General Purpose Antimicrobial Ophthalmic Solutions Evaluated Using New Pharmacokinetic Parameter of Maximum Drug Concentration in Aqueous

Masamichi Fukuda and Kazuyuki Sasaki

Department of Ophthalmology, Kanazawa Medical University, Ishikawa, Japan

Purpose: In order to determine whether the one-component method for calculating drug concentration in the aqueous (AQC_{max}) is useful for selecting an appropriate ophthalmic solution, six general purpose antimicrobial ophthalmic solutions already on the market were investigated.

Methods: The drugs examined were levofloxacin (LVFX), chloramphenicol (CP), erythromycin lactobionate (EM), micronomicin sulfate (MCR), cefmenoxime hydrochloride (CMX), and disodium sulfobenzyl penicillin (SBPC). Fifty microliters of each solution was instilled into the cul-de-sac of New Zealand White rabbit eyes three times at 15-minute intervals. The drug concentrations in the aqueous humor 10, 30, 60, 120, and 240 minutes after the final instillation were examined by high performance liquid chromatography (HPLC) and/or bioassay. The AQC_{max} was calculated using the one-compartment method.

Results: The calculated AQC_{max} was 2.5 µg/mL (HPLC method) and 2.28 µg/mL (bioassay method) for LVFX, 2.17 µg/mL for CP, and 0.45 µg/mL for EM. The AQC_{max} for CMX, MCR, and SBPC could not be calculated by the one-compartment method.

Conclusions: The AQC_{max} of LVFX was higher than that of the two other general purpose antimicrobial ophthalmic solutions. The AQC_{max} of these drugs might be a useful parameter for selecting an appropriate ophthalmic solution for the treatment of infected eyes. **Jpn J Ophthalmol 2002;46:384–390** © 2002 Japanese Ophthalmological Society

Key Words: Albino rabbit, antimicrobial drug, maximum concentration in the aqueous, ophthalmic solution, pharmacodynamics.

Introduction

The development of antibiotics and synthetic antimicrobials that have a broad antimicrobial spectrum and strong antimicrobial activity has greatly contributed to the progress of the treatment of infectious diseases. However, the increase of resistant microbes due to the indiscriminate use of these drugs has become a serious problem throughout all fields of medicine. In the ophthalmological field, because the topical instillation of antibiotics and synthetic antimicrobials is the primary treatment for infectious diseases, a number of ophthalmic solutions are on the market. In the existing situation, each ophthalmologist selects the appropriate ophthalmic solution and determines the dosage regimen based only on his/her own clinical experience in using minimum growth inhibiting concentrations of the drug as parameters of antimicrobial efficacy.

We previously proposed the maximum concentration in the aqueous (AQC_{max}), which indicates the degree of ocular penetration of drugs, as a new parameter in the treatment of infectious eye diseases.[1,2] In Japan, many antimicrobial ophthalmic solutions are used in general ophthalmological clinical practice, but the ocular penetration of these solutions has

not necessarily been evaluated using the same criteria.[3,4] In addition, not all the widely used ophthalmic solutions have been developed recently, and some of them have been used for a long time. However, there have been no studies in which the ocular penetration of these ophthalmic solutions is compared and evaluated using identical criteria. In the present study, six drugs from different chemical families were selected from widely used ophthalmic solutions. The intraocular drug dynamics of these drugs was calculated as AQC$_{max}$ (μg/mL), in order to provide basic data for more appropriate selection of drugs for the treatment of bacterial ocular infection.

Materials and Methods

Materials

Ophthalmic solutions. Ophthalmic solutions used in the present study were as follows (Figure 1).

