Entry of the New Quinolone Antibacterial Agents of Ofloxacin and NY-198 into the Central Nervous System in Rats

Hitoshi SATO,* Eiichi OKEZAKI,** Setsuko YAMAMOTO,* Osamu NAGATA,** Hideo KATO** and Akira TSUJI*,***

Faculty of Pharmaceutical Sciences, Kanazawa University, * Takara-machi, Kanazawa, 920, Japan and Central Research Laboratory, Hokuriku Seiyaku Co., Ltd., ** Katsuyama, Fukui, 911, Japan

(Received November 13, 1987)

The present study describes quantitatively the pharmacokinetics of the antibacterial agents, ofloxacin and NY-198 (quinolonecarboxylic acid derivatives), in the central nervous system in rats by physiological modeling of the penetration, distribution and sequestration processes. The simulation curves corresponded well with the observed concentrations in the cerebrospinal fluid (CSF) and various brain regions after intravenous bolus administration. The estimated cerebrovascular diffusion clearances were considerably small compared with reported serum flow rates and similar among the brain parenchymal tissues examined. The distribution volume of each drug in each brain region was almost the same as the brain extracellular space (15 to 25% of the wet weight). It was also found that the K_p values of these drugs were similar among the various brain regions. These lines of evidence suggest that the antibacterial agents, ofloxacin and NY-198, localized only in the brain extracellular space and exhibited little region-specificity in distribution into the brain. Moreover, it was suggested from unexpectedly low CSF: serum concentration ratios after intravenous administration that these quinolones, which once diffused into CSF, could be sequestered from CSF to blood *via* some transport system.

Keywords — quinolonecarboxylic acid; antibacterial agent; blood-brain barrier; cerebrospinal fluid; brain region; cerebrovascular permeability; pharmacokinetics; ofloxacin; NY-198

Introduction

Since the introduction of nalidixic acid into therapy in 1963, as an effective anti-Gram-negative bacterial agent, a number of its analogues, *i.e.*, quinolonecarboxylic acids (quinolones), have been developed and used in the treatment of a wide variety of infections. Some quinolones, however, are known to exhibit epileptogenic neurotoxicity as a side effect. Most recently, we demonstrated that several quinolones inhibited the specific [^3H] γ -aminobutyric acid (GABA) binding to rat brain membranes in a concentration-dependent manner *in vitro*,¹⁾ which may be involved in the induction of adverse reactions toward the central nervous system (CNS). As indicated from the CNS effects and measurable concentrations in the brain,²⁻⁵⁾ quinolones are thought to penetrate the blood-brain barrier or blood-cerebrospinal fluid (CSF) barrier. However, pharmacokinetic studies on the penetration of quinolones into

CNS have not been comprehensively performed. Therefore, in the present study, we chose ofloxacin and NY-198⁶⁾ (Fig. 1) which is a newly developed difluorinated quinolone, to quantitatively study their distribution into CSF and various brain regions after intravenous administration in rats.

Materials and Methods

Materials — Ofloxacin was kindly supplied by Daiichi Seiyaku, Co., Ltd. (Tokyo, Japan).

Fig. 1. Chemical Structures of Ofloxacin and NY-198

*** Author to whom correspondence should be addressed.

NY-198 and [^{14}C]NY-198 (specific activity, 9.3 $\mu\text{Ci}/\text{mg}$) were synthesized at the Central Research Laboratory, Hokuriku Seiyaku Co., Ltd. (Fukui, Japan). All other reagents were commercially available and of analytical grade.

Drug Administration and Sampling — Male Sprague-Dawley rats (Nihon Clea, Tokyo, Japan) weighing 230–280 g were used. Food and water were available *ad libitum*. Under light ether anesthesia, each rat received through the jugular vein, a single dose of 20 mg/kg of ofloxacin or NY-198 (containing 90 $\mu\text{Ci}/\text{kg}$ of [^{14}C]NY-198) which was dissolved in physiological saline. After a rapid closure of the incision by medical adhesive (Bond Alonalpha, Tooa Gosei Kagaku, Japan), rats were put into cages for free walking until sampling. At designated times after an injection (15, 30 min, 1, 2, 4 and 6 h), each rat was lightly anesthetized with ether and the CSF was obtained by cisternal puncture as described previously.⁷⁾ Immediately after CSF collection, each rat was sacrificed by exsanguination *via* the carotid artery to obtain blood, of which a portion was hemolyzed with an equal volume of distilled water for the determination of whole blood concentration of drug. After standing another portion of blood at room temperature for 1 h, serum was separated by centrifugation for 1 min in a microcentrifuge (Centrifuge 5412, Eppendorf GmbH, West Germany). Blood-to-serum concentration ratio (R_b) was calculated by dividing the concentration in whole blood by that in serum. The brain was rapidly excised and placed on a filter paper wetted with saline. After the dural and subarachnoidal vessels were carefully removed, the brain was dissected into various brain regions (cerebral cortex, hippocampus, corpus striatum, inter-midbrain, pons-medulla, and cerebellum) according to the procedure described previously,⁸⁾ which were weighed.

