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Comparison of Ofloxacin, Gentamicin, and Tobramycin Concentrations in Tears and In Vitro MICs for 90% of Test Organisms

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Concentrations of three anti-infective agents in tear film were monitored after one topical application in rabbits. Ofloxacin concentrations exceeded the MIC for 90% of the organisms tested (MIC_{90}) (gram-negative and gram-positive organisms) for 240 min. Tobramycin concentrations exceeded the MIC_{90} for 10 min. Gentamicin concentrations exceeded the MIC_{90} for 20 min for gram-positive organisms and 120 min for gram-negative organisms.

The increasing number of bacterial strains resistant to widely used antibiotics has given impetus to the search for new anti-infective agents, including drugs suitable for topical use in the treatment of ocular infection. The fluoroquinolones are a class of compounds possessing bactericidal activity against gram-negative and gram-positive organisms and some anaerobic bacteria. Ofloxacin is a fluoroquinolone that shows promise in treating ocular infections (1, 2). Ofloxacin is active against a broad spectrum of bacterial organisms (9), including many species isolated from ocular sources (4, 5, 10–15). Bacterial resistance to ofloxacin rarely arises, and ofloxacin-resistant strains are generally unable to compete with nonresistant strains (4, 7).

The value of ofloxacin in treating ocular infections depends in part on the maintenance of effective concentrations in the eye for sufficient periods. The MIC against 90% of bacterial strains tested in vitro (MIC_{90}) of an anti-infective compound is a useful gauge of effective concentration. In this study, the durations at effective concentrations of ofloxacin and the commonly used aminoglycosides gentamicin and tobramycin were determined.

(Results of this study were reported as a poster presentation at the 61st Annual Meeting of the Association for Research in Vision and Ophthalmology, Sarasota, Fla., May 1989.)

Drug concentrations were measured at multiple time points after a single topical application. The test formulations were 0.3% ofloxacin, 0.3% gentamicin (Genoptic Liquifilm; Allergan Pharmaceuticals, Irvine, Calif.), and 0.3% tobramycin (Tobrex Solution; Alcon Laboratories, Fort Worth, Tex.). Healthy female New Zealand albino rabbits (Vista Rabbitry, Vista, Calif.) weighing approximately 2.0 to 3.5 kg were each treated with 50 μl of test formulation placed in the lower conjunctival cul-de-sac of the left eye with a micropipette. After drug application, the eyelids were held closed for 30 s.

Tear samples were collected from the lower conjunctival cul-de-sac with a Schirmer strip before treatment and from 1 to 360 min after treatment. At each time point, tear specimens were obtained from 10 rabbits and specimen weights were recorded. The Schirmer strips containing tear samples were dried immediately under a stream of N_2 and stored at −20°C until they were assayed.

Ofloxacin concentrations were measured by high-performance liquid chromatography (HPLC) with fluorometric detection. Samples were introduced into the HPLC system with an Intelligent Sample Processor (model 710B; Waters Associates, Inc., Milford, Mass.). Aqueous solvent consisting of 40% (vol/vol) CH_3CN, 1.0% (vol/vol) H_3PO_4, and 0.2% (wt/vol) sodium lauryl sulfate was passed through an Ultrasphere ODS HPLC column (4.6 mm by 25 cm; Beckman Instruments, Inc., Berkeley, Calif.) of 5-μm particle size at a flow rate of 1.2 ml/min with a 110A solvent delivery system (Beckman). The retention time of tear ofloxacin in this system was 6.7 min, compared with 8.1 min for triamterene, the internal standard (U.S. Pharmacopeial Convention, Inc., Rockville, Md.). The average recovery of sample ofloxacin from the HPLC system was 93.3%.

Ofloxacin was detected with a Hitachi model F1000 fluorescence spectrophotometer (EM Industries, Inc., Cherry Hill, N.J.) set at an excitation wavelength of 358 nm and an emission wavelength of 495 nm. Ofloxacin was quantified with a 3392A integrator (Hewlett-Packard Co., Santa Clara, Calif.). Assay results were determined as micrograms of ofloxacin per gram of tears. The limit of detection of the assay for tear film ofloxacin was 10 ng.