1. 0.5% levofloxacin (LVFX) ophthalmic solution (5 mg/mL) (Santen, Osaka), pH 6.2–6.8. Molecular formula: $C_{18}H_{20}FN_3O_4 \cdot 1/2\ H_2O$, molecular weight: 370.38

2. 0.5% Chloramphenicol (CP) ophthalmic solution (5 mg/mL) (Nitto Medic, Toyama), pH 6.0–8.0. Molecular formula: $C_{11}H_{12}Cl_2N_2O_5$, molecular weight; 323.13

3. 0.5% Erythromycin lactobionate (5 mg/mL)—Colistin sodium methanesulfonate (5 mg/mL) ophthalmic solution (Santen), pH 6.0–8.0
   Molecular formula of erythromycin (EM): $C_{37}H_{67}NO_{13} \cdot C_{12}H_{22}O_{12}$, molecular weight of EM: 1092.22. Molecular formula of colistin (CL) type-A: $C_{58}H_{105}N_{16}Na_5O_{28}S_5$, molecular weight of CL type-A: 1749.84. Molecular formula of CL type-B: $C_{57}H_{103}N_{16}Na_5O_{28}S_5$, molecular weight of CL type-B: 1735.82

4. 0.3% Micronomicin sulfate (MCR) ophthalmic solution (3 mg/mL) (Santen), pH 6.8–7.8. Molecular formula: $C_{20}H_{41}N_5O_7 \cdot 2\ 1/2\ H_2SO_4$, molecular weight: 708.77
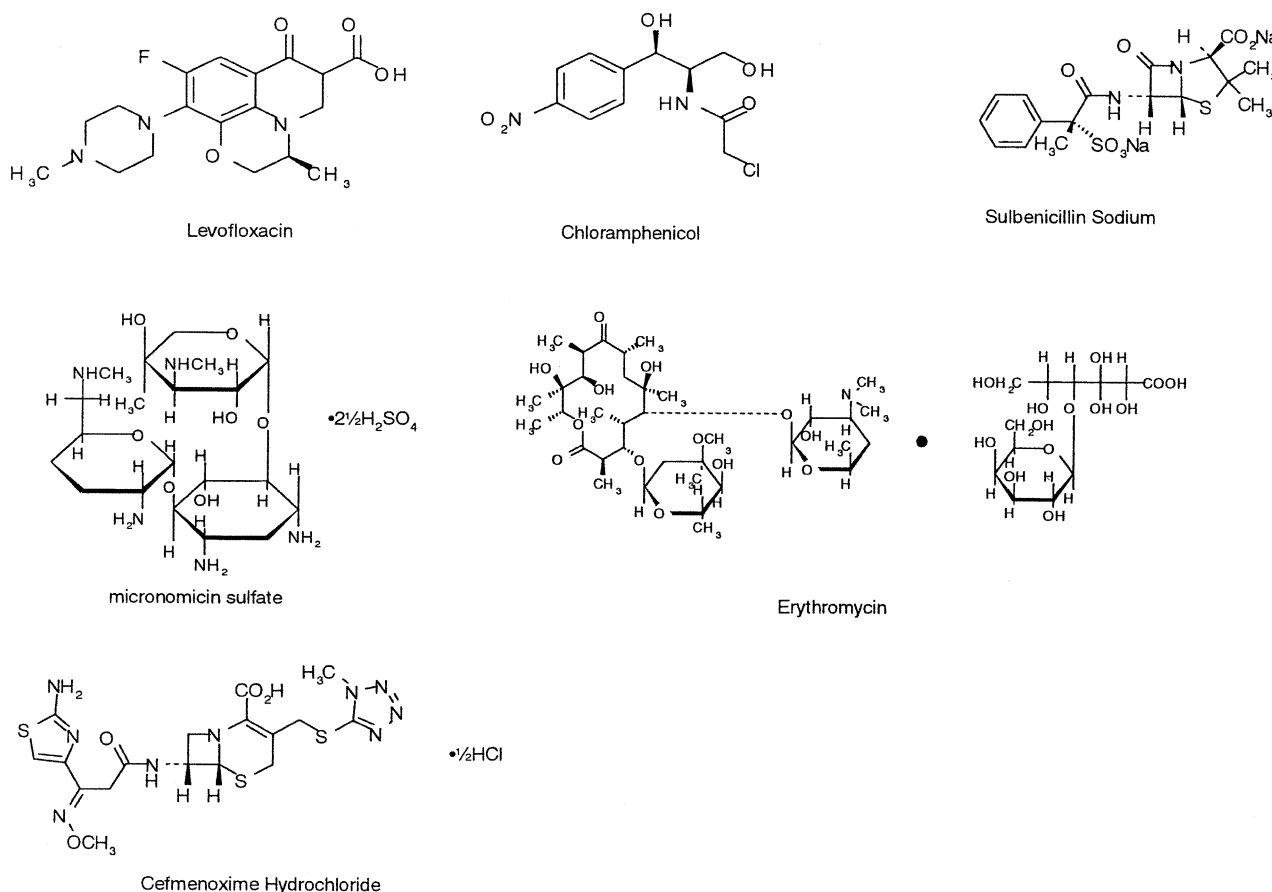

