Comparative Study of Permeability into Rat Cerebrospinal Fluid of the Quinolones: Dependency on Their Lipophilicities

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Pharmacokinetic behavior involved in the entry of four quinolone antibacterial agents, norfloxacin (NFLX), ciprofloxacin (CPFX), ofloxacin (OFLX) and nalidixic acid (NA), into cerebrospinal fluid (CSF) was comparatively investigated in rats.

Periodically, after the bolus i.v. dose of each quinolone (10 mg/kg), aliquots of CSF were collected by cisternal puncture and blood samples were then withdrawn from the jugular vein. CSF and serum (total and unbound) levels of the drugs were determined by HPLC method. Transport parameters for three new quinolones (NFLX, CPFX, OFLX) into CSF were obtained by physiological model analysis.

Serum levels of OFLX and NFLX declined bi-exponentially with time, whereas the serum levels of NA and CPFX declined in mono-exponential and tri-exponential fashion, respectively. Fractions of each quinolone unbound to serum protein (approximately 0.7 for NFLX, CPFX, and OFLX, 0.12 for NA) were almost the same at any point in time. The CSF levels of these quinolones rose quite rapidly after drug administration, and then declined, along with their serum levels. Both the CSF level and the ratio of CSF concentration to serum unbound concentration were the highest for NA, followed by OFLX, CPFX and NFLX. These values of the four quinolones were almost proportional to the apparent partition coefficient \( P_{app} \) between \( n \)-octanol and phosphate buffer (pH 7.0) values of each reported in a previous paper [Tsuiji et al., Antimicrob. Agents Chemother., 32, 190 (1988)]. In the three new quinolones, OFLX had a larger value of apparent diffusional clearance between blood and CSF (\( P_{A} \)) than CPFX and NFLX. We found that the values of \( P_{A} \) for CPFX, OFLX and NFLX correlated well with the reported values of \( P_{app} \) for these quinolones.

The present results suggest that the permeability of these quinolones between blood and CSF may depend predominantly on the lipophilicities of the drugs.

Keywords quinolone antibacterial agent; cerebrospinal fluid; permeability; pharmacokinetics; lipophilicity; rat

Since the introduction of nalidixic acid (NA) as a highly effective antibacterial agent in 1962, many other drugs, including new quinolones with chemical structures related to NA, have been developed and used clinically. These quinolones belong to an important class of antimicrobials, the potential clinical usefulness of which continues to expand because of their broad and strong anti-bacterial activities against both gram-positive and gram-negative bacteria.

The first and prototypic quinolone, NA, has been reported to induce slight toxicity, such as convulsions, above the recommended dose. In general, the quinolones have been known to be distributed into the central nervous system (CNS) and to produce CNS toxicity as a side effect. The reactions include headache, insomnia, dizziness and seizures. Recently, severe convulsions have been reported to occur in patients treated with some of the new quinolones, i.e., enoxacin, norfloxacin (NFLX), ciprofloxacin (CPFX) and lomefloxacin, when coadministered with some non-steroidal anti-inflammatory drugs.

The distribution of these drugs into the CNS may be an important factor in the occurrence of these neurotoxic side effects. Penetration into cerebrospinal fluid (CSF) of some of the quinolones has been studied in animals and in human patients. Despite these studies, very little information is available concerning the comparative distribution behavior in CSF of these drugs in either normal animals or in patients.

The purpose of the present study was to compare the permeability into rat CSF of these quinolones, i.e. CPFX, ofloxacin (OFLX), NFLX and NA, for the evaluation of safety in the clinical use of these quinolones.

MATERIALS AND METHODS

Materials CPFX, NFLX and OFLX were kindly supplied by Bayer AG (Leverkusen, Germany), Kyorin Yakuhin Co., Ltd. (Tokyo, Japan) and Daiichi Seiyaku Co., Ltd. (Tokyo, Japan), respectively. NA of analytical grade was purchased from Nacalai Tesque, Inc. (Kyoto, Japan). All other chemicals were commercial products of analytical or liquid chromatographic grade.

Animals Male Wistar rats (Japan SLIC, Inc., Hamamatsu, Japan) weighing 270 to 360 g, 10 to 11 weeks old, were used. Animals were cannulated in the right jugular vein according to the previous paper at least 15 h before drug administration, and were then housed in individual cages with free access to food and water.

Drug Administration and Sample Collection Each of the quinolones (NA, OFLX and NFLX) was dissolved in 154 mm phosphate-buffered saline containing 0.1 M sodium hydroxide (about pH 11). CPFX hydrochloride was dissolved in distilled water. A bolus dose of 10 mg/kg of each quinolone (CPFX hydrochloride: 10 mg/kg as CPFX) was injected through the cannula to the rats. The volume of solution injected was 2 ml/kg. At designated times after drug administration, each rat was lightly anesthetized with ether, and a CSF specimen (approximately 50–100 µl) was obtained by cisternal puncture.
according to our previous paper. Immediately after CSF collection, blood (approximately 1—2 ml) was withdrawn through the jugular vein cannula. The serum was immediately separated from the blood by centrifugation with a serum separator (Fibrichin; Takazono Sangyo Co., Ltd., Tokyo, Japan), and a portion of it was ultrafiltered with Amicon Centrifree (Amicon Div., W. R. Grace & Co., Danvers, MA) by centrifugation at 4000 rpm for 15 min (model KR-20000T, Kubota, Tokyo, Japan).