Intraday and interday assay precisions were determined with six sets of control samples at four different drug concentrations. The intraday coefficient of variation for tear sample assays was 2.4 to 3.8%; the interday coefficient of variation was 1.4 to 5.8%.

Gentamicin and tobramycin were measured by commercial radioimmunoassays (Diagnostic Products, Inc., Los Angeles, Calif.). The limit of detection on Schirmer tear strips was 10 ng for gentamicin and 5 ng for tobramycin. According to the manufacturer, the cross-reactivity of the antibody used in each of the two assays was negligible for a large number of antibiotics.

The gentamicin interday coefficient of variation for quality control tear sample assays at three concentrations ranged from 12.4 to 14.6% (n = 15). For tobramycin, the interday coefficient of variation was 6.0 to 27.6% (n = 5) for three concentrations in the low range and from 12.2 to 17.8% (n = 9) for three concentrations in the high range.

The concentrations (mean ± standard deviation) of ofloxacin, gentamicin, and tobramycin in tears following topical administration are shown in Fig. 1. The patterns were similar for the three drugs; all three exceeded 2,000 μg/g at 1 min

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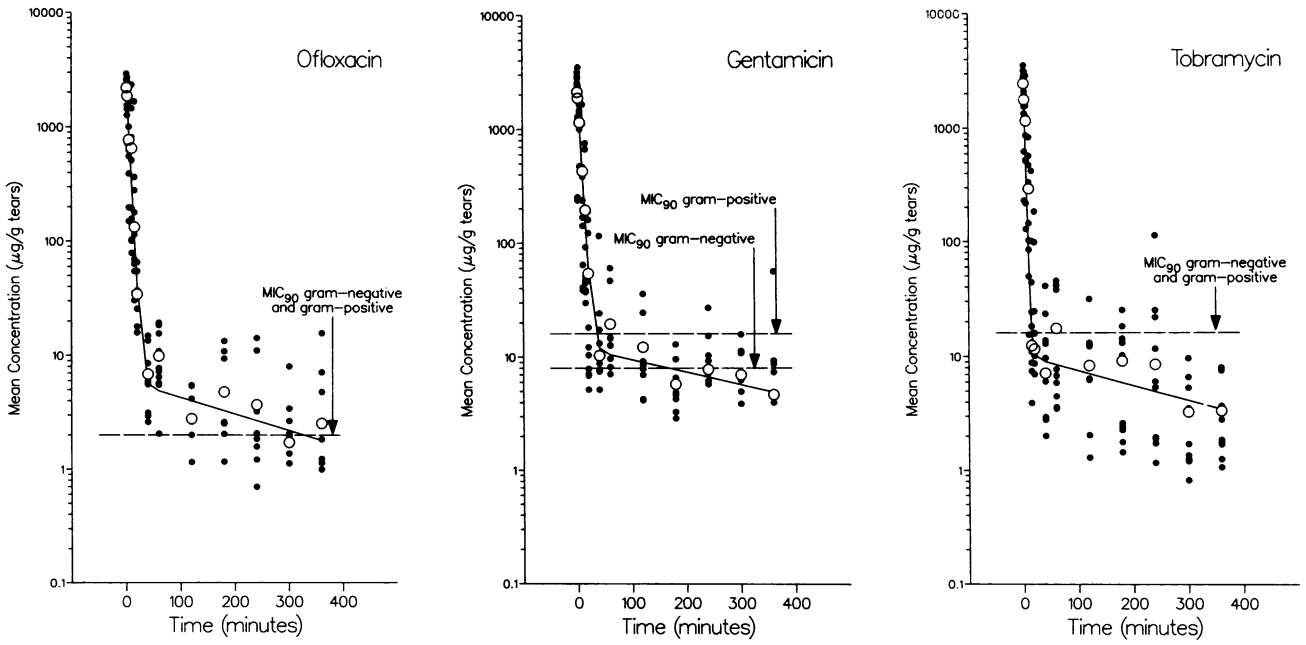