Figure 1. Chemical structures of the six antibacterial ophthalmic solutions.

5. 0.5% Cefmenoxime hydrochloride (CMX) ophthalmic solution (5 mg [titer]/mL) (Senju, Osaka), pH 6.0–8.0. Molecular formula: $C_{16}H_{17}N_9O_5S_3 \cdot$ 1/2 HCl, Molecular weight: 529.80

6. 1% Disodium sulfobenzyl penicillin (SBPC) ophthalmic solution (50 mg [titer]/5 mL) (Senju), pH 6.7. Molecular formula: $C_{16}H_{16}N_2Na_2O_7S_2$, molecular weight: 458.43

Animals

Ninety-five male New Zealand White rabbits (SPF) (body weight: 2.5–3.0 kg) were used in the present study. They were reared and sacrificed according to the Criteria for Use of Laboratory Animals in Kanazawa Medical University, and the ARVO Resolution on the Care and Use of Animals in Vision Research.

Measurement of Drug Concentration

The drug concentration was measured using the high performance liquid chromatography (HPLC) method and/or the bioassay method. LVFX[1,5] was measured using both methods. CP,[6] CMX,[7,8] and SBPC[9] were measured by the HPLC method, and EM[10] and MCR[10] were measured by the bioassay method.

HPLC Method. The instruments used were a pumping system (LC-10AD), an ultraviolet (UV) spectrophotometric detector (SPD-10AV), a fluorescence spectrophotometric detector (RF-10A), an automatic injector (SCL-10A), and a data processor (C-R7A). The column used for drug analysis was Shimpack CLC-ODS (all instruments were produced by Shimadzu, Kyoto).

HPLC conditions for each ophthalmic solution were as follows.

LVFX: Column temperature: 50°C, Mobile phase: a mixture of acetonitrile and 0.04 M phosphoric acid (1:4) (v/v), flow rate: 1.0 mL/min, detection: at 296 mn of excitation wave length (Ex) and at 504 nm of fluorescent wave length (Em).

CP: Column temperature: 35°C, Mobile phase: a mixture of methanol and distilled water (4:6) (v/v), flow rate: 1.0 mL/min, detection: at UV 278 nm.

CMX: Column temperature: 45°C, mobile phase: a mixture of acetonitrile and 0.05 M ammonium acetate (1:4) (v/v), flow rate: 1.0 mL/min, detection: at UV 278 nm.

SBPC: Column temperature: 45°C, Mobile phase: 0.05 M tetra-n-butylammonium containing a mixture of 0.04 M phosphoric acid (pH 2.5) and

acetonitrile (70:30) (v/v), flow rate: 1.0 mL/min, detection: at UV 236 nm.

The quantification limit was 0.078 µg/mL in LVFX, 0.078 µg/mL in CP, 0.039 µg/mL in CMX, and 0.156 µg/mL in SBPC.

Bioassay Method. Bioassay was performed using the cylinder agar plate method (the cup method), and quantification procedure was conducted according to the Japan Antibiotics Drug Research Association Standards.

Preparation of inocula suspension. Approximately 1×10^8 cfu/mL (stock solution) of *Bacillus subtilis*, ATCC6633, was diluted five-fold (approximately 2×10^7 cfu/mL). Then 1 mL each of this suspension was added to each 100 mL of medium to prepare approximately 2×10^5 cfu/mL of inocula suspension.

