ABSTRACT:

Brain interstitial fluid (ISF) concentrations, which regulate the toxicodynamic effect of quinolone antimicrobial agents (quinolones) in the central nervous system, have been determined for norfloxacin, ofloxacin, fleroxacin, and pefloxacin using a quantitative brain microdialysis technique. Steady-state brain ISF concentrations of the quinolones were 7–30 times lower than the unbound serum concentrations due to restricted distribution in the brain. Cerebrospinal fluid concentrations of the quinolones were approximately twice as high as the brain ISF concentrations, except for norfloxacin. Thus, it seems that an active efflux transport system across the blood-brain barrier is responsible for maintaining brain ISF concentrations lower than unbound serum concentrations at steady-state. A good correlation was observed for norfloxacin, ofloxacin, fleroxacin, and pefloxacin between brain ISF concentrations and total brain concentrations. Moreover, a relatively small difference was observed among the quinolones for the in vitro brain slice-to-medium concentration ratio, compared with an 11-fold difference in the in vivo brain-to-unbound serum concentration ratio after intravenous infusion. These results indicate that the different quinolones studied all exhibit similar apparent binding and/or uptake by brain parenchyma, with an average brain ISF-to-total brain concentration ratio of 0.688.

Epileptogenic neurotoxicity is a well known CNS side effect of quinolone antimicrobial agents (quinolones) seen during chemotherapy (1). Nevertheless, quinolones with a wide and powerful spectrum of antimicrobial activity against Gram-negative and Gram-positive bacteria are used to treat a variety of infectious diseases (2). To develop new quinolones with low CNS side effects, it is important to devise a strategy based on toxicodynamic and toxicokinetic principles. As the GABA receptor binding of quinolones is believed to play an important role in these CNS side effects (3), minimizing the GABA receptor binding is one promising strategy (4). Moreover, it is also important to minimize the in vivo brain ISF concentration of quinolones after administration, because the brain ISF concentration directly affects GABA receptor binding in vivo.

Because the in vivo total brain concentrations of quinolones have been reported to be significantly lower than unbound plasma concentrations (5–7), the brain distribution of quinolones seems to be restricted. Although it has been suggested that quinolones are removed from CSF to the circulating blood via an unknown transport system (8, 9), one possible explanation is that this active efflux system on the blood-CSF barrier plays an important role in limiting the brain distribution of quinolones. However, because the surface area of the BBB is known to be 5,000 times greater than that of the blood-CSF barrier (10), we could not exclude the possibility that an efflux transport system on the BBB may play a role in reducing the brain ISF concentration of quinolones in the brain with the result that the apparent brain concentration is lower than that of the unbound serum concentration.

The purpose of present study is to clarify the restricted cerebral distribution mechanism of quinolone antibiotics. Quantitative brain microdialysis (11, 12) and brain slice uptake studies have been performed to determine the in vivo and in vitro distribution of quinolones, respectively.

Materials and Methods

All of the quinolones (NFLX, FLRX, OLFX, SPFX, PFLX, and AM-1155) were synthesized at the Central Research Laboratories of Kyorin Pharmaceutical Co., Ltd. (Tochigi, Japan). Xylazine hydrochloride and ketamine hydrochloride (Ketaral 50) were purchased from Sigma Chemical Co. (St. Louis, MO) and Sankyo Co., Ltd. (Tokyo, Japan), respectively. [carboxyl-14C]Inulin was purchased from Du Pont-New England Nuclear (Boston, MA). All other chemicals were commercial products and of analytical grade. Male Wistar rats weighing 270–300 g were used throughout the experiments. The animals all had free access to food and water.

