Kinetics of Quinolone Antibiotics in Rats: Efflux from Cerebrospinal Fluid to the Circulation

Tsuyoshi Ooie,[1,2] Hiroshi Suzuki,[1] Tetsuya Terasaki,[1] and Yuichi Sugiyama[1,3]

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Purpose. An active transport system, which pumps quinolone antimicrobial agents (quinolones) from cerebrospinal fluid (CSF) to systemic blood, exists at the choroid plexus, an epithelial tissue that forms the blood-CSF barrier (BCSFB). The present study was carried out to clarify the contribution of this transport system to the disposition of quinolones in the central nervous system.

Method. Six quinolones were administered intracerebroventricularly to rats and their elimination from the CSF was examined. The inhibitory effect of probenecid and quinolones on the efflux of fleroxacin from the CSF was also examined. Probenecid or two types of quinolone (AM-1155, pefloxacin) were co-administered intracerebroventricularly with fleroxacin.

Results. The elimination clearance from the CSF for norfloxacin, AM-1155, fleroxacin, ofloxacin, sparfloxacin and pefloxacin was 14, 22, 21, 20, 47 and 35 μl/min/rat, respectively. An approximately 3.5-fold difference was thus observed between norfloxacin and sparfloxacin. These values were 4- to 14-fold larger than the [^{14}C]mannitol clearance. Furthermore, the elimination clearance of quinolones from the CSF was 7- to 60-fold larger than the active efflux clearance at the BCSFB estimated from our previous *in vitro* data. Co-administration of AM-1155, pefloxacin and probenecid did not inhibit the elimination of fleroxacin from the CSF.

Conclusions. The active transport system at the BCSFB plays only a small part in the elimination of quinolones from the CSF. Passive diffusion *via* the BCSFB and diffusion across the ependymal surface into brain extracellular fluid, followed by efflux across the blood-brain barrier, may be the predominant pathway for quinolone elimination from the CSF.

KEY WORDS: quinolone antibiotics; cerebrospinal fluid; blood-CSF barrier; choroid plexus; blood-brain barrier.

INTRODUCTION

The clinical importance of quinolone antimicrobial agents (quinolones) is due to their high level of activity against Gram-positive and Gram-negative pathogens (1). Studies on tissue penetration show that concentrations exceeding plasma levels are obtained in most tissues, except the central nervous system (CNS) (2). It has been reported that steady-state concentrations of these quinolones in rat brain and cerebrospinal fluid (CSF) are 1/10 to 1/2 lower than the unbound serum concentrations (3) and, thus, the CNS distribution of quinolones seems to be restricted. In our previous study using quantitative brain microdialysis (4), we reported that brain extracellular fluid concentrations of quinolones are 1/7 to 1/30 lower than the unbound serum concentrations. In addition, we demonstrated that the extracellular fluid concentrations of quinolones are about 1/2 lower than that of CSF, which suggested that an active efflux transport system at the blood-brain barrier (BBB) is responsible for maintaining brain concentrations lower than unbound serum concentrations at steady-state.

CSF is a nearly protein-free fluid that surrounds the brain. It is continuously secreted by the choroid plexus and reabsorbed into venous blood at the arachnoid villi (bulk flow) (5). There is free passage of molecules between the CSF and extracellular fluid of the brain. At the choroid plexus, which forms the blood-CSF barrier (BCSFB), there is an exchange of molecules between the CSF and systemic circulation. Therefore, at least three possible mechanisms can be postulated to explain the lower concentration of drugs in CSF compared with that in circulating blood, *i.e.*, (i) an active efflux system at the BCSFB is efficient enough to reduce the CSF concentration, (ii) clearance for the drug entering the CSF is significantly lower than the formation rate of the CSF and (iii) an active efflux system at the BBB is efficient enough to reduce the CSF concentration of drugs (6). We have demonstrated that active transport located on the choroid plexus (BCSFB) is a major factor in determining the pharmacokinetics of some β-lactam antibiotics in CSF (6–8).

In our previous study (9), we demonstrated that some quinolones are accumulated by the isolated choroid plexus due to an active transport system shared by benzylpenicillin. The present study, therefore, was undertaken to clarify whether and to what extent this active transport system contributes to the elimination of quinolones from the CSF *in vivo*. Six quinolones were administered intracerebroventricularly to rats and their elimination from the CSF was determined. Furthermore, in order to characterize the active efflux of quinolones from the CSF, we examined the inhibitory effect of probenecid and several quinolones on the elimination of quinolones from the CSF. Fleroxacin (FLRX), a fluorinated quinolone, was used as a model compound. We co-administered probenecid or two different quinolones (AM-1155, pefloxacin (PFLX)) intracerebroventricularly with FLRX.