Drug concentrations in blood serum, its ultrafiltrate and CSF were determined according to the high performance liquid chromatographic methods developed previously for each quinolone.

Data Analysis  After i.v. injection of the quinolones, serum total concentration (C_s) vs. time (t) data were represented by the following equations:

\[ C_s = D/V_d e^{-\lambda t} \]  
\[ C_s = A e^{-\mu} + B e^{-\nu} \]  
\[ C_s = A e^{-\mu} + B e^{-\nu} + C e^{-\gamma} \]

where \( t \) is the time after drug administration, \( \lambda \) is the elimination rate constant, \( D \) is the dose of the drug, \( V_d \) is the apparent volume of distribution and \( A, B, C, \alpha, \beta, \gamma \) and \( \gamma \) are hybrid parameters.

The transport of CPFX, OFLX and NFLX into CSF was analysed based on a physiological model which included passive diffusion, convective removal by CSF turnover, and unidirectional active efflux processes. On the basis of a few assumptions, i.e. no bio-transformation in the CSF and no exchange between brain tissue and CSF, the rate of change of the quinolone concentration in the CSF is given as follows:

\[ V_e \cdot dC_e/dt = PA_e (C_s - C_e) - Q_e \cdot C_e - CL_{eff} \cdot C_e \]

where \( C_s \) and \( C_e \) are CSF and serum unbound concentrations of the quinolone, respectively; \( V_e \) is the volume of CSF; \( PA_e \) is the apparent diffusional clearance of the quinolone between blood and CSF; \( Q_e \) is the rate of CSF turnover and \( CL_{eff} \) is the active efflux clearance of the quinolone from CSF to blood. The value of \( V_e \) was estimated to be 0.18 ml from the relationship between the estimate by the previous paper and the body weight of the animals. \( Q_e \) was reported to be 0.0022 ml/min in rats. The data on concentrations of quinolone in serum and CSF were fitted to the model equations given above by a non-linear least squares regression program, MULTI, to estimate the kinetic parameters, i.e. \( k_e, V_d, A, B, C, \alpha, \beta, \gamma, PA_e \) and \( CL_{eff} \). A weighing factor of the reciprocal of the concentration was used in all of the regression analysis. In the present study, we didn't take the protein binding of these drugs in CSF into consideration, for it has been known that the protein concentration in CSF was less than 1% of the protein content of plasma under normal physiological conditions.

The analysis of NA transport into CSF with the present model could not be performed, since the assumptions described above were not applicable to a drug of such extremely high lipophilicity, which leads to substantial metabolic instability.

RESULTS AND DISCUSSION

Penetration into CSF of some of the quinolones has been investigated in animals and humans. In dogs with both healthy meninges and experimental meningitis, enoxacin penetrated into the CSF. Similar results have been reported with CPFX or OFLX in patients with blood—brain barrier dysfunction. In a recent report, a comparative study of some of the new quinolones (NFLX, OFLX, CPFX, enoxacin and floxacin) was performed in patients with urological diseases. It was reported that these quinolones penetrated into the CSF in patients without any cerebrospinal disease.

Despite such excellent research, information on the comparative distribution into CSF of these quinolones in healthy animals without cerebrospinal disease is poorly documented. The aim of the present work is to compare the permeability into CSF of the quinolones in normal rats.

Figure 1A shows the semilogarithmic plot of the mean serum total concentration of these quinolones vs. time after i.v. injection to rats. The serum concentration of the drugs declined rapidly. The mean serum concentration

![Fig. 1. Serum Total Concentration vs. Time Data (A), and CSF Concentration vs. Time Data (B) of the Quinolones after Intravenous Administration (10 mg/kg) in Rats](image_url)

Each point and vertical bar represents the mean and S.D. of 5—7 rats. Each line [except for NA in (B)] indicates the simulation curve for the mean data by the computer program MULTI. Symbols: ■, NFLX; ○, OFLX; ▲, CPFX; ▽, NA.
Fig. 2. Ratio of CSF Concentration to Serum Unbound Concentration of the Quinolones after Bolus Intravenous Administration (10 mg/kg) in Rats

Each column and vertical bar represents the mean and S.D. of 5–7 rats. Columns: ■ NFLX, □ OFLX, □ CPFX, □ NA.

vs. time data were fitted to the exponential functions by the program MULTI. Selection of a model for kinetic analysis was based on Akaike's Information Criterion (AIC). The model yielding the lowest AIC value in each drug was considered to be the best representation of the experimental data. Based on the minimum AIC estimation, it was determined that a bi-exponential fit should be used to analyze the serum concentration vs. time profiles of OFLX or NFLX. On the other hand, serum concentration vs. time profiles of CPFX and NA were fitted to the equations which expressed tri-exponential and mono-exponential functions, respectively.