Media

Agar medium for seed layer and that for base layer were prepared according to Japan Antibiotic Drug Standards: 6.0 g peptone, 1.0 g glucose, 3.0 g yeast extract, 13.0–20.0 g agar, 1.5 g meat extract
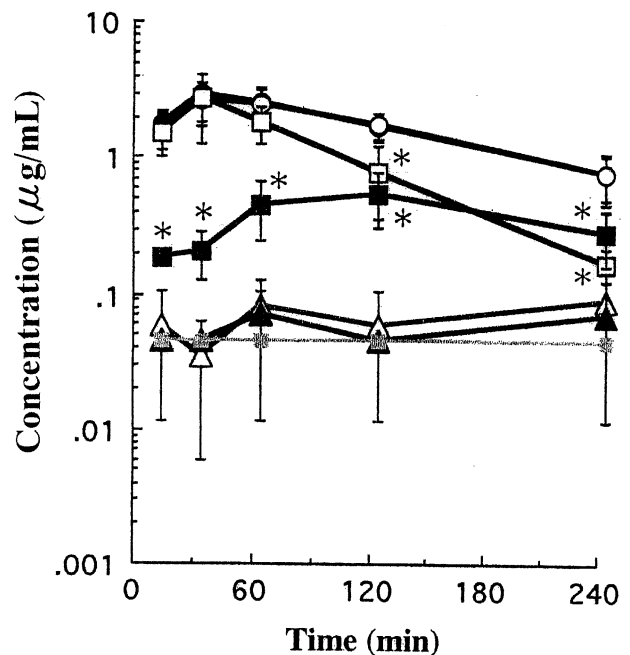

Figure 2. Drug concentrations of each ophthalmic solution in rabbit aqueous. •: levofloxacin (LVFX)-high-performance liquid chromatography, ○: LVFX-bioassay, □: chloramphenicol, ■: erythromycin lactobionate, △: cefmenoxime hydrochloride (CMX), ▲: micronomicin sulfate (MCR), ■: disodium sulfobenzyl penicillin (SBPC). $*P < .005$. The results of comparing drug concentration of LVFX with that of other drugs. Statistical analysis could not be performed for CMX, MCR, or SBPC.

Table 1. Drug Dynamics in Aqueous of Ophthalmic Solutions*

Ophthalmic Solution (%)[†]	10 Minutes	30 Minutes	60 Minutes	120 Minutes	240 Minutes
LVFX (0.5)(H)	1.53 ± 0.38	2.57 ± 1.00	2.33 ± 0.60	1.47 ± 0.34	0.67 ± 0.24
LVFX (0.5)(B)	1.38 ± 0.42	2.26 ± 0.78	2.11 ± 0.62	1.49 ± 0.33	0.66 ± 0.27
CP (0.5)	1.30 ± 0.44	2.33 ± 1.23	1.57 ± 0.48	1.67 ± 0.36	0.15 ± 0.04
EM (0.5)	0.16 ± 0.00	0.18 ± 0.07	0.39 ± 0.18	0.47 ± 0.20	0.25 ± 0.11
CMX (0.5)	0.05 ± 0.04	0.03 ± 0.03	0.07 ± 0.02	0.05 ± 0.04	0.08 ± 0.03
MCR (0.3)	0.04 ± 0.00	0.04 ± 0.00	0.06 ± 0.05	0.04 ± 0.00	0.06 ± 0.05
SBPC (1.0)	< 0.039	< 0.039	< 0.039	< 0.039	< 0.039

*Values are mean ± SD μg/mL.

[†]n = 6. LVFX: levofloxacin, H: high-performance liquid chromatography method, B: bioassay (cup) method, CP: chloramphenicol, EM: erythromycin lactobionate, CMX: cefmenoxime hydrochloride, MCR: micronomicin sulfate, SBPC: disodium sulfobenzyl penicillin.

were weighed and added to water to make 1,000 mL of media at pH 6.5–6.6.

Preparation of sample solutions and quantification limit of each drug. Ten milligrams (titer) was adjusted to 100 mL using artificial aqueous humor (BSS Plus®), and 100 μg (titer)/mL solution was prepared. This solution served as a standard stock solution. The standard stock solution was sequentially diluted by a factor of double with artificial aqueous humor, and 10, 5, 2.5, 1.25, 0.625, 0.312, 0.156 and 0.078 μg (titer)/mL of solutions were prepared. The collected aqueous humor was used undi-

luted as test samples. Quantification limit was 0.312 μg/mL in EM, 0.078 μg/mL in MCR, and 0.312 μg/mL in LVFX.