Microdialysis Cannula. The transcranial-type microdialysis cannula was prepared as described previously (11, 13) using Cuprophan hollow-fiber (Kawasumi Laboratories, Inc., Tokyo, Japan) and stainless-steel tubing (outer diameter: 0.2 mm; MT Giken, Tokyo, Japan). The molecular weight cut-off of this dialysis fiber is 30,000, and its inner diameter is 200 μm. The length of dialysis fiber implanted was 8 mm. To perfuse RHB [NaCl, 141 mM; KCl, 4 mM; CaCl2, 10 mM; and 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid, 10 mM; pH 7.4] through the microdialysis cannula at a constant flow rate, a 5.0 ml Luer-Lock tip syringe (model 1005TLL; Hamilton Apparatus, Dover, MA) was used.
anesthesia with ketamine (235 mg/kg im) and xylazine (2.3 mg/kg im). Cannulation with polyethylene tubing (PE-50, Becton Dickinson & Co., Parsippany, NJ) was performed on a femoral artery and vein to allow blood to be sampled and drugs to be administered. The rat was placed in a stereotaxic frame (KN-398, Natsume Seisakusyo Ltd., Tokyo, Japan), and 1.0 mm holes were made on both sides of the skull 3.4 mm posterior to the bregma and 3.5 mm below the dura using a dental drill. The microdialysis cannula was passed through the holes perfusing RHB into the microdialysis cannula at a constant flow rate of 10 μl/min.

Thirty minutes after probe implantation, the loading and maintenance doses of antipyrene and each quinolone were administered through the indwelling cannula in the femoral vein. The loading doses and following maintenance doses were 75 mg/kg and 8.4 mg/kg/hr for antipyrene, 15.6 mg/kg and 5.40 mg/kg/hr forNFLX, 5.73 mg/kg and 2.75 mg/kg/hr for OFLX, 3.91 mg/kg and 1.05 mg/kg/hr for FLRX, and 12.9 mg/kg and 2.71 mg/kg/hr for PFLX, respectively. The dosage of quinolones was the same as in our previous report (14), in which the steady-state concentration of quinolones in whole brain and CSF was determined without implanting a dialysis probe. The plasma quinolone concentration at steady-state would be expected to be 2–10 μg/ml, which should allow us to obtain a detectable CNS concentration. Immediately after implantation, the dialysate was collected at 10-min intervals. Blood samples were collected from the femoral artery at 0.5, 1, 2, 3, and 4 hr after intravenous injection. At the end of the brain microdialysis (i.e. 4 hr), 50-μl aliquots of crystal-clear CSF were obtained by cisternal puncture using the sharp point of a 24-gauge needle (15). Then, rats were immediately decapitated and the cerebrospinal fluid removed for analysis.

**Serum Unbound Fraction.** The in vivo serum unbound fractions of the quinolones were determined by the centrifugal ultrafiltration method. Portions (1 ml) of serum collected from animals at 4 hr were loaded onto a membrane (20 kDa cut-off; Millipore, Bedford, MA). Ultrafiltration was performed with RHB at 10 ml/min, and the dialysate was collected every 10 min.

**Extrapolation of Brain ISF Concentration.** The concentration in the ISF was determined from that in the dialysate as reported previously (11) and described herein. The in vivo permeability clearance (PA\textsubscript{in vivo}) of a drug and reference compound was determined from the following equation:

\[
PA_{\text{in vivo}} = - F \times \ln(1 - C_{\text{dial}}/C),
\]

where \(C_{\text{dial}}\) and \(C\) denote the dialysate and reservoir concentrations in the in vitro study, respectively, and \(F\) is the dialysis flow rate. The in vivo permeability clearance (PA\textsubscript{in vivo}) was also determined from the following equation:

\[
PA_{\text{in vivo}} = - F \times \ln(1 - C_{\text{dial}}/C_{\text{ISF}}),
\]

where \(C_{\text{ISF}}\) is the concentration in the ISF. The effective dialysis coefficient (\(Rd\)) was defined as follows:

\[
Rd = PA_{\text{in vivo}} / PA_{\text{in vivo}}
\]

To evaluate \(Rd\), antipyrene was used as the in vivo reference compound. As in a previous study (11), the PA\textsubscript{in vivo} value of antipyrene was determined from eq. 2, making the assumption that the concentration of antipyrene in brain ISF is identical to that in serum at steady-state (i.e. \(C_{\text{ISF}} = C_{\text{serum}}\); thus, \(Rd_{\text{ant}}\) can be obtained from eq. 3). Assuming \(Rd_{\text{drug}} = Rd_{\text{ant}}\) and rearranging eq. 1–3, the drug concentration in the ISF (\(C_{\text{ISF,drug}}\)) is obtained as follows:

\[
C_{\text{ISF,drug}} = C_{\text{dial,drug}} \times \exp(-Rd_{\text{drug}} \times PA_{\text{in vivo}} \times \text{drug}) / F
\]

where \(C_{\text{dial,drug}}\) is the drug concentration in dialysate obtained from in vivo microdialysis.