FIG. 1. Concentrations (mean ± standard deviation) of gentamicin, tobramycin, and ofloxacin during the 6 h following a single topical application. Symbols: ○, mean concentration at sampling time point; ●, concentration from individual animal.

after treatment, declined rapidly to approximately 10 μg/g or less by 40 min after treatment, and then diminished gradually. The half-lives for the slow-elimination phase were approximately 210 min for ofloxacin, 274 min for gentamicin, and 231 min for tobramycin. The areas under the concentration curve, which measure both the drug level and the period over which the drug level is maintained, were 13.7 ± 1.4 mg · min/g (mean ± standard error of the mean) for ofloxacin, 16.2 ± 1.2 mg · min/g for gentamicin, and 13.7 ± 1.4 mg · min/g for tobramycin over the 360 min after treatment.

Concentrations of the tested drugs in tears were compared with the MIC_{90}s of the drugs (11). The reported MIC_{90} of ofloxacin against 190 gram-negative and 229 gram-positive organisms from ocular sources is 2 μg/ml (11). This is substantially lower than reported MIC_{90}s for gentamicin against gram-negative (8 μg/ml) and gram-positive (16 μg/ml) organisms and for tobramycin against gram-negative (16 μg/ml) and gram-positive organisms (16 μg/ml) (11). As shown in Fig. 2, mean concentrations of ofloxacin in tear films remained higher than the MIC_{90} for both gram-negative and gram-positive organisms for 240 min after treatment. In contrast, tobramycin concentrations exceeded the MIC_{90} for only 10 min. Gentamicin concentrations in the tear film remained above the MIC_{90} for gram-positive organisms over a period of 20 min and for gram-negative organisms over a period of 120 min.

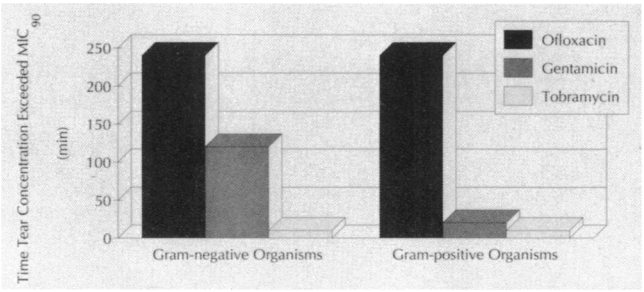

FIG. 2. Periods during which ofloxacin, gentamicin, and tobramycin levels exceeded their respective MIC_{90}s following a single topical application.

In this study, a single topical dose of ofloxacin yielded concentrations greater than the MIC_{90} for 4 h in rabbit tear films. Antibiotic concentrations were maintained at a level higher than the MIC_{90} substantially longer for ofloxacin than for gentamicin and tobramycin. Although differences were seen between gentamicin and tobramycin, because of the similarity in their MIC_{90}s, these differences cannot be considered clinically relevant.

The relationship of the ofloxacin tear concentration-time profile to the in vitro MIC_{90}s may partly account for the efficacy of this agent in treating external ocular infections. Recent clinical studies demonstrate that ofloxacin significantly reduces the signs and symptoms of infection and eradicates the causative organism in the majority of patients treated (8). Maintenance of effective ofloxacin levels (i.e., above the MIC_{90}) in the tear film should provide satisfactory therapeutic activity during the entire dosing period.

Also, the presence of effective ofloxacin levels in tears over a longer period may facilitate clinical management of patients with severe ocular infections that cannot be satisfactorily treated with the aminoglycosides tested (6).

