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Comparison of the effects of fourth-generation fluoroquinolones on corneal re-epithelialization in rabbit eyes

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Abstract
Background Gatifloxacin and moxifloxacin ophthalmic solutions are frequently prescribed for antimicrobial prophylaxis following cataract and corneal refractive surgeries, although the use of topical antibiotics is likely to interfere with wound healing in the immediate postoperative period. A potential factor that may influence rates of wound healing or corneal re-epithelialization is how the solutions are preserved. Gatifloxacin is preserved with 0.005% benzalkonium chloride, whereas moxifloxacin is unpreserved. The purpose of this study was to evaluate the effects of commercially prepared topical gatifloxacin and moxifloxacin on corneal re-epithelialization in rabbit eyes.
Methods In this randomized, prospective, controlled study, 17 New Zealand white rabbits underwent bilateral corneal de-epithelialization procedures using 20% alcohol contained within a 6 mm trephine. Postoperatively, eyes were randomly assigned to receive either gatifloxacin 0.3%, moxifloxacin 0.5%, or balanced salt solution (BSS) four times daily. Each 6 hours during the first 2 days, and every 12 hours thereafter slit-lamp measurements and corneal photography were performed, enabling de-epithelialized surface areas to be calculated via EPCO 2000 computer analysis.
Results Gatifloxacin (n=12) and moxifloxacin (n=13) treated eyes had a statistically significant (p=0.036) delay in epithelial healing relative to controls (BSS, n=8). Healing rates of gatifloxacin and moxifloxacin treated eyes were not significantly different (p=0.545).
Conclusions We found no significant difference in re-epithelialization rates following topical application of gatifloxacin 0.3% and moxifloxacin 0.5%. Both antibiotic solutions delayed healing compared to BSS. Our analysis suggests that there was no apparent added epithelial toxicity due to the presence of BAK in the gatifloxacin preparation.

Keywords Gatifloxacin · Moxifloxacin · Cornea · Re-epithelialization

Introduction

Fluoroquinolones are synthetic fluorinated analogues of nalidixic acid, the first antibacterial quinolone. Since its introduction in 1963, nalidixic acid has undergone modifications which have led to newer agents with enhanced potency and breadth of spectrum [1]. With emerging fluoroquinolone resistance in bacterial keratitis, we are now at a time when the new fourth generation quinolones, gatifloxacin and moxifloxacin, are at the forefront of our antibiotic arsenal [2]. The topical ophthalmic formulations of these are Zymar™ (gatifloxacin 0.3%, Allergan Laboratories, Irvine, CA, USA), and Vigamox™ (moxifloxacin 0.5%, Alcon Laboratories, Fort Worth, TX, USA).

Until recently, fluoroquinolone preparations have all contained the preservative benzalkonium chloride (BAK).

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Gatifloxacin contains a relatively low dose of BAK 0.005% as preservative, while moxifloxacin contains no BAK or other preservative. Gatifloxacin may benefit from the advantageous properties of BAK, such as prevention of microbial growth in the product due to contamination, and protection against drug degradation [3]. Another benefit was observed in a study which showed that gatifloxacin 0.3% exerted better in vitro antimicrobial efficacy than moxifloxacin 0.5% against Staphylococcus strains [4]. This may suggest a synergistic contribution by the BAK. The manufacturer of moxifloxacin, however, claims that it acts as its own preservative, therefore not requiring the addition of BAK. A potential significant difference between the two products lies in the toxic potential of BAK. Whether or not gatifloxacin may be at a disadvantage in certain therapeutic circumstances due to delayed epithelial healing has yet to be determined.

Both gatifloxacin and moxifloxacin ophthalmic solutions are frequently prescribed for antimicrobial prophylaxis post-cataract and corneal refractive surgeries. Wound healing and re-epithelialization are key elements in reducing the vulnerability of the eye to microbial pathogens. Other potential adverse outcomes associated with reduced healing rates include stromal scarring, astigmatism, persistent epithelial defect, pain, poor optical vision, epithelial ingrowth, diffuse lamellar keratitis, and flap slippage [5].

