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Effect of topical netilmicin on the reduction of bacterial flora on the human conjunctiva

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PURPOSE. *To investigate the effects of topical netilmicin on human conjunctival bacterial flora.*
METHODS. *Fifty-six patients' eyes with cataract were treated three times a day with netilmicin 1 day before the surgery. The fellow eyes of the patients were not treated. After 1 day of netilmicin application cultures of the inferior palpebral conjunctiva were performed in both eyes. Bacterial evaluation is qualitative. McNemar test was used for statistical analyses.*
RESULTS. *The number of eyes with positive culture for coagulase negative staphylococcus (CNS) in untreated eyes was 43 (76.8%) and in treated eyes it was 5 (8.93%) and the difference was statistically significant (p=0.000). In untreated eyes number of negative cultures with no bacterial growth was 8 (14.3%) and it was 47 (83.9%) in the treated eyes. The difference among groups was statistically significant (p=0.000). Netilmicin is found very effective for CNS as the percentage of positive cultures (n=43) in untreated eyes to positive cultures (n=4) in the treated fellow eye was 90.7%. Likewise, all patients with Staphylococcus aureus positive cultures in untreated eyes (n=5) had negative culture for this microorganism in their treated fellow eyes (100%).*
CONCLUSIONS. *The Endophthalmitis Vitrectomy Study demonstrated that the most common causes of postoperative endophthalmitis are coagulase negative microorganisms, S aureus and Streptococcus species. As netilmicin was found effective to reduce the number of positive cultures for these bacteria, it can be used to lower the incidence of postoperative endophthalmitis before surgery. (Eur J Ophthalmol 2008; 18: 512-6)*

KEY WORDS. *Netilmicin, Endophthalmitis, Conjunctival flora*

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INTRODUCTION

Postoperative endophthalmitis is a rare but potentially devastating complication of intraocular surgery. The incidence of endophthalmitis after intraocular surgery is 0.093% (1). The organism responsible for this ocular infection has been shown to be genetically identical to organisms present on the ocular surface and ocular adnexa of the infected patient (2, 3). Therefore, it has been theorized that reducing the bacterial load on the ocular surface at the time of surgery may reduce the risk of endophthalmitis.

Treatment of the ocular surface with topical antibiotics and instillation of povidone-iodine 5% into the conjunctival cul-de-sac before surgery are the two main techniques to reduce bacterial flora on the ocular surface. Povidone-iodine has been shown to be an effective antiseptic for use in ophthalmic surgery (4, 5). The application of a topical antibiotic in addition to povidone-iodine further reduces the number of bacteria recovered from the conjunctiva (6).

The choice of antibiotic for surgical prophylaxis is critical. It must be broad spectrum to eradicate the organisms most likely to cause endophthalmitis (7). It must also work quickly and achieve a high level in tears and maybe in aqueous humor with low minimum inhibitory concentra-

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tion (MIC_{90}) against a broad spectrum of bacteria.
Netilmicin is a derivative of a dehydrogenated C_{1a} gentamicin. Although its activity is similar to that of gentamicin and tobramycin, it also has good activity against many gentamicin and tobramycin resistant strains, depending on the N-ethyl substitution of the 2-deoxystreptamine ring. Netilmicin and the other aminoglycosides are bactericidal by binding to intracellular ribosomes and interfering with bacterial protein synthesis (8).
This study sought to determine how topical ophthalmic netilmicin (Netira®) affects the bacterial flora on the ocular surface of the patients' eyes that will undergo cataract surgery with respect to their fellow eyes.