LITERATURE CITED

1. **Borrmann, L., D. Tang-Liu, J. Kann, J. Nista, P. Akers, and J. Frank.** 1989. Tear levels and systemic absorption of ofloxacin eyedrops in humans. Invest. Ophthalmol. Visual Sci. **30**(Suppl.):247.
2. **Borrmann, L. R., and I. H. Leopold.** 1988. The potential use of quinolones in future ocular antimicrobial therapy. Am. J. Ophthalmol. **106**:227–229.
3. **Chau, P. Y., Y. K. Leung, and W. W. S. Ng.** 1986. Comparative in vitro antibacterial activity of ofloxacin and ciprofloxacin against some selected gram-positive and gram-negative isolates. Infection **14**(Suppl. 4):S237–S241.
4. **Crumplin, G. C., and M. Odell.** 1987. Development of resis-

tance to ofloxacin. Drugs **34**(Suppl. 1):1–8.

5. **Debbia, E., S. Mannelli, G. Gianrossi, and G. C. Schito.** 1987. Susceptibility *in vitro* of gram-positive aerobe and anaerobe bacteria to ofloxacin. Drugs Exp. Clin. Res. **13**:213–217.
6. **Gilbert, M. L., K. R. Wilhelmus, and M. S. Osato.** 1987. Comparative bioavailability and efficacy of fortified topical tobramycin. Invest. Ophthalmol. Visual Sci. **28**:881–885.
7. **Kresken, M., and B. Wiedemann.** 1988. Development of resistance to nalidixic acid and the fluoroquinolones after the introduction of norfloxacin and ofloxacin. Antimicrob. Agents Chemother. **32**:1285–1288.
8. **Mitsui, Y., S. Sakuragi, O. Tamura, M. Abe, I. Watanabe, M. Ueno, K. Choshi, H. Sakata, T. Suehiro, N. Ohba, S. Fujita, Y. Miyazono, K. Sasaki, T. Yamamura, N. Watanabe, M. Uyama, K. Kanai, T. Tokura, H. Miyatani, M. Itoi, Y. Kodama, H. Tasaka, R. Manabe, Y. Ohashi, Y. Shimomura, K. Segawa, K. Nishiyama, K. Norose, S. Inoue, K. Matsumura, M. Ooishi, F. Sakaue, A. Oomomo, J. Hara, S. Harino, J. Tsutsui, H. Kimura, S. Inoue, M. Higashitsutsumi, M. Sakamoto, T. Miwatani, and T. Onoda.** 1986. Effect of ofloxacin ophthalmic solution in the treatment of external bacterial infections of the eye. Folia Ophthalmol. Jpn. **37**:1115–1140.
9. **Monk, J. P., and D. M. Campoli-Richards.** 1987. Ofloxacin: a review of its antibacterial activity, pharmacokinetic properties and therapeutic use. Drugs **33**:346–391.
10. **Nagayama, A., T. Nakao, and H. Taen.** 1988. In vitro activities of ofloxacin and four other new quinoline-carboxylic acids against *Chlamydia trachomatis*. Antimicrob. Agents Chemother. **32**:1735–1737.
11. **Osato, M. S., H. G. Jensen, M. D. Trousdale, J. A. Bosso, L. R. Borrmann, J. Frank, and P. Akers.** 1989. The comparative *in vitro* activity of ofloxacin and selected ophthalmic antimicrobial agents against ocular bacterial isolates. Am. J. Ophthalmol. **108**:380–386.
12. **Periti, P., T. Mazzei, and P. Nicoletti.** 1987. Comparative in vitro activity of ciprofloxacin, ofloxacin and perfloxacin against resistant clinical isolates. Chemioterapia **6**:75–78.
13. **Sato, K., Y. Inoue, T. Fujii, H. Aoyama, and S. Mitsuhashi.** 1986. Antibacterial activity of ofloxacin and its mode of action. Infection **14**(Suppl. 4):S226–S230.
14. **Sato, K., Y. Matsuura, M. Inoue, T. Une, Y. Osada, H. Ogawa, and S. Mitsuhashi.** 1982. In vitro and in vivo activity of DL-8280, a new oxazine derivative. Antimicrob. Agents Chemother. **22**:548–553.
15. **Speciale, A., S. Stefani, R. Caccamo, V. M. Nicolosi, and G. Nicoletti.** 1987. The sensitivity of gram-negative and gram-positive bacteria to ofloxacin. Drugs Exp. Clin. Res. **12**:555–561.