This study investigated the effects of the active ingredients of commercially formulated gatifloxacin and moxifloxacin ophthalmic solutions on the corneal epithelial healing rates in rabbits. As the gatifloxacin commercial formulation contains BAK, a potential side effect of delayed re-epithelialization from this preservative was investigated.

Materials and methods

Animals

This study complied with the "Principles of laboratory animal care" (NIH publication No. 85–23, revised 1985), the OPRR Public Health Service Policy on the Humane Care and Use of Laboratory Animals (revised 1986) and the U.S. Animal Welfare Act, as amended. Seventeen New Zealand white rabbits (mixture male/female) of the same age, weighing between 2.8 and 3.2 kg, underwent corneal de-epithelialization procedures in both eyes. The surgery was performed by one surgeon masked to the postoperative therapeutic regimen (JC).

Anesthesia and surgical procedure

The rabbits were anesthetized via an intramuscular injection with 1.2–1.8 ml of a ketamine/xylocaine mixture (7:1). Following the placement of a wire lid speculum, approximately 0.5 ml of 20% alcohol was placed on the central cornea within the barrel of a 6 mm marking trephine. After 60 seconds, the alcohol solution was removed with cellulose sponges, and the surface of the eye irrigated with balanced salt solution (BSS®, Alcon Laboratories, Fort Worth, TX, USA) for another 60 seconds. The ethanol-exposed epithelium was removed with BSS moistened sponges or spatula when necessary. The same procedure was then repeated in the fellow eye. The lesions of both eyes were photographed, and measured immediately postoperatively under a slit lamp with cobalt light, after application of fluorescein (FLUOR-I-STRIP® A.T., Bausch & Lomb, Rochester, NY, USA).

Treatment

The rabbits were assigned to one of two groups. Group 1 consisted of eight rabbits; each received BSS as their drop medication in one eye, and either gatifloxacin 0.3% or moxifloxacin 0.5% ophthalmic solutions in the fellow eye. Group 2 consisted of nine rabbits. Each of these rabbits had gatifloxacin 0.3% as the drop medication randomly assigned to one eye, and moxifloxacin 0.5% to the fellow eye. Scheduling of the designated drop medication was every 6 hours postoperatively, up to 72 hours, or until complete epithelial healing had occurred. The first dose was applied immediately after de-epithelialization and lesion size/appearance documentation.

Evaluation

Postoperative examinations occurred every 6 hours postoperatively for the first 48 hours, then every 12 hours until 72 hours post de-epithelialization, or until complete epithelial healing had occurred. This consisted of application of fluorescein into the eye, without anesthesia, followed by slit-lamp examination under cobalt blue light. Measurements of the vertical and the horizontal diameters of the epithelial defects were initially taken under the slit-lamp view. This represented the first set of data used to compare the groups of eyes. Digital images were then obtained with a Nikon™ Coolpix 990, 3.34 megapixel camera (Nikon Corporation, Tokyo, Japan) coupled to a Zeiss™ SL-120 slit lamp (Carl Zeiss International, Jena, Germany). The digital images allowed epithelial defect surface areas of each eye, at each postoperative time point, to be calculated through the EPCO 2000© (Mannheim, Germany) computer analysis system, which has greater precision than slit-lamp measurements. This was the second set of data used to compare the groups of eyes. Repeated measures analysis of variance (ANOVA) testing was used to determine if a difference in re-epithelialization existed among the three

treatment groups. A post-hoc power calculation was performed using the observed data, to estimate the standard deviation and correlation between the repeated observations of the eyes over time. Statistical calculations were performed using Stata software (Stata, version 9.0, College Station, TX, USA).

Image analysis system

The EPCO computerized system developed by Tetz et al. is an established system that has been used for posterior capsule opacification (PCO) research [6]. Using this software system, standardized digital slit-lamp photographs can be imported into the program and analyzed by measuring the area and degree of opacification to calculate a PCO-index. In this context, details on its methods are described in a previous publication by Auffarth et al. [7].