Bindings of Drugs in Serum and CSF — Each drug (ofloxacin or NY-198) at the concentrations of 0.4–40 $\mu\text{g}/\text{ml}$ was incubated with rat pooled serum or CSF at 37 °C for 5 min. Ultrafiltration of the serum or CSF was performed at 37 °C using a micropartition system MPS-1 (Amicon Corp., Danvers, MA). Drug concentrations in the residue (total concentration) and

in the filtrate (unbound concentration) were determined by the methods described below.

Determination of Ionization Constant (pK_a) — The apparent pK_a value of NY-198 was determined by a potentiometric titration⁹⁾ of 0.01 M aqueous solution of NY-198 at 25 °C.

Analytical Procedures — Concentrations of the quinolones were determined by a high-performance liquid chromatographic (HPLC) assay for ofloxacin or by scintillation counting of the radioactivity for [^{14}C]NY-198 as follows.

(1) Ofloxacin: Each brain tissue was homogenized in 0.1 M phosphate buffer (pH 11) using a homogenizer (Ultra-Turrax, Ika-Werk, Janke & Kunkel) to obtain 33% w/v homogenate. To 0.2 ml of the homogenate, 3 ml of diethyl ether and 0.4 ml of 0.01 M phosphate buffer (pH 11) containing the internal standard, (1-ethyl-6-fluoro-1,4-dihydro-7-(3,4-dimethyl-1-piperazinyl)-4-oxo-3-quinolinecarboxylic acid), were added. The solution was shaken for 5 min and centrifuged at 3000 rpm for 5 min at 4 °C. Into the lower aqueous phase, 0.1 ml of 0.3 N HCl, 0.1 ml of 0.2 M phosphate buffer (pH 7.0), 0.7 g of ammonium sulfate and 5 ml of a mixture of organic solvents (chloroform-isopropyl alcohol 1:1 v/v) were added, shaken for 30 min and centrifuged at 3000 rpm for 5 min at room temperature. The upper organic phase (approximately 4 ml) was evaporated and reconstituted in 0.3 ml of the carrier solution, the composition of which will be described later, and filtered through a membrane filter (0.45 μm , Nihon Millipore Kogyo, Yonezawa, Japan). The resultant filtrate was loaded onto a reversed-phase HPLC column (TSK-gel ODS-80TM, Toyosoda Industry Co., Tokyo, Japan). The solvent delivery system (BIP-I, Japan Spectroscopic Co., Tokyo, Japan) was equipped with a spectrofluorometer (FP110, Japan Spectroscopic Co.) set at 340 nm for excitation and 460 nm for emission, and with an integrator, Chromatopac C-R3A (Shimadzu Corp., Kyoto, Japan). A guard column C₁₈/CORASIL (Waters Associates Inc., Milford, MA) was placed between the injector and the analytical column. The mobile phase was acetonitrile–0.05 M citric acid–1 M ammonium acetate (16:83:1 v/v) and the flow rate was 1.0 ml/min. Serum samples were deproteinized by adding an

equal volume of methanol, mixing thoroughly and then the mixture was centrifuged in a microcentrifuge for 5 min. The supernatant fluid was filtered through a membrane filter and loaded onto the HPLC column as described above. CSF samples were directly applied onto the column without deproteinization. Calibration curves were generated by the above procedures using corresponding samples (from untreated rats) containing known quantities of ofloxacin. The recovery of ofloxacin in the extraction from the serum and tissue samples was within the range of 66–69% and 42–76%, respectively. However, the fluctuation of the recovery ratio of ofloxacin was ignored, because an internal standard method was employed in the present analysis. Moreover, the adsorption of ofloxacin to the membrane filter was negligible.

(2) [^{14}C]NY-198: The serum, CSF and tissue samples were oxidized with a sample oxidizer (model ASC-113, Aloka Co., Tokyo, Japan) to $^{14}\text{CO}_2$, and the radioactivity was determined by a liquid scintillation counter (model LSC-700, Aloka Co.). NY-198 was metabolized only to a negligible extent in rats (unpublished observation), so that the radioactivity found in the sample was considered to be associated with unchanged NY-198. The concentration of NY-198 ($\mu\text{g}/\text{ml}$) in each sample was calculated as [fraction of the radioactive dose injected per ml sample] $\times$ [dose administered (μg)].