**Brain Slice Uptake Study.** Brain slices were prepared as reported previously with some modification (16). Rats were killed by decapitation; the brain was removed, dissected in ice-cold buffer at pH 7.3, and placed on ice. The buffer used throughout the brain slice experiment contained: NaCl, 122 mM; NaHCO\textsubscript{3}, 25 mM; glucose, 10 mM; KCl, 3 mM; CaCl\textsubscript{2}, 1.4 mM; MgSO\textsubscript{4}, 1.2 mM; K\textsubscript{2}HPO\textsubscript{4}, 0.4 mM; and 4-(2-hydroxyethyl)-l-piperazine ethanesulfonic acid, 10 mM. The experiment was conducted in an atmosphere of prehumidified 95% O\textsubscript{2}/5% CO\textsubscript{2} gas.

A hypothalamic slice, 300 μm thick, was cut using a microslicer (DTK-2000; Dosaka EM Co., Ltd., Kyoto, Japan) and was kept in the ice-cold buffer until required. Slices were transferred by wide-bore pipette to the preincubation buffer (50 ml) maintained at 37°C. After preincubation for 5 min, the brain slice (~150 mg) was transferred to 50 ml of the same medium containing quinolone (0.5 μg/ml) or \(^{14}C\)inulin (1 Kbq/ml) at 37°C. Initial uptake of \(^{14}C\)inulin was measured at 5, 10, and 30 min, whereas the uptake of FLRX into the brain slice was determined at 30 min and at 1, 2, 3, and 4 hr. Steady-state distribution of quinolones for the slice was compared at 4 hr. Incubations were terminated by removing the slice from the incubation medium. The brain slice and a portion of the incubation medium were stored at −20°C for the determination of drug concentrations.

**Determination of Drug Concentration.** Brain tissues or slices were homogenized with 0.067 M phosphate buffer (pH 7.0). The homogenate was centrifuged at 3,000 rpm for 10 min to separate the supernatant. In the in vitro brain slice uptake study, the concentrations of quinolones were determined by HPLC as described previously (17), with some modification. For the analysis of NFLX, FLRX, OFLX, SPFX, and PFLX, a portion of the supernatant or incubation medium (0.2–0.5 ml) was mixed with 0.5 internal standard solution and extracted with chloroform (1 × 5 ml for FLRX, OFLX, SPFX, and PFLX; 2 × 5 ml for NFLX). The internal standard solution was prepared by dissolving pipemidic acid in 0.5 M phosphate buffer (2 μg/ml for NFLX; 20 μg/ml for FLRX; 40 μg/ml for OFLX; 4 μg/ml for SPFX and PFLX). The organic phases were evaporated to dryness and the resulting residues dissolved in 0.1 ml acetonitrile/0.04 M phosphoric acid (30:70, v/v). A portion of each solution (5–20 μl) was injected into the HPLC system (model 655A-11; Hitachi Ltd., Tokyo, Japan) equipped with a TSK gel ODS-120T columna (particle size, 5 μm; 4.6 × 250 mm; Tosoh Co., Tokyo, Japan). The eluting mobile phase and flow rate were as follows: NFLX; acetonitrile/10 mM KH\textsubscript{2}PO\textsubscript{4} (20:80, v/v, containing 0.005% 1-octane sulfonate sodium; pH 3.0), 1.0 ml/min; FLRX, OFLX, and PFLX; methanol/5 mM tetra-n-butylammonium hydrogen sulfate (28:72, v/v), 0.8 ml/min; SPFX; and acetonitrile/10 mM KH\textsubscript{2}PO\textsubscript{4} (3:10, v/v, containing 0.012% 1-octane sulfonate sodium; pH 3.0), 1.2 ml/min. To quantitate the concentration of AM-1155, samples were diluted with an equal volume of methanol to precipitate proteins and then centrifuged at 3,000 rpm for 10 min. The supernatants (5–10 ml) were applied to the HPLC column, TSK gel ODS-80T columna (particle size, 5 μm; 4.6 × 150 mm; Tosoh Co.).