We were able to use EPCO for the analysis of corneal epithelial defect surface areas (Figs. 1 and 2). In the EPCO program, firstly the desired area of evaluation (the initial corneal epithelial defect photographed immediately after the surgery, or time zero) was marked. This function was initially designed to mark the edge of an intraocular lens, and requires that the epithelial defect be round. Once this reference circle was established, its size/shape could be transferred to other images by using the same relative pixel coordinates. After placement of the reference circle into another image, e.g., from a later postoperative time of the same eye, the epithelial defect was outlined with the mouse and marked. The program evaluated the area outlined and gave a "PCO" score. This score was the proportion of the area within the outline compared to the area within the reference circle. It therefore represented the remaining epithelial defect surface area compared to its initial size at time zero, which could be expressed as a percentage. The application of the EPCO program to this type of analysis requires that all images be taken with the same camera and slit-lamp settings, as well as the same computer image file type, size, and format.

Results

Figures 3 and 4 show the mean values of diameter (vertical + horizontal/2) and percentage surface area of the epithelial defects obtained with the slit lamp and EPCO analysis respectively, performed at each time point for each group. Repeated measures ANOVA analysis indicated that the healing rate was slower for both the gatifloxacin and moxifloxacin groups relative to the BSS group in both direct slit-lamp diameter measurements ($p=0.049$) as well as EPCO % surface area ($p=0.036$). Moreover, the p-value ($p=0.001$ in diameter measurements, and $p<0.001$ in EPCO analysis) from the interaction between time (hour) and treatment groups also indicated that the difference between the groups varied with time. When comparing only gatifloxacin and moxifloxacin eyes, there was no significant difference between the two groups with regard to diameter measurements ($p=0.949$) or to EPCO % surface area analysis ($p=0.545$).

One eye in the gatifloxacin group had some spillover of the alcohol solution at the time of surgery. This resulted in an irregularly (non-round) shaped epithelial defect, so a reference circle could not be well-established. It also made the initial defect significantly larger than the others. Due to this, surface area analysis could not be considered accurate, and the data collected from this eye were excluded from statistical analysis. One of the 13 eyes in the moxifloxacin group (7.6%) had mild diffuse corneal haze after 72 hours. This resolved over the course of 1 week. Another eye from

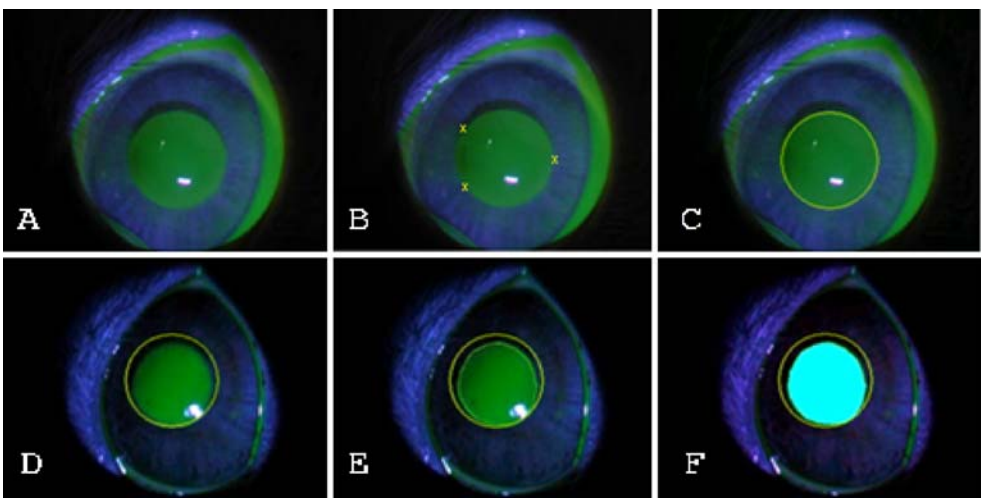

Fig. 1 Use of the EPCO 2000© system for analysis of the corneal epithelial healing. **a** Fluorescein stained epithelial defect at time zero. **b** and **c** Three points were marked on the desired circle to obtain an outline of the reference circle (seen here in *yellow*). **d** The reference circle was transferred to the next image using pixel coordinates from **b**. **e** The partially healed epithelial defect was outlined with the computer mouse. **f** The space for surface area analysis was then designated