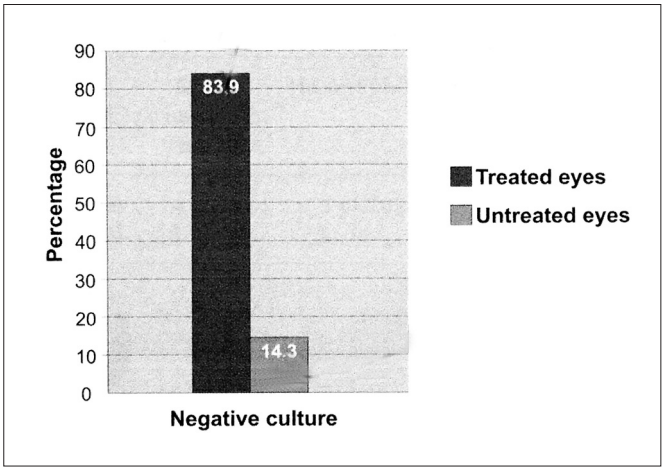

Fig. 1 - *The percentage of negative culture in treated and untreated eyes.*

METHODS

This was a prospective nonrandomized comparative study approved by the local ethics committee at Ulucanlar Eye Hospital. Patient volunteers with different types of cataract and who would undergo cataract surgery, ages 24 to 86, were recruited to participate in the study. Exclusion criteria included use of systemic and topical ocular antibiotics 2 weeks before entry into the study and active conjunctivitis in one or both eyes. All patients signed an informed consent form approved by the local ethics committee.
Fifty-six patients' eyes that would undergo cataract surgery were treated three times with two drops of netilmicin (8.00 AM, 16.00 PM, and 24.00 PM) within 24 hours prior to the surgery. The fellow eyes of the patients were not treated. Patients did not receive any other medication except cyclopentolate 1.0% for pupil dilatation during this preoperative period. All cultures were performed at 08.00 AM the day after the last application of netilmicin.
Just before the surgery cultures of the inferior palpebral conjunctiva were performed in both eyes respectively and no cleaning agent was used like povidone iodine prior to the procedure. Conjunctival cultures were obtained using a sterile cotton swab moistened with sterile serum physiologic. The swab was gently rolled four times across the inferior palpebral conjunctiva and immediately inoculated into 1 mL tryptic soy broth. Swabs were transferred to the laboratory without delay. All samples were incubated at 37 °C for 6 hours; after that they were inoculated onto chocolate agar, eosin methylene blue agar, and two 5% sheep blood agar plates. Chocolate agar plates were incubated with 5% carbon dioxide to encourage microaerophilic bacterial growth and one of the blood agar plates was incubated in an anaerobic bag for anaerobic isolation; those plates were evaluated after 48 hours incubation. Eosin methylene blue agar and second blood agar plates were incubated in aerobic conditions at 37 °C and evaluated after 24 and 48 hours incubation. Cultures inoculated into plates were observed for bacterial growth and all bacterial species from isolated colonies were identified by standard microbiologic methods. The surgeon who obtained the culture samples was not masked as he was the one who treated the patients but the microbiologist responsible for isolating and identifying the bacteria was masked.
McNemar test was used to determine the significance of differences in numbers of positive cultures between the groups. A p value of less than 0.05 was considered to be statistically significant.

RESULTS

The mean age of patients participating the study was 64.1. The bacteria cultured in treated and untreated eyes of the patients included coagulase negative staphylococcus (CNS), *Staphylococcus aureus*, *Corynebacterium* species, streptococci (alpha-hemolytic streptococci and *S pneumoniae*), micrococci, Gram-negative bacilli (*Pantoea* species, *Pseudomonas luteola,* and orzihabitans), haemophilus, and anaerobic microorganism (peptostreptococci). Their numbers and the rates of culture positivity for different bacteria are listed in Table I.

TABLE I - TOTAL NUMBERS AND THE RATE OF CULTURE POSITIVITY IN ALL UNTREATED AND TREATED EYES

	CNS	S aureus	Coryne-bacterium	Strepto-cocci	Micro-cocci	Gram-negative bacilli	Haemophilus	Anaerobic	No bacterial growth
Untreated eyes	43 (76.8)	5 (8.93)	11 (19.6)	5 (8.93)	3 (5.36)	4 (7.14)	1 (1.79)	1 (1.79)	8 (14.3)
Treated eyes	5 (8.93)	1 (1.79)	2 (3.57)	2 (3.57)	1 (1.79)	1 (1.79)	0	0	47 (83.9)