MATERIALS AND METHODS

Materials

D-[^{14}C]Mannitol (1.85–2.29 GBq/mmol) was purchased from Amersham International (Buckinghamshire, UK). All the quinolones (norfloxacin (NFLX), FLRX, AM-1155 [(±)-1-cyclopropyl-6-fluoro-1,4-dihydro-8-methoxy-7-(3-methyl-1-piperazinyl)-4-oxo-3-quinolinecarboxylic acid], ofloxacin (OFLX), sparfloxacin (SPFX) and PFLX) were synthesized at the Central Research Laboratories of Kyorin Pharmaceutical Co., Ltd. (Tochigi, Japan). Probenecid was purchased from Sigma Chemical Co. (St. Louis, MO). All other chemicals were commercial products of analytical grade.

Animals

Male Wistar rats weighing 300–320 g were used in all experiments, which were carried out according to the guidelines

[1] Department of Pharmaceutics, Faculty of Pharmaceutical Sciences, The University of Tokyo, Hongo, Bunkyo-ku, Tokyo 113, Japan.

[2] Present address: Central Research Laboratories, Kyorin Pharmaceutical Co., Ltd., Nogi, Nogi-machi, Shimotsuga-gun, Tochigi 329-01, Japan.

[3] To whom correspondence should be addressed.

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provided by the Institutional Animal Care Committee (Faculty of Pharmaceutical Sciences, The University of Tokyo).

Efflux of [^{14}C]mannitol and Quinolones from the CSF

The efflux of [^{14}C]mannitol and quinolones after intracerebroventricular administration was studied by a method described previously (8). Rats were anesthetized with urethane (ethylcarbamate, 1.5 g/kg, i.p.) and their heads were fixed in a stereotaxic apparatus. An 18-gauge needle connected to silastic tubing was inserted into the right ventricle through a hole drilled in the skull. An intracerebroventricular dose of [^{14}C]mannitol (1.78 kBq) or quinolones (12.5 nmol) dissolved in 10 μl artificial CSF was administered through the cannula. The prepared artificial CSF contained NaCl, 122 mM; $NaHCO_3$ 25 mM; glucose, 10 mM; KCl, 3 mM; $CaCl_2$, 1.4 mM; $MgSO_4$, 1.2 mM; K_2SO_4, 0.4 mM; and 4-(2-hydroxyethyl)-n-piperazine ethanesulfonic acid, 10 mM, pH 7.3, equilibrated with 95 % O_2–5 % CO_2 gas at 37°C (8). Crystal-clear CSF samples were obtained by cisternal puncture with a 24-gauge needle at 2.5, 3.5, 5, 7, 12, 15, 30, 45 min after dosing. We collected the CSF specimens (50–80 μl) just before sacrificing the rats at the different time points. At 5 and 30 min, the CSF was collected in triplicate, whereas a single collection was made for the other time points.

The inhibitory effect of probenecid, AM-1155 and PFLX on the elimination of FLRX from the CSF was also studied. Probenecid (75 nmol), AM-1155 (500 nmol) and PFLX (125 nmol and 500 nmol) were simultaneously administered intracerebroventricularly with FLRX (12.5 nmol) to determine the FLRX concentration in cisternal CSF 30 min after administration. The dose-dependence of FLRX elimination from the CSF was examined by administering an intracerebroventricular dose of 500 nmol. The dose of probenecid and quinolones was determined by considering their inhibitory effect on the accumulation of [^{14}C]FLRX by the isolated choroid plexus (3,9), as well as the distribution volume of each compound in the CSF (Vd_{CSF}, see Results); after administration of 75 nmol probenecid, the initial probenecid concentration in the CSF can be calculated to be 300 μM, which was approximately 6 times higher than its IC_{50} value determined *in vitro* (9). This amount of probenecid was high enough to reduce the CSF elimination of benzylpenicillin after intracerebroventricular administration (6). In the same manner, the intracerebroventricular administration of 500 nmol FLRX, PFLX and AM-1155 results in initial CSF concentration of these quinolones of 1200 μM, 1700 μM and 1300 μM, respectively, which is comparable or higher than their IC_{50} values determined *in vitro* (3,9).

Determination of Drug Concentration

Concentrations of quinolones in CSF were determined by HPLC as described previously (4). The [^{14}C]mannitol radioactivity was determined using a liquid scintillation spectrophotometer (model LS6000SE, Beckman Instruments Inc., Berkeley, CA). The counting efficiency and crossover correction were determined by the external standard technique.