Study Methods

Drug concentrations in aqueous. Exactly 50 μL of each ophthalmic solution was measured using a micropipette, and was instilled into the cul-de-sacs of rabbits. This treatment was repeated three times at intervals of 15 minutes. After anesthetizing the rabbits with 0.4% topical oxybuprocaine hydrochloride (Benoxil®), a 27-G × 1/2 needle was inserted into the anterior sac and approximately 100 μL of aque-
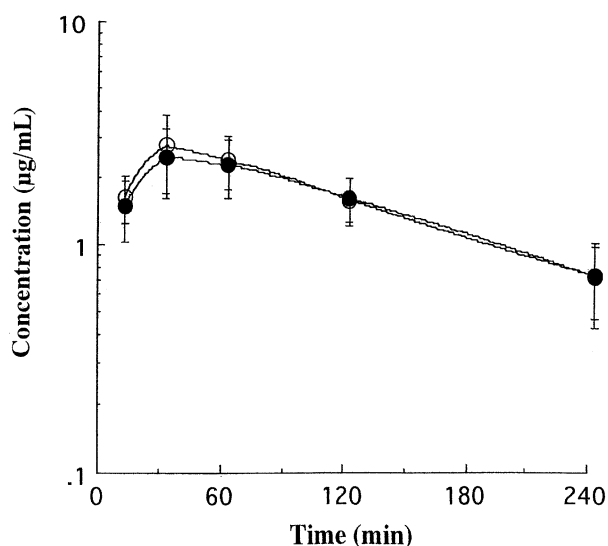

Figure 3. Comparison of measured drug concentrations between high-performance liquid chromatography (HPLC) and bioassay methods. ○: HPLC method, ●: bioassay method. The drug concentration in aqueous after treatment with 0.5% levofloxacin ophthalmic solution was measured using both methods, and no significant differences were observed at any time point.
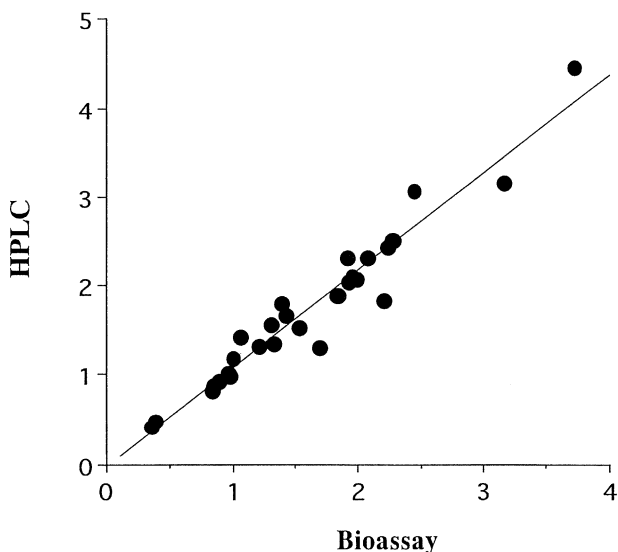

Figure 4. Correlation of measured drug concentrations by high-performance liquid chromatography (HPLC) and bioassay methods. R^2 = 0.936. A strong correlation of measured drug concentrations between HPLC and bioassay methods was observed.

Table 2. Pharmacokinetic Parameters of Three
Ophthalmic Solutions

Ophthalmic Solution (%)*	AQC_{max}[†] (μg/mL)	t_{max}[‡] (h)	AUC[§] (μg/h/mL)	$t_{1/2}$[∥] (h)
LVFX (0.5)(H)	2.5	0.59	7.39	1.58
LVFX (0.5)(B)	2.28	0.64	7.42	1.75
CP (0.5)	2.17	0.51	3.46	0.63
EM (0.5)	0.43	1.76	2.07	1.22

*LVFX: levofloxacin, CP: chloramphenicol, EM: erythromycin lactobionate.
[†]ACQ_{max}: maximum drug concentration in aqueous.
[‡]t_{max}: maximum time to peak concentration.
[§]AUC: area under the concentration–time curve in aqueous.
[∥]$t_{1/2}$: elimination half-life.

ous was collected 10, 30, 60, 120, and 240 minutes after the final instillation to be used as samples.