Values are n (%).
CNS = Coagulase negative staphylococcus

In untreated eyes number of negative cultures with no bacterial growth was 8 (14.3%) and it was 47 (83.9%) in the treated eyes (Fig. 1). The difference among groups was statistically significant (p=0.000). The number of eyes with positive culture for CNS in untreated eyes was 43 (76.8%) and in treated eyes it was 5 (8.93%) and the difference was statistically significant (p=0.000). *S aureus* was positive in only 5 eyes (8.93%) of the untreated and in 1 eye (1.79%) of the treated group. The difference among groups was not statistically significant (p=0.219). The number of *Corynebacterium* positive cultures in untreated eyes was 11 (19.6%) and 2 (3.57%) in treated eyes. This difference was also statistically significant (p=0.000). Streptococci were positive in 5 eyes (8.93%) in the untreated group and positive in 2 eyes (3.57%) of the treated group. The difference was statistically insignificant (p=0.250).

Netilmicin is found to be very effective for CNS as the percentage of positive cultures (n=43) in untreated eyes to positive cultures (n=4) in the treated fellow eyes was 90.7%. We had five positive cultures for *S aureus* in untreated eyes of the patients and all cultures were negative in their treated fellow eyes. So the percentage of effectiveness is 100%. The number of positive cultures in untreated eyes for *Corynebacterium* was 11 but their treated fellow eyes had only two positive cultures for this microorganism. This means netilmicin is 81.82% potent on *Corynebacterium*. Likewise the number of positive cultures for streptococci was reduced from five to two with a percentage of 60% (Tab. II and Fig. 2).

DISCUSSION

The Endophthalmitis Vitrectomy Study (EVS) demonstrated that bacteria are the most common cause of infectious endophthalmitis, with *Staphylococcus epidermidis* being

TABLE II - NUMBER OF POSITIVE CULTURES FOR DIFFERENT BACTERIA IN UNTREATED AND TREATED FELLOW EYES OF SAME PATIENTS

	Culture-positive in untreated eyes	Culture-positive in treated fellow eyes
CNS	43	4
S aureus	5	0
Corynebacterium	11	2
Streptococci	5	2

CNS = Coagulase negative staphylococcus

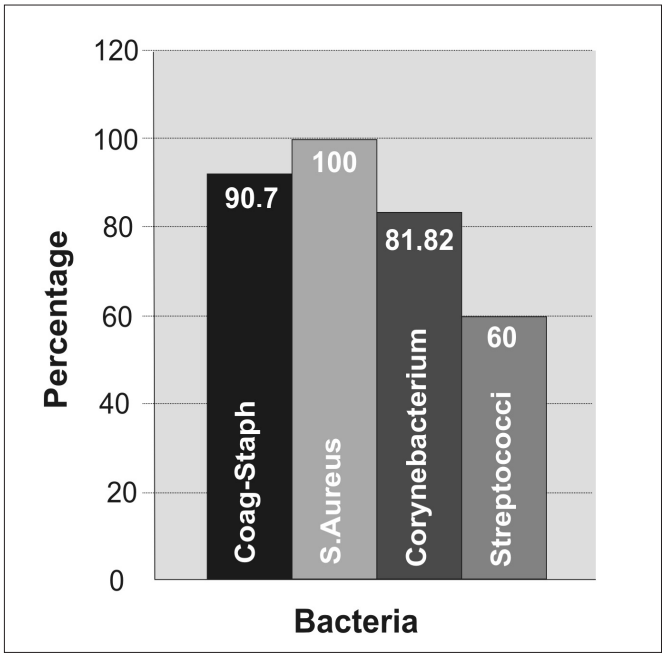

Fig. 2 - *Percentage of positive culture in untreated eyes to negative culture of the fellow treated eyes.*

the most likely organism. Approximately 70% of patients with positive cultures are infected with coagulase-negative microorganisms (mostly *S epidermidis*), 10% *S aureus*, 9% streptococcus species, 6% with Gram-negative species, 3% with other Gram-positive species, and finally 2% with enterococcus (7, 9-11). The most common sources of bacteria causing postoperative endophthalmitis are the eyelid and conjunctival areas. Speaker et al demonstrated that bacterial isolates from the vitreous genetically matched bacterial species isolated from the eyelid and conjunctiva or nose in 14 of 17 cases of endophthalmitis (3). By lowering the number of bacteria on the eyelids and conjunctiva at the time of surgery, the risk of developing endophthalmitis may decrease.