Kinetic Analysis

The dose-normalized CSF concentration (C_{CSF} (% of dose/ml CSF)) of [^{14}C]mannitol and quinolones versus time (t) data were fitted to Equation 1 using nonlinear regression analysis (10);

$$C_{CSF} = 100 / Vd_{CSF} \times \exp(-CL_{CSF} / Vd_{CSF} \times t) \quad (1)$$

where, Vd_{CSF} is the distribution volume in CSF and CL_{CSF} denotes the elimination clearance from CSF, respectively.

Results are shown as means ± calculated s.d. except when noted otherwise.

Estimation of Active Efflux Clearance at BCSFB

To determine the contribution of the choroid plexus to the elimination of intracerebroventricularly administered quinolones from the CSF, the active efflux clearance of quinolones across the choroid plexus (PS_{act}) was calculated from the kinetic parameters obtained *in vitro* (3,9) described as follows; *in vitro*, [^{14}C]FLRX was accumulated by the rat choroid plexus *via* a saturable process (K_m = 664 μM, V_{max} = 240 pmol · min^{-1} · μl tissue^{-1}). We can calculate the uptake clearance of FLRX by the choroid plexus as V_{max} / K_m. Taking into account the tritiated water space of the rat choroid plexus (6 μl/rat; Ref. 6), we extrapolated the active efflux clearance of FLRX from CSF *in vivo* ($PS_{act,FLRX}$). Based on the assumption that the maximum velocity of transport (V_{max}) is similar for all quinolones, the efflux clearance of other quinolones by the choroid plexus ($PS_{act,quinolones}$) was calculated as follows:

$$PS_{act,quinolones} = PS_{act,FLRX} \times K_{m,FLRX} / IC_{50,quinolones} \quad (2)$$

where $IC_{50,quinolones}$ is the inhibition constant of quinolones for the accumulation of [^{14}C]FLRX by the choroid plexus.

RESULTS

Figure 1 illustrates the time profiles for the dose-normalized CSF concentrations (C_{CSF}) of six quinolones and [^{14}C]mannitol after intracerebroventricular administration to rats. All quinolones examined were eliminated from the CSF faster than

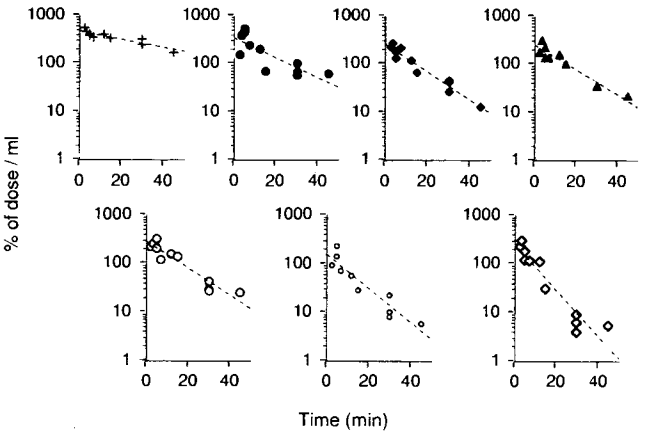

Fig. 1. CSF concentration—time profiles for quinolones and [^{14}C]mannitol after intracerebroventricular administration. Quinolones (12.5 nmol) or [^{14}C]mannitol (1.78 kBq) were administered intracerebroventricularly. At 2.5, 3.5, 5, 7, 12, 15, 30, 45 min after dosing, CSF was obtained from each rat by cisternal puncture. Each point represents a measurement obtained from one rat. The CSF concentrations at 5 and 30 min after administration were determined in triplicate, whereas a single determination was performed for other time points. Lines were calculated by non-linear least squares regression analysis: (+) Mannitol, (●) NFLX, (♦) AM-1155, (▲) FLRX, (○) OFLX, (○) SPFX, (◇) PFLX.

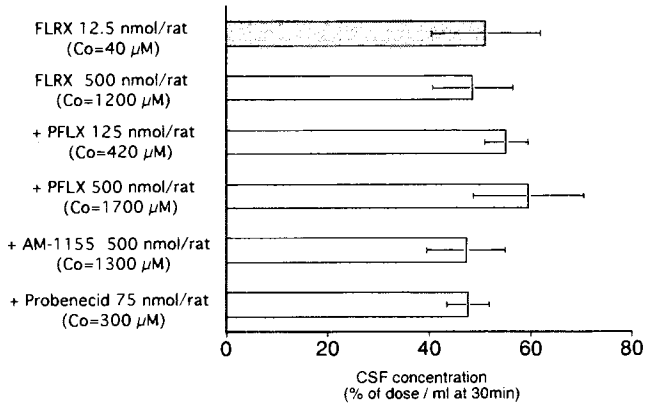

Fig. 2. Inhibitory effect of probenecid and quinolones on the elimination of FLRX from rat CSF *in vivo*. An intracerebroventricular dose of FLRX (12.5 nmol), with or without ligands, was administered to rats. After 30 min, the concentration of FLRX in the cisternal CSF was determined. Each column represents the mean ± s.e. of six experiments. Numbers in parenthesis represent the initial CSF concentrations (Co) of ligands calculated from their dose and distribution volume in CSF.