Comparison of measured values between HPLC and bioassay methods. The LVFX ophthalmic solution (0.5%) was instilled into rabbit eyes in the same manner as above, and intraocular drug concentration was measured through HPLC and bioassay methods using the obtained aqueous samples.

Calculation of pharmacokinetic parameters. AQC_{max}, time to peak concentration (t_{max}), area under the concentration-time curve in aqueous (AUC), and elimination half-life ($t_{1/2}$) were calculated using the nonlinear least-square method according to the one-compartment model,

$$C_P = P_1*[P_2/(P_2 - P_3)*(e^{p3t} - e^{p2t})].[11]$$

Correlation between instillation dose and AQC_{max}. The AQC_{max} of each ophthalmic solution calculated using the one-compartment model was divided by the instillation dose to obtain the penetration ratio to aqueous. As the value for CMX, SBPC, and MCR, the highest of actual measurement values was used.

Table 3. Correlation Between Instillation Dose and Maximum Concentration in the Aqueous (AQC_{max})

Ophthalmic Solution (%)*	Instillation Dose (μg)	AQC_{max} (μg/mL)	AQC_{max}/Instillation Dose Ratio ($\times 10^{-3}$)
LVFX (0.5)	750	2.5	3.33
CP (0.5)	750	2.17	2.89
EM (0.5)	750	0.43	0.57
SBPC (1.0)	1500	0.04	0.03
MCR (0.3)	450	0.06	0.13
CMX (0.5)	750	0.08	0.11

*LVFX: levofloxacin, CP: chloramphenicol, EM: erythromycin lactobionate, SBPC: disodium sufobenzyl penicillin, MCR: micronomicin sulfate, CMX: cefmenoximehydrochloride.

Results

Drug Concentrations in Aqueous Humor

The maximum drug concentration of LVFX measured in the aqueous humor was 2.57 ± 1.00 μg/mL (HPLC method), and that of CP was 2.33 ± 1.23 μg/mL (HPLC method). The concentration of EM was 0.47 ± 0.20 μg/mL (bioassay method). A very small quantity of drug concentration of CMX and MCR was detected in the aqueous. The concentration of SBPC (HPLC method) was below the detection limit of 0.04 (Figure 2, Table 1). The maximum drug concentration of LVFX and CP in the aqueous were observed 30 minutes after the instillation, and that of EM, 120 minutes after the instillation.

Comparison of measured values between HPLC and bioassay methods. The LVFX ophthalmic solution (0.5%) was instilled in rabbit eyes, and intraocular drug concentration was measured by HPLC and bioassay methods using the aqueous samples. The maximum concentration 30 minutes after instillation was 2.57 ± 1.00 μg/mL using the HPLC method and 2.26 ± 0.78 μg/mL using the bioassay method. The concentration gradually decreased to 0.67 ± 0.24 μg/mL in the HPLC method and 0.66 ± 0.27 μg/mL in the bioassay method 240 minutes after the instillation. The values measured at each time point using the HPLC method were not statistically significantly different from those using the bioassay method. In addition, strong correlation was seen between the values measured using these two methods; the correlation coefficient (R^2) was 0.936 (Figures 3 and 4).

Calculation of pharmacokinetic parameters. The AQC_{max} was 2.50 μg/mL (HPLC method) and 2.28 μg/mL (bioassay method) for LVFX, 2.17 μg/mL for CP, and 0.45 μg/mL for EM. The AQC_{max} for CMX, MCR, and SBPC could not be calculated. Other parameters of each ophthalmic solution including t_{max}, AUC, and $t_{1/2}$ are shown in Table 2.