The brain parenchymal concentration (C_b) was calculated in terms of measured brain concentration ($C_{b,\text{obs}}$), serum concentration (C_p) and the blood-to-serum concentration ratio (R_b) as follows:

$$C_b = C_{b,\text{obs}} - C_p \times R_b \times [\text{regional blood volume (\%)}] / 100 \quad (1)$$

where the regional blood volume ranged from 1.5 to 3.5% of the brain.¹⁰⁾

Pharmacokinetic Analysis — In order to elucidate the behaviors of drugs in CSF and various brain regions, we performed a hybrid model analysis¹¹⁾ in which the compartments of CSF and each brain region were independently connected with the serum compartment by apparent diffusion clearances (PA) defined as follows:¹²⁾

$$PA = Q(1 - e^{-PA_{\text{int}}/Q}) \quad (2)$$

where PA_{int} and Q represent the capillary permeability coefficient-surface area product and capillary serum flow, respectively. The following assumptions were made: 1) only the unbound drug is subject to the transcapillary diffusion which is a linear process; 2) cell membrane equilibration is rapid compared with capillary exchange rate; 3) no chemical conversion of the drug occurs in the brain and CSF; and 4) the extraction ratio of the drug across the brain is so small that the concentration of drug in the cerebral venous blood equilibrating with CNS is almost equal to that in the artery.

The analysis for the uptake of drugs into CSF was performed based on the diffusion and flow model,¹³⁾ except that a unidirectional efflux from CSF to blood, *i.e.*, sequestration process, was involved in the present analysis. The penetration and distribution of drugs into various brain regions were analyzed based on the theory described previously.^{14,15)} The changes in the concentration of drug in CSF and i -th region of the brain can be expressed as follows:

$$V_c \frac{dC_c}{dt} = PA_c(f_p C_p - C_c) + Q_c(f C_p - C_c) - CL_{\text{eff}} C_c \quad (3)$$

$$V_{bi} \frac{dC_{bi}}{dt} = PA_{bi}(f_p C_p - C_{bi}/K_{pi}) \quad (4)$$

where V_c and V_{bi} are the volumes of CSF and a brain region, respectively; f_p is the fraction of unbound drug in serum; C_p , C_c and C_{bi} represent the concentrations of drug in serum, CSF and brain parenchyma ($\mu\text{g}/\text{ml}$), respectively; Q_c is the bulk flow rate of production at the choroid plexus and removal at the subarachnoid villi of CSF; f is the fraction of drug in serum that appears in choroidally formed CSF as a result of filtration at the choroid plexus; CL_{eff} represents unidirectional efflux (sequestration) of drug from CSF to blood; PA_c and PA_{bi} are the apparent diffusion clearances (ml/min) between blood and CSF and between blood and a brain parenchymal region, respectively; and K_{pi} represents the volume fraction of brain into which

the drug distributes in the absence of drug binding to brain tissue. The value of V_c was estimated to be 0.15 ml for a 250-g rat from an allometric relationship between the body weight of animals and the CSF volume reported in dogs¹³⁾ and humans.¹⁶⁾ The value of Q_c was reported in rats to be 0.0022 ml/min.¹⁷⁾ The unbound quinolones removed in the filtration process for CSF formation at the choroid plexus is assumed to be negligible, so that f in Eq. 3 is subsequently regarded as equal to f_p . Verification of this assumption will require further investigation, such as direct measurement of drug concentrations in choroidally formed CSF, and is beyond the scope of this study. With regard to the exchange between brain tissues and CSF, the exchange of drugs occurs only within a short distance adjacent to the ependymal surface and its influence is small compared with capillary exchange, so that a CSF concentration is not necessarily indicative of parenchymal tissue concentration.¹⁵⁾ Therefore, we omitted the tissue-CSF exchange in the present study and focused on the effective processes among all details present in the complex anatomy of the brain.