Many techniques can be used to reduce ocular surface flora. Povidone-iodine 10% or 5% for the skin and conjunctival preparation and preoperative topical antibiotics reduce the number of pathogenic organisms on the ocular surface and adnexa (6). Povidone-iodine is a highly effective agent for bacteria, viruses, fungi, protozoa, and spores as a part of the preoperative preparation (4, 5). It kills the microorganisms by disrupting the cell membranes on contact. Because of its rapid onset of action instillation immediately before surgery is enough (5, 6, 12, 13). Scuderi et al showed that a single netilmicin ocular administration in healthy volunteers determines pharmacologic concentrations of the drug better than the MIC_{90} of most important pathogens after 5, 10, 20, and 60 minutes after instillation (14). Netilmicin remains on the ocular surface 100 times better than the MIC_{90}, of ocular strains such as *S aureus*. Netilmicin is also used to treat acute bacterial conjunctivitis and found to be well tolerated and effective for all the isolates, especially Gram-positive, suggesting that it can be effectively used as a first-line agent in the treatment of such infections (15).

Isenberg et al found that when topical polymyxin B sulfate-neomycin sulfate-gramicidin ophthalmic solution and povidone-iodine 5% solutions were used together, there was a greater reduction in conjunctival flora than when either was used alone (6). Mino de Kaspar et al, who studied with topical ofloxacin and povidone-iodine, concluded that combination of topical antibiotic and povidone-iodine resulted in very few bacteria present on the ocular surface, presumably reducing the risk of postoperative endophthalmitis (16). It is therefore logical that a preoperative topical antibiotic combined with povidone-iodine immediately before surgery is the ideal method to reduce the conjunctival surface flora of the eye. Netilmicin, because of its effectiveness to reduce the number of positive cultures for different bacteria responsible for postoperative endophthalmitis, can be used for prophylaxis of endophthalmitis together with povidone-iodine.

Snyder-Perlmutter et al found that reduction in bacterial colony-forming units achieved by ciprofloxacin 0.3% was significantly greater than that of ofloxacin 0.3% (17). Ciprofloxacin has been shown to be effective against coagulase negative microorganisms *S epidermidis*, the most common cause of endophthalmitis, and *P aeruginosa*, the organism that causes the most serious ocular infections. Bonfiglio and et al demonstrated that netilmicin has a 2.4 hour postantibiotic effect (PAE) for *P aeruginosa* and a PAE of 1.5 hour for *S aureus*. Also they found that the PAE values of netilmicin were higher than those of ofloxacin (18). We also found netilmicin to be very effective for coagulase negative staphylococcus and *S aureus*. Although the difference between the groups was statistically insignificant, all patients with positive culture for *S aureus* in their untreated eyes had negative culture for the same microorganism in their treated fellow eyes. As these are the most common causes of postoperative endophthalmitis, netilmicin may be effective in prophylaxis of endophthalmitis. We could not reach any result about the effect of netilmicin on *P aeruginosa* as we did not have any positive culture for this bacteria. We had only one positive culture in an untreated eye having *P luteola* and *Pseudomonas orzihabitans* growth and in the treated eye of this patient no bacterial growth was detected.

Ta et al demonstrated that application of topical ofloxacin for 3 days before surgery is more effective than an application given only 1 hour before surgery in reducing the number of positive bacterial cultures at several time points in the perioperative period (19). We instilled netilmicin three times within 24 hours before the surgery and observed that it is very effective in reducing the number of positive cultures with respect to fellow eyes of patients in which netilmicin was not used. Having a rapid onset of action is an important feature for an ideal antibiotic. This short duration for application of netilmicin before surgery also reduces the cost of medication.

In summary, the results of this study suggest that netilmicin having a rapid onset of action is very effective in reducing the conjunctival surface flora of the eye and

can be used for the prophylaxis of endophthalmitis before intraocular surgery. Further research is needed to compare netilmicin with other topically used antibiotics on reducing the bacterial flora of ocular surface.

None of the authors has a financial or proprietary interest in any material or method mentioned.

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