NOTES

Neurotoxicodynamics of the Interaction between Ciprofloxacin and Foscarnet in Mice

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The potential for convulsions induced by the coadministration of ciprofloxacin (CPFX) and foscarnet (PFA) may be due not to a change in the distribution of CPFX to the brain but to a potential CPFX-induced inhibition of γ-aminobutyric acid (GABA)-GABA_A receptor binding in the presence of PFA.

Since the finding that nalidixic acid has the structure of a pyridon carboxic acid, quinolone, in 1962 (12), the development of new quinolone antimicrobial agents (NQs) has been extensively investigated. NQs are oral agents having broad and effective antimicrobial spectra and activity (7, 13, 19). At present, they are used in many fields, including treatment of bronchopulmonary and urinary tract infections (15, 20). The pyrophosphate analog foscarnet (PFA) selectively inhibits the DNA polymerase of human herpesviruses including cytomegalovirus and the reverse transcriptase of human immunodeficiency virus (HIV); therefore, it has been used as an antiviral agent in HIV patients in Europe (4).

However, a great number of clinical cases of NQ-induced adverse events in the central nervous system involving convulsions have been particularly serious. A case of convulsions after coadministration of enoxacin (ENX) and fenbufen, a prodrug of felbital, was reported in 1986 (5). The mechanism of convulsion based on the interaction of NQs and nonsteroidal anti-inflammatory drugs (NSAIDs) has been investigated since then. Recently, it has been reported that the dominant mechanism of convulsion was the NQ-induced blockade of specific binding of GABA to GABA_A receptors in inhibitory neurons and their synergistic enhancement in the presence of NSAIDs (1, 21).

Recently, a clinical case of tonic-clonic convulsion after administration of ciprofloxacin (CPFX) to AIDS patients receiving PFA therapy for treatment of infections was reported, and the neurotoxic effects were abated after PFA therapy was stopped (6, 14). The authors suggested that convulsions may have been due to the concomitant use of both drugs. However, the mechanism of convulsion induced by coadministration of CPFX and PFA is still unclear.

In this study, to clarify the mechanism of convulsion induced by the concomitant use of CPFX and PFA, we tried to reproduce these phenomena in a mouse model and to examine the effects of PFA on NQ-induced neurotoxicological alteration, the pharmacokinetic behavior of the NQs, and the NQ-induced blockade of specific binding of GABA to GABA_A receptors.

ENX, CPFX, PFA, and FLB were kindly supplied by Dai-nippon Pharmaceutical Co., Ltd. (Osaka, Japan), Bayer Yaku-hin, Ltd. (Tokyo, Japan), Astra Japan (Tokyo, Japan), and Le-derle Japan, Ltd. (Tokyo, Japan), respectively. ENX and CPFX were prepared as aqueous solutions of the sodium salt by adding an equimolar amount of NaOH, and PFA was dissolved in saline. Pipemidic acid (PFA), used as an internal standard in the determination of NQ concentrations, was given by Dai-nippon Pharmaceutical Co., Ltd. GABA and [3H]GABA were purchased from Sigma Chemical Co. (St. Louis, Mo.) and New England Nuclear (Boston, Mass.), respectively. Clear-sol I, as a scintillation cocktail, was obtained from Nacalai Tesque (Kyoto, Japan). All other reagents were of commercial analytical grade. Male ddY mice (4 weeks old) were purchased from Seac Yoshitomi (Fukuoka, Japan) and were fed commercial food pellets and tap water ad libitum.

Under freely moving conditions, 130 mM CPFX (0.69 to 0.96 g/kg of body weight) or 144 mM ENX (1.22 to 1.29 g/kg) was infused into mice with or without 67 mM PFA (0.69 to 1.22 g/kg) through the tail veins at a constant rate of 0.03 ml/min with a syringe infusion pump (Model 11; Harvard Apparatus, S. Natick, Mass.). The time from the beginning of infusion to the occurrence of a clonic convulsion was measured. At the time of convulsion, the cervix was dislocated and the mouse was guillotined; the blood and cerebrum samples were then collected. The blood samples were centrifuged at 1,620 × g for 5 min to obtain plasma. The concentrations of the NQs in the blood and brain were determined by high-performance liquid chromatography (HPLC).

For the CPFX concentration-time profiles, 65 mM CPFX (0.15 to 0.60 g/kg) was infused through the tail veins at a constant rate of 0.03 ml/min in the presence or absence of 67 mM PFA (0.12 to 0.48 g/kg) with a syringe infusion pump. The samples of blood and cerebrum were collected at 5, 10, and 20 min after infusion. The blood samples were centrifuged at 1,620 × g for 5 min to obtain plasma. The concentrations of the NQs in the blood and brain were determined by HPLC.
The plasma protein binding of 0.78 mM CPFX in the presence or absence of 3.35 mM PFA was examined by equilibrium dialysis. In brief, 0.2 ml of plasma containing drugs and 0.1 M phosphate buffer (pH 7.0) were injected into cells on a dialysis membrane (Spectra/Por membrane; Spectrum Medical Industries, Inc., Houston, Tex.) and incubated at 37°C for 6 h. After incubation, the volume of each cell was measured and 0.1 ml of samples was withdrawn. The concentration of CPFX was determined by HPLC.

