interpretation of Drug Interactions

The antibiotic/corticosteroid combinations that did not cause weakened antibacterial outcomes are summarized in the Table. The co-administration of either fluorometholone or loteprednol with any of the tested antibiotics produced lower antibacterial effects in almost cases. Neither dexamethasone nor

prednisolone affected the effect of the antibiotics in some combinations. The cellular concentrations were changed for some combinations. It was found that *Staphylococcus epidermidis* and *Streptococcus pneumoniae* could be stably eradicated when a dexamethasone was co-administered to tobramycin, and the concentration of intracellular tobramycin also increased when used in combination with dexamethasone. When tobramycin and prednisolone were used together, it did not affect the antibacterial outcomes themselves for *S. aureus* and *S. pneumoniae*, but the concentration of prednisolone decreased in the cell. Fluorometholone reduced the antibacterial effects of gatifloxacin on all of the bacterial strains we tested in our analyses and the concentration of this antibiotic in the cells. The combination of moxifloxacin and fluorometholone also negatively affected the antibacterial effects against all of the bacteria and increased the concentration of fluorometholone in the cells.

Discussion

In the treatment of various eye diseases, corticosteroids are typically used to reduce inflammation and tissue damage or to preserve vision, so they are often co-administered with antibiotics. There are, however, differing views on how corticosteroids may affect the effect of an antibiotic when used in combination. Although not an ophthalmic study, a prior report has indicated that dexamethasone is effective in the treatment of acute bacteriological meningitis without increasing side effects during early treatment.[21] On the other hand, corticosteroids have been reported to increase the infection period of conjunctivitis and some researchers have thus urged caution in their use.[22] Hence, although there was evidence that corticosteroids may affect the surrounding environment and thereby change the effectiveness of an antibiotic, we sought to examine this directly in our present study as this had not been done to any great extent previously.

This study investigated the interactions between adjuvant corticosteroids and antibiotics using an in vitro system, which has a low risk of bias. Whether the added corticosteroids weaken the efficacy of antibiotics at concentrations above the MIC was investigated. However, it is difficult to determine whether the efficacy of antibiotics can be truly improved. This is because the antibacterial effect was measured over a fixed period of 48 hours, and therefore the experimental design could not determine whether the antibacterial effect was achieved more quickly due to the use of adjuvant corticosteroids at concentrations above the MIC of antibacterial agents. In other words, this study focused on whether adjuvant corticosteroids weaken the efficacy of antibacterial agents.

With regard to eye drops combining an antibiotic and a corticosteroid, fixed combinations have several advantages over the use of single components, including better patient compliance, lower costs, and reduction of potential washout effects and ocular toxicity by reducing exposure to preservatives.[23–25] Based on our current results, the levofloxacin-prednisolone and ofloxacin-prednisolone combinations had stable antibacterial effects against Gram-negative bacteria, and the neomycin-prednisolone, ofloxacin-dexamethasone, ofloxacin-prednisolone, and polymyxin-dexamethasone combinations maintained a stable antibacterial effect against all of the Gram-positive bacterial strains we tested. In the case of the commercially available tobramycin and dexamethasone ophthalmic ointment, a fixed combination medication, we found a reduced antibacterial effect except in the case of *S. epidermidis*. Notably, however, this reduced effect of this preparation was never more than 10%, and the intracellular tobramycin concentration was found in our analyses to be increased by 43%. There are complex interactions involved therefore, and these should be confirmed in further clinical studies. In clinical practice, neomycin and polymyxin B sulfates and dexamethasone ophthalmic ointment are applied with a pressure patch within 1 day after intraocular surgery. In our current analyses of these medications, we found that the dexamethasone reduced the antibacterial effects of neomycin by 4.95% against *P. aeruginosa* and by 5.07% in the case of *S. aureus*. In addition, dexamethasone reduced the antibacterial effects of polymyxin on *P. aeruginosa* by 5.84%. Overall, therefore, dexamethasone did not reduce the effect of the two different antibiotics toward Gram-positive bacteria. The interaction of neomycin and polymyxin contained in eye drops may also be an important consideration in this regard. In previous studies, neomycin and polymyxin showed additive effects against *P. aeruginosa* and *S. aureus*, and a synergistic effect was reported for *Enterococcus faecalis*, which is not used in this study, but can infect the cornea although with a low probability (0.9%).[18,26] In addition, another combination eye drop contains 1% prednisolone acetate, 0.5% gatifloxacin, and 0.075% bromfenac sodium.[26] The gatifloxacin-prednisolone combination had reduced antibacterial effects except against *S. pneumoniae*.

On the basis of our in vitro interaction data, we believe that the ophthalmologist can easily become aware of the interactions between antibiotics and

corticosteroids at a glance. Because this study dealt with clinical topics through laboratory analyses, there were some notable limitations that must be considered. In the first instance, the analyses were performed on a very large number of combinations, so the experiments were not repeated many times, which necessarily reduces the reliability of the statistical analysis. Of note in particular, although the same pattern appeared for the average intracellular drug concentrations in each experiment shown in Figure 3, there were quite a few statistically insignificant results when the deviation was large. In addition, too many combinations make it very difficult to interpret only the statistically significant analyses. Four types of bacteria identified in Korean patients were evaluated in our current report. However, the characteristics of these strains may not reflect those of all bacteria of the same species that cause corneal infections worldwide. The validity and reliability of our current findings will therefore be increased by future studies of other strains. We note also that it is difficult to fully reproduce complex interactions in the body using in vitro systems, and the activities of hormones and neurotransmitters are also difficult to measure this way. In addition, the three-dimensional structures and interaction of cells may also not be reliably reproduced in vitro, so that again the entirety of the system in vivo will not be fully reflected. Hence, it should be considered that the results of this present study may not reflect the actual disease situation.

Nevertheless, we believe that our results which incorporate pharmacodynamic and pharmacokinetic interactions between antibiotics and corticosteroids, can be applied to further in vivo studies and will eventually be helpful in clinical practice. We expect that reference to our results will enable more detailed and accurate design when clinical studies are conducted in the future.

Conclusion

This study investigated the interactions between seven antibiotics and four corticosteroids against four types of bacteria that commonly cause human corneal infection by measuring the antibacterial effects and intracellular concentrations of the drugs. Fluorometholone and loteprednol significantly reduced the effect of all of our tested antibiotics when used in combination. Dexamethasone and prednisolone did not change antibacterial effects in some of the combinations. There were also four combinations in our hands that produced changes in the intracellular drug concentrations, some of which may well have affected the antibacterial outcomes.

Acknowledgments

The authors thank Jeon Han Park and Kyungwon Lee (Department of Microbiology and Laboratory Medicine, respectively) at Yonsei University College of Medicine for providing four bacteria (*Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Staphylococcus epidermidis*, and *Streptococcus pneumoniae*) used in this study.

Supported by the National Research Foundation of Korea (NRF) grants funded by the Korean government (MSIT) (2019R1F1A1062468 and 2021R1F1A1062702) and a grant (2022IL0032) from the Asan Institute for Life Sciences, Asan Medical Center, Seoul, Korea.

Disclosure: **H. Lee**, None; **S.M. Kim**, None; **M.I. Rahaman**, None; **D.J. Kang**, None; **C. Kim**, None; **T. Kim**, None; **S.W. Kim**, None

* HL and SMK contributed equally to this work.

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