Serum concentration vs. time curve was fitted to the following biexponential equation by a nonlinear least squares regression analysis using the computer program NONLIN:¹⁸⁾

$$C_p = A \cdot e^{-\alpha t} + B \cdot e^{-\beta t} \quad (5)$$

Substitution of Eq. 5 into 3 and integration gives:

$$C_c = A \cdot \frac{(PA_c + Q_c)f_p}{Z - \alpha V_c} [e^{-\alpha t} - e^{-(Z/V_c)t}] \\ + B \cdot \frac{(PA_c + Q_c)f_p}{Z - \beta V_c} [e^{-\beta t} - e^{-(Z/V_c)t}] \quad (6)$$

where Z represents:

$$Z = PA_c + Q_c + CL_{\text{eff}} \quad (7)$$

Similarly, substitution of Eq. 5 into 4 and integration gives:

$$C_{bi} = A \cdot \frac{PA_{bi}f_p}{PA_{bi}/K_{pi} - \alpha V_{bi}} [e^{-\alpha t} - e^{-(PA_{bi}/K_{pi} + V_{bi})t}] \\ + B \cdot \frac{PA_{bi}f_p}{PA_{bi}/K_{pi} - \beta V_{bi}} [e^{-\beta t} - e^{-(PA_{bi}/K_{pi} + V_{bi})t}] \quad (8)$$

In order to estimate the kinetic parameters (PA_c , CL_{eff} , PA_b and K_p), the data on the concentration of drug in CSF and brain parenchymal tissues were fitted to Eqs. 6 and 8, respectively, by a nonlinear least squares regression analysis¹⁸⁾ using a digital computer (FACOM-M360AP) at the Information Processing Center, Kanazawa University.

TABLE I. CSF and Various Brain Parenchymal Region Concentrations of Ofloxacin after Intravenous Bolus Administration at a Dose of 20 mg/kg in Rats

Tissue	Time (h)					
	0.25	0.5	1	2	4	6
CSF	2.12 ± 0.15 ^{a)}	2.19 ± 0.57	0.91 ± 0.02	0.59 ± 0.07	0.16 ± 0.03	0.10 ± 0.05
Cerebral cortex	1.23 ± 0.24	1.30 ± 0.27	1.06 ± 0.10	1.02 ± 0.21	0.31 ± 0.128	n.d. ^{b)}
Cerebellum	1.17 ± 0.11	1.34 ± 0.16	0.95 ± 0.05	1.08 ± 0.13	0.30 ± 0.10	n.d.
Pons-medulla	1.06 ± 0.17	1.15 ± 0.13	0.87 ± 0.03	0.61 ± 0.09	0.35 ± 0.08	n.d.
Hippocampus	1.03 ± 0.09	1.10 ± 0.13	0.90 ± 0.04	1.05 ± 0.04	0.49 ± 0.06	n.d.
Corpus striatum	0.86 ± 0.11	1.04 ± 0.08	0.74 ± 0.04	0.90 ± 0.11	0.29 ± 0.03	n.d.
Inter-midbrain	1.07 ± 1.18	1.18 ± 0.10	0.88 ± 0.05	0.96 ± 0.14	0.25 ± 0.06	n.d.

^{a)} Mean ± S.E.M. ($n = 4-7$). The unit is $\mu\text{g/ml}$.

^{b)} Not determined.

TABLE II. CSF and Various Brain Parenchymal Region Concentrations of NY-198 after Intravenous Bolus Administration at a Dose of 20 mg/kg in Rats

Tissue	Time (h)					
	0.25	0.5	1	2	4	6
CSF	1.16 ± 0.10 ^{a)}	1.15 ± 0.16	0.63 ± 0.02	0.37 ± 0.03	0.17 ± 0.03	0.102 ± 0.025
Cerebral cortex	0.75 ± 0.04	0.91 ± 0.07	0.79 ± 0.07	0.61 ± 0.08	0.23 ± 0.02	0.070 ± 0.013
Cerebellum	0.89 ± 0.06	0.95 ± 0.07	0.87 ± 0.11	0.50 ± 0.03	0.22 ± 0.02	0.065 ± 0.012
Pons-medulla	0.60 ± 0.04	0.70 ± 0.14	0.51 ± 0.06	0.38 ± 0.05	0.15 ± 0.02	0.057 ± 0.013
Hippocampus	0.68 ± 0.04	0.71 ± 0.05	0.67 ± 0.04	0.45 ± 0.03	0.23 ± 0.03	0.113 ± 0.032
Corpus striatum	0.56 ± 0.01	0.54 ± 0.02	0.51 ± 0.04	0.34 ± 0.02	0.18 ± 0.02	0.074 ± 0.013
Inter-midbrain	0.60 ± 0.04	0.65 ± 0.04	0.57 ± 0.05	0.37 ± 0.02	0.27 ± 0.02	0.054 ± 0.008

^{a)} Mean ± S.E.M. ($n = 3-6$). The unit is $\mu\text{g/ml}$.