The fractions of the four quinolones unbound to rat serum protein after i.v. injection were also investigated. The unbound fractions of the three new quinolones were about 0.7, whereas the fraction of NA was about 0.12. These values for all of the quinolones were almost the same, regardless of the point in time.

CSF concentration vs. time profiles for these quinolones after i.v. injection to rats is shown in Fig. 1B. The CSF concentration of these drugs rose quite rapidly after drug administration, then declined in concert with their serum concentrations. The mean CSF concentration of NA was found to be remarkably higher than that of the three new quinolones. In the three new quinolones, the mean CSF levels were as follows: OFLX > CPFX, NFLX.

CSF-serum unbound concentration (C_{CSF}/C_{serum}) ratios after i.v. injection are shown in Fig. 2. The C_{CSF}/C_{serum} ratios for these quinolones showed essentially the same tendency as their mean CSF levels. The ratio for NA was the highest, followed by OFLX, CPFX and NFLX. Thus, it is considered that NA penetrates into the CSF more readily and intensely than the others. Of the three new quinolones, OFLX had the largest values of both mean CSF concentration and C_{CSF}/C_{serum} ratio, followed by CPFX and NFLX. Penetration of the drugs into the CNS was known to relate closely to their lipophilicity. Tsuji et al. reported that the apparent partition coefficients (P_app) between n-octanol and the phosphate buffer (pH 7.0) of NA, OFLX, CPFX and NFLX were 3.34, 0.391, 0.115 and 0.069, respectively. The values of the mean CSF concentration and C_{CSF}/C_{serum} ratio for these quinolones were almost proportional to their reported P_app values.

It has been reported that drugs unbound to serum protein and in a non-ionic form are those most likely to be distributed in CNS. NA is an organic acid and its pK_a value has been reported to be 6.12. NA was highly bound to serum protein and it was considered that the fraction of its non-ionic form was smaller than that of the ionic form at a physiological pH. Despite these facts, NA was more easily transported into CSF than the other quinolones. These results suggest that the lipophilicity of the quinolones might be a predominant factor in their penetration into CSF.

OFLX, NFLX and CPFX are amphoteric compounds which have two pK_a values associated with their carboxylic acid function and piperazinyl nitrogen. It has been reported that these drugs have similar isoelectric points near physiological pH. In addition, they showed maximum partition coefficients at their isoelectric points in the octanol/water partition experiments because their non-ionic forms maximally existed at these pHs. Thus, on these three drugs with similar physicochemical properties, a physiological model analysis was carried out.

Previous papers have presented a physiological model for the analysis of drug transport between blood and CSF. Sato et al. developed a physiological model that consisted of the sequestration process added to the diffusion and CSF bulk flow, and they reported that the transport mechanism for two quinolones, OFLX and lomefloxacin, across the blood-CSF barrier might involve a sequestration process from CSF into blood. Therefore, in the present analysis of CSF concentration vs. time data, the model equation was constructed by adding the active efflux process from CSF to blood to the “diffusion and flow” model described by Karol et al.
estimated by fitting the mean data to the model equation. OFLX had a larger $P_Ae$ value than did CPFX and NFLX. The $P_Ae$ is a parameter related to the passive diffusion between blood and CSF at the choroid plexus, and is presumed to be affected by the lipophilicity of the drug diffused. Therefore, the relationship between the $P_Ae$ values and the lipophilicity of these quinolones was examined (Fig. 3). We found that the $P_Ae$ values for CPFX, OFLX andNFLX correlated well with the reported values of $P_{app}$ for these quinolones, as described above ($r=0.984$). This aspect suggests that the diffusibility of these three quinolones between blood and CSF at the choroid plexus may depend mainly on the lipophilicity of each drug.

The value of $CL_{eff}$ for OFLX was the smallest among these quinolones (CPFX>NFLX>OFLX). The $C_v/C_f$ ratios of OFLX were remarkably larger than those of CPFX or NFLX. The ratios of CPFX and NFLX revealed maximum values at 15—20 min after injection, which tended to decline in a time-dependent manner, whereas the ratios of OFLX were maintained at high values up to the last time of sampling. The ratios of $CL_{eff}$ to $P_Ae$ for these drugs obtained by the present model were 23.1 and 5.2 for NFLX, CPFX and OFLX, respectively. It is suggested that the remarkably small ratio of $CL_{eff}$ to $P_Ae$ for OFLX may explain the accumulation of OFLX in the CSF.

While the mechanism of CNS toxicity caused by the quinolones has not yet been elucidated, it has been postulated that its interaction with $\gamma$-aminobutyric acid (GABA) receptors in the CNS may play an important role in the enhanced seizures potential observed. Tsuji et al. reported that various quinolones inhibited the specific binding of GABA to its receptor sites in rat brain membranes in a concentration-dependent manner. Therefore, it is important to evaluate the CNS toxicity of the quinolones to elucidate the penetrability of the drugs into CSF. The present study indicates that the permeability of these quinolones between blood and CSF may be predominantly dependent on the lipophilicity of each drug, and that the $P_Ae$ and $CL_{eff}$ for these quinolones may be excellent marker-parameters for the transport and elimination of these drugs, respectively.

REFERENCES