In the microdialysis study, HPLC was also used to quantify the quinolones and antipyrene. Brain homogenate and serum samples were deproteinized with methanol (1:3, v/v) and centrifuged at 3,000 rpm for 10 min. An aliquot of supernatant or dialysate (5–50 μl) was injected directly onto the HPLC column, TSK gel ODS-80T columna (particle size, 5 μm; 4.6 × 150 mm; Tosoh Co.).

A fluorescence detector was used for NFLX, AM-1155, FLRX, and OFLX. The excitation and emission wavelengths were 290 and 460 nm for NFLX, 295 and 485 nm for AM-1155, 290 and 450 nm for FLRX, and 290 and 500 nm for OFLX, respectively. To detect SPFX and PFLX, UV absorption was used. The analytical wavelengths were 298 nm for SPFX and 280 nm for PFLX. Antipyrene was monitored using its UV absorption at 254 nm.

Calibration standards were prepared by adding authentic standards of each compound (quinolones: 0.0001–0.25 μg/ml for the microdialysis study and 0.01–0.5 μg/ml for the brain slice study; antipyrene: 0.1–100 μg/ml) to blank materials (i.e. serum and tissue homogenates). To analyze dialysate, we added quinolone and antipyrene to RHB and used these for calibration. The concentration of quinolones and antipyrene was calculated from the calibration curve by linear regression of the peak intensity (peak height ratios to internal standard or peak heights) as a function of the spiked concentrations. The detection limit of quinolones in this procedure was 0.01 μg/ml in serum, 0.05 μg/g in tissues, and 0.0001 μg/ml for dialysate. The coefficient of variation for the quinolones assays in serum was <4.8%.
Total \[ ^{14}\text{C} \] inulin-related radioactivity was determined in a liquid scintillation spectrophotometer (model LS6000SE; Beckman Instruments, Inc., Berkeley, CA).

**In Vivo Brain Concentration.** Because the observed brain concentration in the in vivo microdialysis study included drug in the vascular space of the brain capillary, the quinolone concentration in the brain parenchyma was used after subtracting the drug in the vascular space. The blood vascular volume was found to be 0.020 ml/g from the reported plasma vascular volume (0.011 ml/g) (18) using a hematocrit of 0.45.

**Results**

Figure 1 illustrates the time course of the serum and brain dialysate concentrations of NFLX, OFLX, FLRX, and PFLX during the combination of brain microdialysis and constant intravenous infusion via the femoral vein in rats. The dialysate concentration reached a steady-state at 2 hr after the start of infusion (fig. 1). The steady-state concentrations of serum and brain dialysate are given in table 1. The total brain concentrations (\( C_{\text{Brain}} \)) and CSF concentrations (\( C_{\text{CSF}} \)) of the quinolones were determined at the end of brain microdialysis (i.e., 4 hr after infusion). The \( C_{\text{Brain}} \) and \( C_{\text{CSF}} \) values determined are also given in table 1.

The steady-state unbound serum concentrations (\( C_{\text{p,u}} \)) and serum unbound fraction (\( f_u \)) of the quinolones were determined by the ultrafiltration method and are given in table 1. The \( K_{\text{p,uBrain}} \) and \( K_{\text{p,UCSF}} \) and \( K_{\text{p,uBrain}} \) and \( K_{\text{p,UCSF}} \) were also estimated and given in table 1. An ~11-fold difference in \( K_{\text{p,uBrain}} \) was observed between NFLX and FLRX (table 1).