Correlation between instillation dose and AQC_{max}. The LVFX (0.5%) solution showed the largest value of penetration ratio to the aqueous (AQC_{max}/instillation dose) (3.33 $\times 10^{-3}$), and the other values in decreasing order are 0.5% CP (2.89 $\times 10^{-3}$), 0.5% EM (0.57 $\times 10^{-3}$), MCR, CMX, and SBPC (Table 3).

Discussion

In the present study, the six representative antimicrobial drugs from different chemical families were selected from widely used ophthalmic solutions, and the intraocular drug dynamics of these drugs were
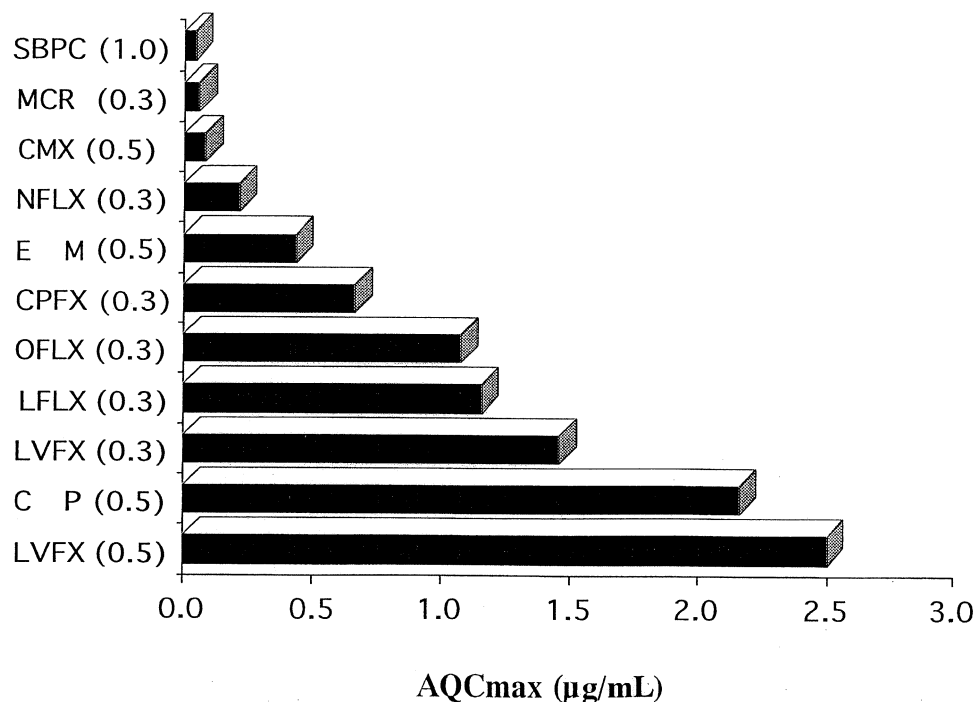
Figure 5. Comparison of maximum drug concentrations in aqueous (AQC$_{max}$) of 11 ophthalmic solutions.

compared using a new parameter, AQC$_{max}$. The intraocular drug dynamics of all the drugs selected in the present study have already been investigated in the process of drug development. The measurement method used has been mainly the bioassay method for the early generation drugs, while the HPLC method alone, or both the HPLC and bioassay methods were used for the relatively new generation drugs. The bioassay method measures drug concentration considering antibacterial activity, and the measurement of the dynamics of antibiotics and antimicrobials using the bioassay method is always necessary. Only in specialized studies is the bioassay method used together with the HPLC method to investigate the intraocular drug dynamics of antibiotics.

The same holds true for the six drugs used in the present study. We measured the intraocular drug concentration of not all six drugs but only of LVFX using both methods. No significant difference was observed between values obtained for LVFX through the HPLC and bioassay methods. It was the first time these authors attempted to obtain AQC$_{max}$ using values measured through the bioassay method. We methodologically confirmed that both methods are reliable for investigating intraocular drug dynamics, as long as they are performed with highly accurate techniques. Some of the present authors had

previously compared intraocular concentrations of 5 antimicrobial ophthalmic solutions of the same family (fluoroquinolones) using AQC$_{max}$.[1] The physicochemical characteristics of each drug differed, and it could be presumed to some extent that the intraocular drug dynamics of each drug also differed. More objectively, the intraocular drug dynamics of each drug could have been compared by using AQC$_{max}$ as a parameter[1,2] in the previous study.