Results

Bindings of Drugs in Serum and CSF

The bindings of the quinolones in rat serum and CSF were determined by the ultrafiltration method. The unbound fractions of ofloxacin and NY-198 in serum were 0.77 ± 0.01 and 0.72 ± 0.02 (mean ± S.E.M.; $n = 5$), respectively, indicating that the bindings of these drugs to serum proteins were linear over the concentration range studied ($0.4-40 \mu\text{g/ml}$). The unbound fractions of these drugs in CSF

were more than 0.98 and not significantly different from 1 (data not shown); thus the binding in CSF was not considered in the pharmacokinetic analysis as noted in Eq. 3.

Pharmacokinetic Analysis

The R_b values of ofloxacin and NY-198 were determined to be 0.92 ± 0.05 and 0.96 ± 0.06 (mean ± S.E.M.; $n = 6$), respectively, indicating that these agents were distributed into the intracellular fluids of blood cells. Thus, the R_b value of each drug was measured and used to calculate the drug concentrations in brain

TABLE III. Physicochemical^{a)} and Pharmacokinetic^{b)} Parameters of Ofloxacin and NY-198

	Ofloxacin			NY-198		
pK_a	n.d. ^{c)}			$pK_{a1} 5.99 \pm 0.03$, $pK_{a2} 9.01 \pm 0.03$		
P_{app}	0.391 ± 0.054			0.106 ± 0.005		
	PA_c ($\text{ml/min} \times 10^3$)	PA_c/V_c ($\text{min}^{-1} \times 10^3$)	CL_{eff} ($\text{ml/min} \times 10^3$)	PA_c ($\text{ml/min} \times 10^3$)	PA_c/V_c ($\text{min}^{-1} \times 10^3$)	CL_{eff} ($\text{ml/min} \times 10^3$)
CSF	2.92 ± 3.05 ^{b)}	19.5 ± 20.3	19.2 ± 11.6	0.94 ± 1.46	6.27 ± 9.73	17.1 ± 8.3
Brain region	PA_b ($\text{ml/min} \times 10^3$)	PA_b/V_b ($\text{min}^{-1} \times 10^3$)	K_p	PA_b ($\text{ml/min} \times 10^3$)	PA_b/V_b ($\text{min}^{-1} \times 10^3$)	K_p
Cerebral cortex	5.14 ± 1.17 ^{b)}	6.69 ± 1.52	0.230 ± 0.026	5.26 ± 0.43	6.84 ± 0.56	0.178 ± 0.008
Cerebellum	1.57 ± 0.46	6.37 ± 2.17	0.219 ± 0.032	2.24 ± 0.17	9.07 ± 0.70	0.172 ± 0.006
Pons-medulla	1.15 ± 0.37	6.37 ± 2.03	0.181 ± 0.026	1.14 ± 0.16	6.29 ± 0.88	0.117 ± 0.007
Hippocampus	0.46 ± 0.12	4.14 ± 1.04	0.258 ± 0.045	0.64 ± 0.12	5.77 ± 1.09	0.147 ± 0.014
Corpus striatum	0.36 ± 0.10	4.23 ± 1.16	0.188 ± 0.030	0.41 ± 0.08	4.88 ± 0.95	0.110 ± 0.010
Inter-midbrain	1.59 ± 0.44	6.06 ± 1.69	0.194 ± 0.026	1.55 ± 0.14	5.93 ± 0.54	0.120 ± 0.005

^{a)} The apparent ionization constants (pK_a) were determined by potentiometric titration⁹⁾ and expressed as mean ± S.D. ($n = 3$). The apparent partition coefficients (P_{app}), measured in *n*-octanol-50 mM phosphate buffer (pH 7.0) at 37 °C, were cited from Ref. 1 and expressed as mean ± S.E.M. ($n = 4$). ^{b)} Pharmacokinetic parameters in CSF (PA_c and CL_{eff}) and in various brain regions (PA_b and K_p) in rats were estimated by fitting the data (in Tables I and II) to Eqs. 6 and 8, respectively, using the computer program NONLIN.¹⁸⁾ All values are expressed as mean ± S.D. of the estimated parameter.

^{c)} Not determined.

Fig. 2. Ofloxacin Concentrations in Serum (●), CSF (Panel A), Cerebral Cortex (Panel B) and Cerebellum (Panel C) after an Intravenous Bolus Administration in Rats

Each point and vertical bar represent the mean $\pm$ S.E.M. of 4–7 rats. The solid lines indicate simulated concentrations in serum, CSF and brain regions calculated from Eqs. 5, 6 and 8, respectively, using the NONLIN fitted parameters.

parenchymal tissues by Eq. 1.