[^{14}C]mannitol. The CL_{CSF} and the Vd_{CSF} of each ligand are listed in Table I. An approximately 3.5-fold difference in CL_{CSF} was observed between SPFX and NFLX (Table I). The extrapolated PS_{act} value of quinolones from the *in vitro* data was about 1/7 to 1/60 smaller than the CL_{CSF} (Table I).

We examined the characteristics of the elimination of intracerebroventricularly administered FLRX. Figure 2 shows that the C_{CSF} of FLRX 30 min after intracerebroventricular administration was 51 ± 11 % of the dose/ml (mean ± s.e.). This value was unaffected even by administration of a forty-fold higher dose of FLRX (49 ± 8 % of dose/ml, mean ± s.e.) (Fig. 2). Furthermore, simultaneous administration of PFLX (500 nmol/rat), AM-1155 (500 nmol/rat) or probenecid (75 nmol/rat) did not affect the C_{CSF} of FLRX at 30 min (Fig. 2).

DISCUSSION

The transport system located on the choroid plexus has been postulated to play an important role in the elimination of several β-lactam antibiotics (such as benzylpenicillin) from the CSF to the blood (6,11). We have also demonstrated that those quinolones are transported by the isolated choroid plexus *in vitro via* the active transport system shared by benzylpenicillin (9), suggesting that quinolones can be eliminated from the CSF across the choroid plexus. The PS_{act} for quinolones extrapolated from the *in vitro* data (Table I) was comparable with the bulk flow rate of CSF (2.9 μl/min/rat, Ref. 12). The CL_{CSF} of quinolones *in vivo*, however, was 7- to 60-fold greater than PS_{act}, suggesting that this active transport system may play only a small role in elimination of quinolones from the CSF. The fact that no significant inhibitory effect on the elimination of intracerebroventricularly administered FLRX (Fig. 2) was produced by probenecid, PFLX and AM-1155 or an excess of FLRX further supports this hypothesis.

Based on the kinetic model proposed by Collins and Dedrick (13), CL_{CSF} is described by the following equation (6):

$$CL_{CSF} = Q + PS_{BCSFB} + PS_{act} + \sqrt{Ar^2 \times D_{app} \times PS_{BBB} \times Vbr} \quad (3)$$

where, Q is the bulk flow rate of CSF (2.9 μl/min/rat, Ref. 12), PS_{BCSFB} is the passive diffusion clearance across the choroid plexus. The fourth term in Equation 3, which consists of the area of the ependymal surface (Ar), diffusivity of drug in the brain tissue (D_{app}), efflux clearance at the BBB (PS_{BBB}) and volume of distribution in the brain tissue (Vbr), represents ligand diffusion loss across the ependymal surface, *i.e.*, diffusion into the brain extracellular fluid followed by efflux across the BBB.

Figure 3 shows the contribution of PS_{act} (Table I) to the CL_{CSF} of quinolones observed *in vivo*. The CL_{CSF} of quinolones was 3- to 8-fold greater than the sum of the CSF bulk flow rate and the PS_{act}, which was in marked contrast to the situation for mannitol whose elimination was predominantly (85 %) mediated by bulk flow (Table I, Fig. 3). This result could be accounted for by passive elimination across the BCSFB and/or BBB, since the octanol to buffer partition coefficients (expressed as log P′) of the quinolones ranged from −1.34 to 0.06 (3), which are higher than that of mannitol (−3.1, Ref. 14). The passive diffusion of quinolones across the BBB and choroid plexus, which is related to the lipophilicity of these drugs, may be larger than that of

Table I. Elimination Clearance (CL_{CSF}), Distribution Volume of CSF (Vd_{CSF}) and Active Efflux Clearance at the Choroid Plexus (PS_{act}) of [^{14}C]mannitol and Quinolones in Rats

	CL_{CSF} (μl/min/rat)[a]	Vd_{CSF} (ml/rat)	PS_{act} (μl/min/rat)[b]
[^{14}C]Mannitol	3.38 ± 0.50	0.184 ± 0.012	
NFLX	13.6 ± 3.6	0.257 ± 0.067	0.243
AM-1155	22.3 ± 2.2	0.321 ± 0.039	1.05
FLRX	20.5 ± 2.4	0.338 ± 0.043	2.17
OFLX	19.5 ± 2.2	0.287 ± 0.037	3.05
SPFX	46.6 ± 8.1	0.520 ± 0.123	2.73
PFLX	34.5 ± 3.5	0.248 ± 0.040	4.81

[a] Each value represents the mean ± calculated s.d.
[b] Calucurated from kinetic parameters obtained *in vitro* (see text).