To estimate the in vivo brain ISF concentration (\( C_{\text{ISF}} \)) from the dialysate concentration (\( C_{\text{dil}} \)), antipyrine was used as a reference marker. The in vitro dialysate-to-reservoir concentration ratio (\( C_{\text{dil}}/C_{\text{r}} \)) (i.e., the relative recovery) of antipyrine was 0.110 ± 0.003 (mean ± SE, \( N = 12 \)). In the in vivo brain microdialysis experiment, the steady-state unbound serum concentration and the dialysate concentration of antipyrine were found to be 66.3 ± 3.3 \( \mu \text{g/ml} \) and 2.57 ± 0.13 \( \mu \text{g/ml} \) (mean ± SE, \( N = 12 \)), respectively. Based on eqs. 1 and 2, \( P_{\text{Ain vivo}} \) and \( P_{\text{Ain vitro}} \) of antipyrine was determined to be 1.18 ± 0.03 and 0.410 ± 0.012 \( \mu \text{g/min} \) (mean ± SE, \( N = 12 \)), respectively. Thus, the effective dialysis coefficient (\( R_d \)) of antipyrine was found to be 0.349 ± 0.014 (mean ± SE, \( N = 12 \)), which was very similar to the reported value (11). In the study of quinolone analogs, the values of \( C_{\text{dil}}/C_{\text{r}} \) were found to be 0.0476 ± 0.0050, 0.0520 ± 0.0009, 0.0548 ± 0.0064, and 0.0500 ± 0.0019 (mean ± SE, \( N = 3 \)) for NFLX, OFLX, FLRX, and PFLX, respectively. Thus, \( P_{\text{Ain vitro}} \) of NFLX, OFLX, FLRX, and PFLX was found to be 0.496 ± 0.057 \( \mu \text{g/min} \), 0.543 ± 0.009 \( \mu \text{g/min} \), 0.573 ± 0.068 \( \mu \text{g/min} \), and 0.522 ± 0.022 \( \mu \text{g/min} \) (mean ± SE, \( N = 3 \)), respectively.

Accordingly, the \( C_{\text{ISF}} \) values of quinolones were obtained using eq. 4. The \( C_{\text{ISF}} \) values of the quinolones, the ratios of \( C_{\text{ISF}} \) and \( C_{\text{p,u}} \), and the ratios of \( C_{\text{Brain}} \) and \( C_{\text{ISF}} \) are also given in table 1. For all of the quinolones examined, the \( C_{\text{ISF}} \) values were significantly lower than \( C_{\text{p,u}} \) (\( p < 0.05 \), Student’s paired t test). Moreover, the \( C_{\text{CSF}} \) values were approximately twice those of the corresponding \( C_{\text{ISF}} \) values (table 1) for OFLX, FLRX, and PFLX. As illustrated in fig. 2, the \( C_{\text{ISF}} \) values correlated closely with the \( C_{\text{Brain}} \) values for the quinolones studied. The slope of \( C_{\text{ISF}} \) vs. \( C_{\text{Brain}} \) (fig. 2) was found to be 0.459 ±
Brain ISFs were determined by brain microdialysis during the constant infusion of quinolones into the femoral vein. Line represents the result of a least squares regression analysis. The slope was 0.459 ± 0.092 (95% confidence interval). The ratio of CSF/Cm to be 0.141 ± 0.017, 0.768 ± 0.054, 0.683 ± 0.029, and 0.727 ± 0.023 for NFLX, OFLX, FLRX, and PFLX, respectively. Extrapolation of the CSF/Cm to be 0.141 ± 0.017, 0.768 ± 0.054, 0.683 ± 0.029, and 0.727 ± 0.023 for NFLX, OFLX, FLRX, and PFLX, respectively. Extrapolation of the CSF/Cm values were also determined, and these are given in table 2. Although the CSF/Cm values of FLRX, PFLX, and OFLX were significantly smaller than that of NFLX (table 2), these differences were relatively smaller than the difference in the in vivo Kp,u|brain among quinolones (table 1). The mean Cs/Cm value for all of the quinolones studied was found to be 1.56 ± 0.06 ml/g (mean ± SE, N = 24).

### Discussion

Steady-state concentrations of quinolones in brain ISF were 7–30 times lower than the unbound serum concentrations (table 1). In the same manner, the CSF concentrations of quinolones were 2–30 times lower than the unbound serum concentrations (table 1), which is consistent with previous reports for different quinolone analogs (6, 8). To explain these results, one has to assume that an active efflux transport system for quinolones at the BBB (i.e. brain capillary endothelial cells) is responsible for the restricted distribution in the brain (table 1), although at least two other possible mechanisms can be postulated: i) the bulk flow rate of CSF along with an active efflux system on the blood-CSF barrier is efficient enough to reduce the brain ISF concentration of quinolones, and ii) quinolones are significantly metabolized in the brain parenchyma. Because the CSF concentration of quinolones is greater than the brain ISF concentration for OFLX, FLRX, and PFLX (table 1), the first possibility can be ex-

### TABLE 1

Steady-state concentration of quinolones in the serum, brain, brain ISF, and CSF of rats