Tables I and II present the concentrations of ofloxacin and NY-198, respectively, in CSF and various brain parenchymal regions at the specific time intervals after intravenous bolus administration at a dose of 20 mg/kg in rats. The serum concentration *vs.* time curves of the quinolones were very similar to those reported for ofloxacin⁵⁾ and NY-198⁶⁾ and were fitted to Eq. 5 using the computer program NONLIN. The computed values of A , α , B and β were 9.81 ± 0.37 ($\mu\text{g/ml}$), 0.0346 ± 0.006 (min^{-1}), 8.21 ± 0.26 ($\mu\text{g/ml}$) and 0.00802 ± 0.00011 (min^{-1}) for ofloxacin, and 6.99 ± 3.43 ($\mu\text{g/ml}$), 0.100 ± 0.037 (min^{-1}), 10.1 ± 0.3 ($\mu\text{g/ml}$) and 0.00880 ± 0.00019 (min^{-1}) for NY-198, respectively. Using these values together with known physiological parameters (V_c , V_b , Q_c and f_p), the observed drug concentrations in CSF and each brain parenchymal region were fitted to Eqs. 6 and 8, respectively, to estimate the pharmacokinetic parameters (PA_c , CL_{eff} , PA_b and K_p) by a fitting procedure using the computer program NONLIN. The parameters obtained are listed in Table III, together with the physicochemical

constants of the apparent pK_a values and partition coefficient (P_{app}) between octanol and buffer (pH 7.0), where the values of pK_{a1} and pK_{a2} are associated with the ionization of the 3-carboxylic acid group and the piperazine ring nitrogen of the drug, respectively. Figures 2 and 3 illustrate the simulated concentration *vs.* time profiles in CSF and representative two brain regions (cerebral cortex and cerebellum) after intravenous administration of ofloxacin and NY-198, respectively. The simulation curves are in good agreement with the observed data.

Discussion

The present study was designed to quantitatively describe the pharmacokinetics of two antibacterial agents, ofloxacin and NY-198 (quinolones), in the central nervous system in rats by physiological modeling of the penetration, distribution and sequestration processes.

In contrast to similar values of PA_b/V_b between ofloxacin and NY-198, the 3-fold difference in PA_c/V_c observed between these two drugs and shown to coincide with that in P_{app}

Fig. 3. NY-198 Concentrations in Serum (●), CSF (Panel A), Cerebral Cortex (Panel B) and Cerebellum (Panel C) after an Intravenous Bolus Administration in Rats

Each point and vertical bar represent the mean $\pm$ S.E.M. of 3–6 rats. The solid lines indicate simulated concentrations in serum, CSF and brain regions calculated from Eqs. 5, 6 and 8, respectively, using the NONLIN fitted parameters.

(Table III) suggested that the blood-CSF permeability of these drugs may be dependent on lipophilicity. The values of PA_b were much smaller than the regional serum flow rates (Q_b) in rats, *e.g.*, 0.18 ml/min in cerebellum; 0.11 ml/min in hippocampus and 0.16 ml/min in pons-medulla, which were calculated from the regional blood flow rates¹⁹⁾ using the brain tissue hematocrit.²⁰⁾ Accordingly, it is obvious from Eq. 2 that the estimated PA_b values are very close to the cerebrovascular diffusion clearances (PA_{int}) in each brain tissue.

As presented in Table III, the PA_b values of ofloxacin and NY-198 varied widely among the brain regions examined, while the PA_b/V_b values varied in a relatively narrow range, indicating that the values of P (permeability coefficient, $\text{cm} \cdot \text{s}^{-1}$) are similar among these brain regions. The slight difference in PA_b/V_b might be due to varying quantities of gray and white matter in each tissue sample, since A in the white matter was approximately one-half of A in the gray matter.²¹⁾ Using the reported value of $A/V_b = 240 \text{ cm}^2/\text{g}$ ¹⁴⁾ and the averaged PA_b values, the P values of ofloxacin and NY-198

can be calculated as 3.92×10^{-7} and $4.49 \times 10^{-7} \text{ cm} \cdot \text{s}^{-1}$, respectively. These P values are comparable to those of urea, mannitol, methotrexate and *N*-methylnicotinamide, higher than those of sucrose and tetraethylammonium (TEA), and lower than those of ethylene glycol and glycerol.¹⁰⁾