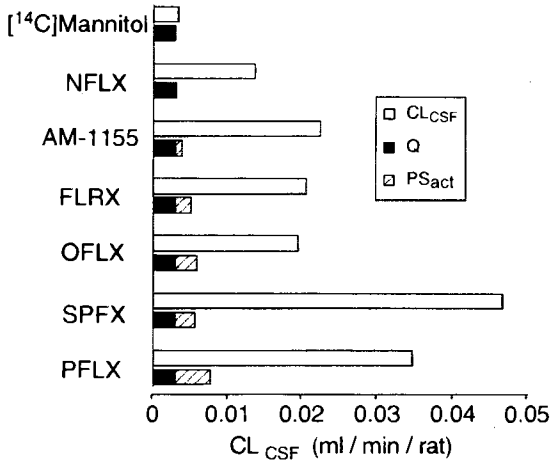

Fig. 3. Contribution of active efflux clearance at the BCSFB (PS_{act}) to the efflux clearance of [^{14}C]mannitol and quinolones from the CSF observed *in vivo* (CL_{CSF}). The contribution of the bulk flow (Q) of CSF (determined by blue dextran, Ref. 13) is also illustrated. PS_{act} was calculated from the kinetic parameters obtained *in vitro* (3,6) (see text).

mannitol and, thus, the second and fourth terms of Equation 3 can play a predominant role in the CL_{CSF} of quinolones.

This hypothesis was also supported by the following *in vivo* experiments; we determined the clearance for the unidirectional influx of quinolones by measuring the initial uptake of quinolones into the CSF after intravenous administration and found a good correlation between log P′ and this influx clearance (unpublished observation). The result suggests that the influx of the quinolones into CSF across the choroid plexus may be accounted for by the passive diffusion. PS_{BCSFB}, in turn, should be related to the lipophilicity. Further analysis based on the distributed model proposed by Collins and Dedrick (13) indicated that 38 % and 37 % of the elimination of FLRX from the CSF can be accounted for by passive diffusion across the BCSFB and by diffusional loss across the ependyma followed by transport across the BBB, respectively (unpublished observation). Although the surface area of the BBB is 5,000 times greater than that of the BCSFB (15), the contribution of the BBB to the elimination on intracerebroventricularly administered FLRX was comparable with that of the BCSFB. The result may be accounted for by assuming that diffusion into the brain parenchyma may be the rate-determining process for ligand elimination from the brain. We also found that there was no significant correlation between the previously reported CSF-to-unbound serum concentration ratio of intravenously administered quinolones at steady-state (3) and CL_{CSF} listed in Table I, suggesting that the difference in the efflux process cannot be the determining factor for the difference in CSF disposition after systemic administration.

In the present study, we took 50–80 μl CSF from each rat to determine the mean ligand concentration in the CSF. This methodology can be justified by previous observations by Seki *et al.* (16) who found that 50–100 μl of rat CSF obtained by cisternal puncture may correspond to the ventricular CSF. Although uneven CSF distribution of ligands has been reported in goats (17), dogs (18) and cats (19) due to a difference in transport ability between the lateral and fourth ventricular choroid plexus, such an uneven distribution may not be present in rats due to the comparable ability of the choroid plexus between these ventricles (20). In our previous study, Ogawa *et al.* (6) carried out a kinetic analysis of the CSF transport of β-lactam antibiotics both *in vitro* and *in vivo*. Kinetic parameters obtained from the cisternal CSF concentration of this ligand after intracerebroventricular administration were comparable with those determined in *in vitro* transport studies (6). These results suggest that CSF concentrations determined in this way may represent the mean CSF concentration.

In conclusion, the elimination of quinolones from the CSF after intracerebroventricular administration exhibited about a 3.5-fold difference among the quinolones studied. The contribution of active transport at the BCSFB may be small, thus, this difference is probably due to differences in passive diffusion at the BCSFB and efflux permeability (passive + active) at the BBB for these compounds.

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