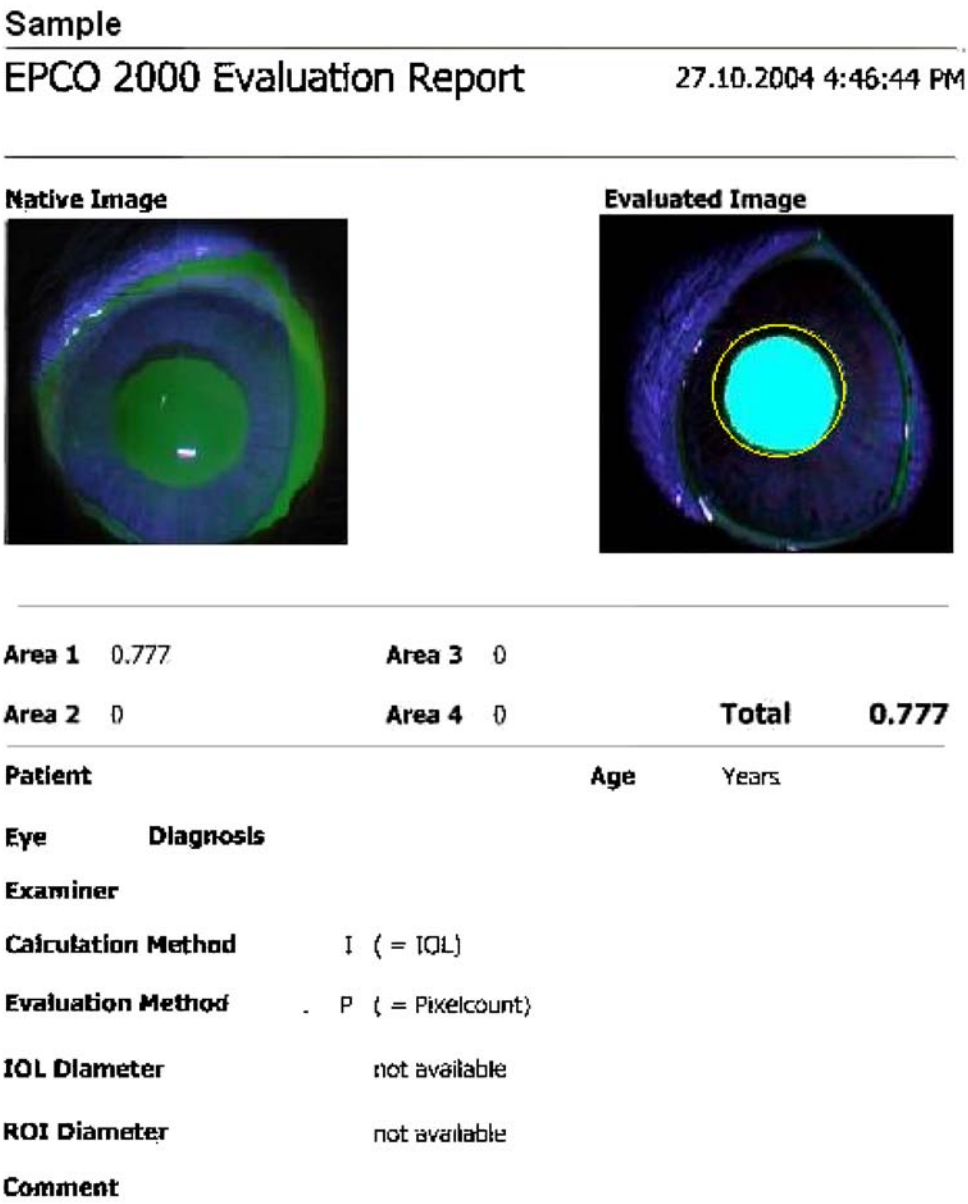

Fig. 2 EPCO compared the remaining defect size to the original defect to calculate the percentage of the original defect that remained. Compared images were obtained at the same magnification and resolution. The remaining defect shown here represents 77.7% of the original defect

the moxifloxacin group also had a few punctate epithelial erosions 72 hours postoperatively, but these were not observed 24 hours later.

The power to detect a mean difference in the healed area between the mean of the baseline measurements and the mean of the follow-up measurements of 0.389 and 0.369 for gatifloxacin and moxifloxacin, respectively, was 10.3%.