<table>
<thead>
<tr>
<th>Serum concentration</th>
<th>Unit</th>
<th>NFLX</th>
<th>OFLX</th>
<th>FLRX</th>
<th>PFLX</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cp</td>
<td>µg/ml</td>
<td>8.97 ± 0.75</td>
<td>6.68 ± 1.40</td>
<td>5.46 ± 0.81</td>
<td>6.72 ± 0.15</td>
</tr>
<tr>
<td>Serum unbound fraction</td>
<td>fu</td>
<td>0.795 ± 0.017</td>
<td>0.768 ± 0.054</td>
<td>0.683 ± 0.029</td>
<td>0.727 ± 0.023</td>
</tr>
<tr>
<td>Serum unbound concentration</td>
<td>Cp,u</td>
<td>7.14 ± 0.65</td>
<td>5.13 ± 0.96</td>
<td>3.76 ± 0.69</td>
<td>4.88 ± 0.21</td>
</tr>
<tr>
<td>Brain concentration</td>
<td>CBrain</td>
<td>0.232 ± 0.084</td>
<td>1.02 ± 0.20</td>
<td>1.47 ± 0.22</td>
<td>1.52 ± 0.03</td>
</tr>
<tr>
<td>Brain dialysate concentration</td>
<td>Cdial</td>
<td>0.0040 ± 0.0010</td>
<td>0.0101 ± 0.00036</td>
<td>0.0093 ± 0.0010</td>
<td>0.0135 ± 0.0015</td>
</tr>
<tr>
<td>Brain ISF concentration</td>
<td>CISF</td>
<td>0.233 ± 0.065</td>
<td>0.603 ± 0.206</td>
<td>0.539 ± 0.071</td>
<td>0.716 ± 0.034</td>
</tr>
<tr>
<td>CSF concentration</td>
<td>CCSF</td>
<td>0.229 ± 0.028</td>
<td>1.21 ± 0.30</td>
<td>1.55 ± 0.21</td>
<td>1.82 ± 0.26</td>
</tr>
<tr>
<td>Brain concentration</td>
<td>Kp,u</td>
<td>Brain(ml/g</td>
<td>0.035 ± 0.014</td>
<td>0.200 ± 0.021</td>
<td>0.394 ± 0.012</td>
</tr>
<tr>
<td>Serum unbound concentration</td>
<td>CSF concentration</td>
<td>Kp,u</td>
<td>CSF</td>
<td>0.033 ± 0.006</td>
<td>0.234 ± 0.021</td>
</tr>
<tr>
<td>Serum unbound concentration</td>
<td>Brain concentration</td>
<td>0.034 ± 0.011</td>
<td>0.118 ± 0.041</td>
<td>0.147 ± 0.012</td>
<td>0.147 ± 0.004</td>
</tr>
<tr>
<td>Serum unbound concentration</td>
<td>Brain concentration</td>
<td>0.975 ± 0.340</td>
<td>1.94 ± 0.37</td>
<td>2.72 ± 0.19</td>
<td>2.13 ± 0.14</td>
</tr>
<tr>
<td>Brain ISF concentration</td>
<td>Brain concentration</td>
<td>1.34 ± 0.48</td>
<td>0.567 ± 0.133</td>
<td>0.371 ± 0.027</td>
<td>0.473 ± 0.029</td>
</tr>
</tbody>
</table>

Each value represents the mean ± SE of three experiments.
TABLE 2

Comparison of brain slice to medium concentration ratio among quinolones

<table>
<thead>
<tr>
<th>Quinolones</th>
<th>Slice-to-Medium Concentration Ratio (Cs/Cm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NFLX</td>
<td>1.91 ± 0.15</td>
</tr>
<tr>
<td>OFLX</td>
<td>1.37 ± 0.02a</td>
</tr>
<tr>
<td>FLRX</td>
<td>1.25 ± 0.15a</td>
</tr>
<tr>
<td>PFLX</td>
<td>1.33 ± 0.04a</td>
</tr>
<tr>
<td>AM-1155</td>
<td>1.71 ± 0.04</td>
</tr>
<tr>
<td>SPFX</td>
<td>1.75 ± 0.04</td>
</tr>
</tbody>
</table>

Each value represents the mean ± SE of four experiments.

*Significantly different fromNFLX, which was examined by multiple comparison testing, Tukey test (p < 0.01).