The $K_{p,f}$ ($=K_p/f_p$) value of ofloxacin or NY-198 (data not shown), which represents the ratio of the tissue volume in which the unbound drug is distributed to the total volume, was close to the brain extracellular space (15 to 25% of the wet weight)¹⁴⁾ in each brain region. It was also found that the K_p values of both quinolones were similar among the various brain regions, indicating that the distributions of these drugs are not region-specific. Recently we suggested that *in vitro* inhibition of [^3H]GABA binding to rat brain membranes by quinolones might be involved in the *in vivo* induction of epileptogenic side effects.¹⁾ It is known that the sodium-independent [^3H]GABA binding activity (or the GABA receptors) in the brain is rich in cerebellum and cerebral cortex as compared to other regions.²²⁾ However, the lack of region-

specificity in the CNS distribution of these two quinolones does not necessarily contradict the above possible mechanism of quinolone-induced CNS effects, because the values of K_i estimated from the inhibition of GABA-receptor binding by quinolones are as high as 0.25–8.5 mM,¹⁾ so that they possess only low affinity with the GABA receptors. Taken altogether, it is suggested that the quinolones, ofloxacin and NY-198, localize only in the brain extracellular space and are thereafter bound to or taken up to a small extent into the brain parenchymal cell membranes. In order to confirm the above interpretation, further studies such as direct measurement of the quinolones appearing in the brain extracellular fluid should be performed by a newly developed technique of regional brain microdialysis.²³⁾

In order to interpret the observed CSF concentration, a unidirectional efflux of drug from CSF to blood was assumed to be involved in the present analysis. However, the reliability in the simultaneously estimated PA_c and CL_{eff} values (listed in Table III) turned out to be relatively low. This is because the concentrations of drug in CSF reached the terminal phase in a short period of time after the intravenous bolus administration. Therefore, we made another verification of the parameters in the CSF pharmacokinetics. Following an intravenous bolus injection, the concentrations in serum and CSF at the terminal phase can be expressed as:

$$C_p = C_p e^{-\beta(t-\tau)} \quad (9)$$

$$C_c = C_c e^{-\beta(t-\tau)} \quad (10)$$

where C_p and C_c are the concentrations in arterial serum and CSF at time τ during the terminal phase. Substitution for C_p and C_c in Eq. 3 according to Eqs. 9 and 10, division by $e^{-\beta(t-\tau)}$ and solving for C_c/C_p (designated as $K_{p,app,CSF}$) yields:

$$K_{p,app,CSF} = \frac{(PA_c + Q_c)f_p}{PA_c + Q_c + CL_{eff} - \beta V_c} \quad (11)$$

Provided that $CL_{eff}=0$, i.e., sequestration of drug from CSF to blood does not occur, the

value of $K_{p,app,CSF}$ in Eq. 11 should fall into the ranges of 0.77 ($PA_c = \infty$) – 1.70 ($PA_c = 0$) for ofloxacin and 0.72 ($PA_c = \infty$) – 1.81 ($PA_c = 0$) for NY-198. On the contrary, the observed $K_{p,app,CSF}$ values, averaged from the terminal sampling points, were 0.169 ± 0.020 and 0.147 ± 0.031 (mean $\pm$ S.E.M.; $n=4$), for ofloxacin and NY-198, respectively, and only 1/4–1/10 of the expected values. Therefore, it is reasonable to assume that a unidirectional efflux process exists from CSF to blood for these quinolones.

In conclusion, we quantitatively described the pharmacokinetics of ofloxacin and NY-198 (quinolones) in the central nervous system by physiological modeling of the penetration, distribution and sequestration processes. Thus obtained simulation curves corresponded well with the observed data. It was suggested that the quinolones cross the blood-brain barrier to small extent and enter the brain extracellular space. Moreover, it was suggested that the quinolones which once diffused into CSF were sequestered from CSF to blood *via* some transport system.

Acknowledgements The authors are grateful to Daiichi Pharmaceutical Co. for the gift of ofloxacin. This work was supported in part by a Grant-in-Aid for Scientific Research from the Ministry of Education, Science and Culture of Japan.

References

- 1) A. Tsuji, H. Sato, Y. Kume, I. Tamai, E. Okezaki, O. Nagata and H. Kato: Inhibitory effects of quinolone antibacterial agents on γ -aminobutyric acid binding to receptive sites in rat brain, *Antimicrob. Agents Chemother.*, **32**, 190–194 (1988).
- 2) M. Hashimoto, N. Morino, H. Miyazaki and A. Kage-moto: Disposition and biotransformation of ^{14}C -pipemidic acid in rats and mice, *Chemotherapy* (Tokyo), **23**, 2693–2705 (1975).
- 3) Y. Tochino, K. Sugeno, M. Doteuchi, H. Okabe, R. Norikura and H. Tanaka: Absorption, distribution, metabolism and excretion of cinoxacin. The first report: absorption, distribution, metabolism and excretion of cinoxacin in rats, *Chemotherapy* (Tokyo), **28** (s-4), 73–89 (1980).
- 4) S. Nakamura, N. Kurobe, S. Kashimoto, T. Ohue and S. Shimizu: absorption, distribution, excretion and metabolism of AT-2266 in experimental animals, *Chemotherapy* (Tokyo), **32** (s-3), 86–94 (1984).