Discussion

It has been widely reported that use of topical antibiotics is likely to interfere with wound healing in the immediate postoperative period [8–10]. However, it is equally understood that the use of antibiotic prophylaxis is needed for the prevention of potentially visually devastating infections. Topical use of these agents has become the standard of care. Since the use of topical antibiotics is critical in the postoperative period, it is important for clinicians to select the solution that is least likely to interfere with wound healing or to produce adverse effects. The results of our study suggest that there were no differences in rates of re-epithelialization between commercially formulated ophthalmic gatifloxacin and moxifloxacin. The healing rates were slower for both antibiotics in comparison with the control group.

A potential factor that may influence rates of wound healing or corneal re-epithelialization is how the solutions are preserved. Gatifloxacin is preserved with 0.005% BAK, whereas moxifloxacin is unpreserved. The presence of

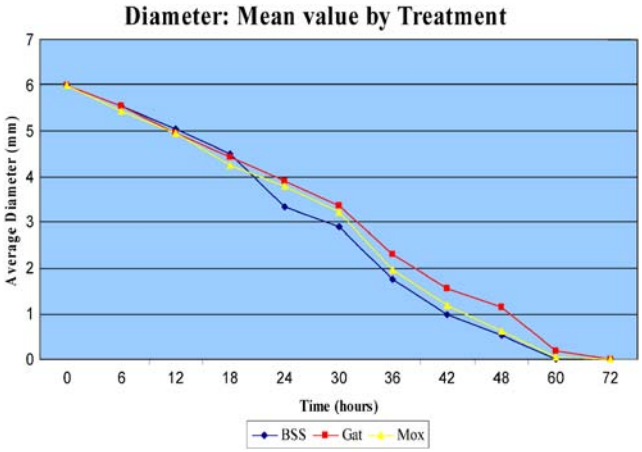

Fig. 3 Average diameter calculation for each group (slit lamp measurements) at each time point (mean +/− standard deviation). *BSS*: balanced salt solution; *Gat*: gatifloxacin; *Mox*: moxifloxacin

BAK in topical ophthalmic medications may be clinically concerning, because some studies have documented its deleterious effects on the ocular surface [11, 12]. Furthermore, BAK may accumulate in the ocular tissues and exert a cytotoxic effect on mammalian cells when used in high concentrations or over an extended period of time [13]. However, in at least one other study, it has been suggested that BAK in a concentration of 0.05% has no effect on corneal wound healing [14].

Our findings suggest that any potentially harmful effects of the gatifloxacin plus BAK solution on re-epithelialization rates are not significant when compared to moxifloxacin. It is possible that the low concentration of BAK preservative in this preparation does not exert a clinically detectable effect when used only for a short period of time, as is the case with post-surgical prophylaxis. This hypothesis is supported by the findings of Herrygers et al. [15], who looked at the potentially damaging effects of the same commercial formulations of gatifloxacin and moxifloxacin on the rabbit corneal epithelium using two dosing protocols: high-frequency dosing for bacterial keratitis and cataract surgery prophylaxis. They concluded in both protocols that there was no statistically significant difference between the antibiotics when looking at mean corneal damage scores. Interestingly, they also did not find a statistically significant difference between either antibiotic and the control (no drops). Other studies on the effects of commercial fluoroquinolones on the intact corneal epithelium found variable results. Farley et al. found that commercial moxifloxacin caused greater disruption of the corneal epithelial barrier function than gatifloxacin in mice, which could be, in part, due to toxic effects on the epithelial tight junctions [16]. Confocal microscopy studies by Kim et al., however, showed greater corneal thinning and damage to the corneal epithelium with ophthalmic gatifloxacin compared to moxifloxacin in rabbits [17].