In the present study, the AQC$_{max}$ of six drugs of various families other than fluoroquinolones was calculated. The AQC$_{max}$ of LVFX was the highest (2.57 μg/mL), followed by that of CP (2.33 μg/mL). The AQC$_{max}$ of EM of macrolides was 0.47 μg/mL, indicating that ocular penetration was relatively favorable. The AQC$_{max}$ of CMX, MCR, or SBPC could not be calculated, and it was confirmed that penetration into the aqueous of these three drugs was remarkably different from that of LVFX, CP, and EM (Figure 5). We previously confirmed that the AUC, which indicates biological availability, was a useful parameter for the evaluation of drug dynamics in the aqueous humor, just as is AQC$_{max}$.[1] Based on this AUC and the penetration ratio to aqueous obtained from AQC$_{max}$, and the instillation dose of each drug (a parameter indicating the extent of penetration of drugs themselves), the penetration of LVFX, fol-
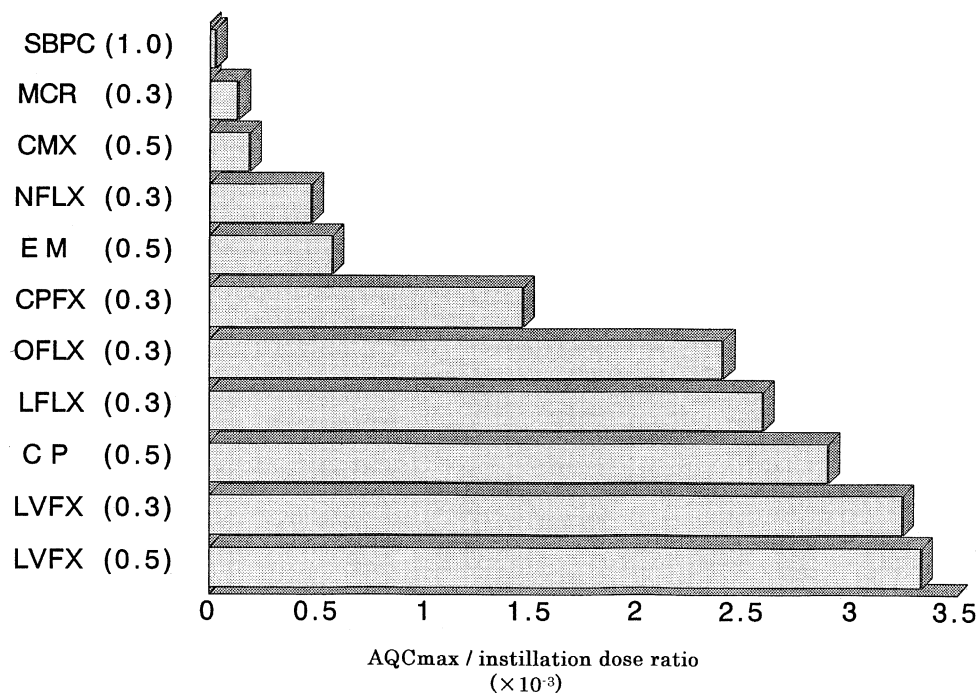
Figure 6. Correlation between instillation dose and maximum drug concentrations in aqueous (AQC_{max}).

lowed by that of CP and then EM, has been confirmed through the present study to be the most favorable (Figure 6). Most bacterial infectious diseases in the ophthalmological field are extraocular infectious diseases, and drugs are not necessarily required to penetrate into intraocular regions unless the infection is severe. Thus, clinical effects by even the ophthalmic solutions with low AQC_{max} can be expected if the drugs have effective antibacterial activity. However, it is needless to say that the ophthalmic solutions with high AQC_{max} are preferable for treating the cases of infectious disease when intraocular penetration of the drug is required. The minimum inhibitory concentration as a parameter of antimicrobial activity should certainly be valued in selecting drugs, but we authors believe that it is necessary also to consider AQC_{max} in the treatment of infected eyes.

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