- 5) O. Okazaki, T. Kurata, K. Hashimoto, K. Sudo, M. Tsumura and H. Tachizawa: Metabolic disposition of DL-8280 (2): absorption, distribution and excretion of ^{14}C -DL-8280 in various animal species, *Chemotherapy* (Tokyo), **32** (s-1), 1185—1202 (1988).
- 6) H. Kato, O. Nagata, E. Okezaki, T. Yamada, Y. Ito, T. Terasaki and A. Tsuji: NY-198, a new antimicrobial agent of quinolone: absorption, distribution and excretion in animals, Program Abstr. Intersci. Conf. Antimicrob. Agents Chemother. 25th, Minneapolis, Minn., 1985, p.195.
- 7) R. C. Chou and G. Levy: Effect of heparin or salicylate infusion on serum protein binding and on concentration of phenytoin in serum, brain and cerebrospinal fluid of rats, *J. Pharmacol. Exp. Ther.*, **219**, 42—48 (1981).
- 8) J. Glowinski and L. L. Iversen: Regional studies of catecholamines in the rat brain I. The disposition of ^3H -norepinephrine, ^3H -dopamine and ^3H -dopa in various regions of the brain, *J. Neurochem.*, **13**, 655—669 (1966).
- 9) A. Albert and E. P. Serjeant: "The Determination of Ionization Constants," Chapman and Hall, London, England, 1971.
- 10) S. I. Rapoport, K. Ohno and K. D. Pettigrew: Drug entry into the brain, *Brain Res.*, **172**, 354—359 (1979).
- 11) R. J. Lutz, R. L. Dedrick and D. S. Zaharko: Physiological pharmacokinetics. An *in vivo* approach to membrane transport, *Pharmacol. Ther.*, **11**, 559—592 (1980).
- 12) G. P. Stec and A. J. Atkinson Jr.: Analysis of the contributions of permeability and flow to intercompartmental clearance, *J. Pharmacokinet. Biopharm.*, **9**, 167—180 (1981).
- 13) M. D. Karol, P. Veng-Pedersen and R. E. Brashear: Diffusion and flow transfer of theophylline across the blood-brain barrier: pharmacokinetic analysis, *J. Pharmacokinet. Biopharm.*, **5**, 513—531 (1983).
- 14) K. Ohno, K. D. Pettigrew and S. I. Rapoport: Lower limits of cerebrovascular permeability to nonelectrolytes in the conscious rat, *Am. J. Physiol.*, **235**, 299—307 (1978).
- 15) S. I. Rapoport, W. A. Klee, K. D. Pettigrew and K. Ohno: Entry of opioid peptides into the central nervous system, *Science*, **207**, 84—86 (1980).
- 16) J. M. Collins and R. L. Dedrick: A distributed model for drug delivery to CSF and brain tissue, *Am. J. Physiol.*, **245**, R303—R310 (1983).
- 17) H. F. Cserr: Potassium exchange between cerebrospinal fluid, plasma and brain, *Am. J. Physiol.*, **209**, 1219—1226 (1965).
- 18) C. M. Metzler, G. L. Flitring and A. J. McEwen: "A user's manual for NONLIN and associated programs," The Upjohn Co., Kalamazoo, Mich., 1974.
- 19) K. Ohno, K. D. Pettigrew and S. I. Rapoport: Local cerebral blood flow in the conscious rat as measured with ^{14}C -antipyrine, ^{14}C -iodoantipyrine and ^3H -nicotine, *Stroke*, **10**, 62—67 (1979).
- 20) W. D. Lo, S. R. Ennis, G. W. Goldstein, D. L. McNeely and A. L. Betz: The effects of galactosamine-induced hepatic failure upon blood-brain barrier permeability, *Hepatology*, **7**, 452—456 (1987).
- 21) C. Crone: The permeabilities of capillaries in various organs as determined by use of the 'indicator diffusion' method, *Acta Physiol. Scand.*, **58**, 292—305 (1963).
- 22) S. J. Enna and S. H. Snyder: Properties of γ -aminobutyric acid (GABA) receptor binding in rat brain synaptic membrane fractions, *Brain Res.*, **100**, 81—97 (1975).
- 23) H. Benveniste, J. Drejer, A. Schousboe and N. H. Diemer: Elevation of the extracellular concentrations of glutamate and aspartate in rat hippocampus during transient cerebral ischemia monitored by intracerebral microdialysis, *J. Neurochem.*, **43**, 1369—1374 (1984).