Different studies have been performed to evaluate the effects of commercial gatifloxacin and moxifloxacin on the corneal re-epithelialization. Gao et al. performed anterior keratectomies using 8.0-mm defects on 36 rabbit eyes [18]. Those animals were randomized to receive gatifloxacin, moxifloxacin, or a vehicle three times daily, starting on the day of surgery. While the incisions in all eyes closed at approximately the same rate during the first 48 hours, during the next 48 hours moxifloxacin-treated eyes healed at a significantly lower rate. The gatifloxacin-treated eyes healed most like the vehicle-treated eyes. An important difference in comparison to our study is that the created anterior keratectomies involved the anterior stroma. Our study looked only at corneal re-epithelialization without assessing stromal wound healing. Two other animal studies that compared fluorescein images of epithelial defects did not find significant differences in re-epithelialization rates between the two drugs [19, 20].

Corneal re-epithelialization with ophthalmic gatifloxacin compared to moxifloxacin has also been evaluated in clinical settings with variable results. Burka et al. compared fellow eyes of 35 patients receiving each antibiotic after photorefractive keratectomy (PRK), and found that eyes treated with moxifloxacin healed faster and had smaller defects than those treated with gatifloxacin [21]. In contrast, Solomon et al. presented an analysis of mean epithelial closure times in 20 patients receiving either antibiotic after PRK, and concluded that moxifloxacin delayed epithelial wound healing compared to gatifloxacin [22]. Besides being PRK protocols, methodological differences between these studies and ours (which may account, at least in part,

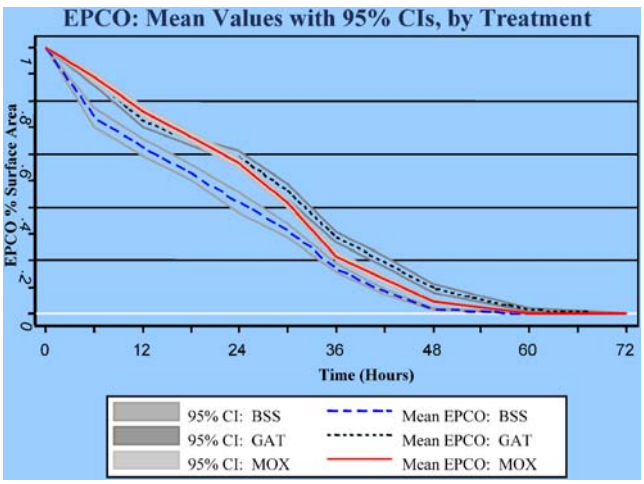

Fig. 4 EPCO percentage surface area calculation for each group at each time point (mean with 95% confidential intervals). *BSS*: balanced salt solution; *Gat*: gatifloxacin; *Mox*: moxifloxacin

for the different results) include differences in the epithelial debridement technique and the use of bandage contact lenses after surgery.

Our experimental study presents some limitations. First, the 6.0 mm defect size inflicted to the rabbit eyes was smaller than what is typically used in human patients. Secondly, this study was conducted in only a small number of rabbit eyes, and the exact congruency with the healing process in human eyes has not been established. Moreover, there was no stromal ablation or deep corneal involvement in the study eyes, which may limit the extrapolation of our findings. Our study design might also have benefited from the inclusion of another control group receiving a vehicle as the treatment, as in the study by Gao et al. [18]. However, the treatment variables, as well as the initial epithelial defect size, were well controlled in our study. Although slit-lamp measurements were taken at time zero as well as all other examination intervals, we consider the data obtained to be limited in value due to its variable accuracy. The error in measuring defects of such small size (sometimes with irregular margins, as they healed) with a slit beam on rabbits could be significant. Therefore, we also used the EPCO program, which allowed a more accurate measure of epithelial defect surface area in the digital photographs. The program does not have the capability of giving absolute values as opposed to surface area percentage relative to a reference size.

Although the slower healing processes in our antibiotic groups were found to be statistically significant compared to the control group, all rabbit corneas were completely healed postoperatively within 72 hours. The eyes had no complications secondary to medications with the exception of one eye with mild corneal haze and one eye with punctuate epithelial erosions in the moxifloxacin group. In both cases, the condition spontaneously resolved.

Questions of whether or not the same conclusions could be made with higher dosages that may be used in a keratitis protocol require further investigation. Also, because the wounds created were only epithelial, we cannot comment on the effects of the new fourth generation fluoroquinolones with respect to corneal wounds that involve the stroma or deeper structures.

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