In order to extract the NQs from plasma samples, a solution of 0.9 ml of 0.1 M phosphate buffer, 0.1 ml of PPA (10 μg/ml), and 5 ml of chloroform containing 1% ethyl chlorocarbonate (Wako Pure Chemicals, Ltd., Osaka, Japan) was added to 0.1 ml of plasma samples, shaken for 10 min, and then centrifuged at 1,620 × g for 5 min. After centrifugation of 4 ml of the organic phase under reduced pressure, the residue was dissolved in 0.1 ml of methanol-0.05 M NaOH (2:1, vol/vol), and 20 μl was injected into the HPLC system. The cerebral samples were homogenized with a fourfold volume of 0.1 M phosphate buffer. One hundred microliters of PPA (10 μg/ml) and 5 ml of dichloromethane was added to 1 ml of the homogenate. Samples were shaken for 10 min and centrifuged at 1,620 × g for 5 min. Then, 4 ml of 1 M NaOH was added to 4 ml of the organic phase and shaken for 10 min. After centrifugation at 1,620 × g for 5 min, 3 ml of the aqueous phase was collected and treated in a manner similar to that for the plasma samples except for the addition of an internal standard. The extraction of ENX from the plasma and cerebrum samples was treated similarly to that of CPFX, and CPFX was employed as an internal standard.

The corrected concentration of the NQs in the brain was obtained by subtracting the amount of the remaining NQs in the intravascular space. The equation used was:

\[ C_{BR,obs} = \frac{C_{BR} \cdot R_p \cdot \frac{C_B}{C_P} - r \cdot R_p \cdot C_P}{(1 - r)} \]

where \( C_{BR} \), \( C_{BR,obs} \), \( R_p \), and \( C_P \) represent the corrected brain concentration of NQs, the uncorrected brain concentration of NQs, the blood-to-plasma concentration ratio of the NQs, the cerebral intravascular volume, and the plasma concentration of the NQs, respectively.

\( C_{BR,obs} \) includes the drug remaining in the cerebral intravascular spaces. Therefore, \( C_{BR} \) was determined by subtracting the drug amount calculated from both the ratio of capillary volume in the brain and the blood concentrations of drugs from \( C_{BR,obs} \), according to the equation given above. Consequently, \( C_{BR} \) shows the corrected drug concentration in the brain tissue.

The HPLC system consisted of a liquid chromatograph (Shimadzu LC-6A; Shimadzu, Kyoto, Japan) and a UV spectrophotometric detector (Shimadzu SPD-6A) operated at 280 nm. The column was a stainless steel tube (inner diameter, 250 by 4.6 mm) packed with Nucleosil 5C18 (Chemco, Osaka, Japan). The mobile phase was methanol-5 mM sodium dodecyl sulfate and was adjusted to pH 2.5 by the addition of phosphoric acid. The flow rate of the mobile phase was 0.8 ml/min. All analyses were performed at room temperature.

The procedure for preparation of the dissociated mouse brain cells was as follows. Male ddY mice were killed by decapitation, and the brains were removed quickly. To prepare the cell suspension, brain tissue was minced with a scalpel blade and passed through nylon mesh (pore sizes, 210, 130, and 80 μm). The cell suspension was centrifuged at 300 × g for 3 min at 4°C. After centrifugation, the pellet was gently resuspended in physiological buffer containing 110 mM NaCl, 5.3 mM KCl, 1.8 mM CaCl2, 1.0 mM MgSO4, 25 mM glucose, 68.3 mM sucrose, and 20 mM HEPES (pH 7.4). The viability of the cells was determined by trypan blue dye exclusion, and the suspensions with greater than 90% viability were used. Protein was measured by the pyrogallol red method (Micro TP-Test; Wako Pure Chemicals, Ltd.). Binding of [3H]GABA to dissociated mouse brain cells was determined as follows. Ninety microliters of cell suspension was incubated with 0.5 nM [3H]GABA and 1 mM CPFX or 1 mM ENX in the presence or absence of 100 μM PFA or 100 μM FLB at 4°C for 60 min. After incubation, unbound [3H]GABA was separated by density gradient centrifugation with a mixture of silicon oil and mineral oil (silicon layer method [d = 1.017]). [3H]GABA bound to the cells was digested in 3 M NaOH and neutralized with 1 M HCl. To determine the effect of 100 μM PFA or 100 μM FLB alone, the binding study was performed as described above. The radioactivities of bound and unbound [3H]GABA were counted with a liquid scintillation counter (LS 6500; Beckman, Fullerton, Calif.). The binding coefficient to the GABA receptor was calculated by the following equation: binding coefficient = bound [3H]GABA (dpm)/unbound [3H]GABA (dpm)/protein (mg/ml). The specific binding of 0.5 nM [3H]GABA alone to dissociated mouse brain cells was used as a control, and results were expressed as percentages of control values.

Statistical significance was evaluated by Student’s t test, and a P value of 0.05 was taken as the minimum level of significance. Data are expressed as means ± standard errors (SE).

The time of onset of the occurrence of clonic convolution after intravenous infusion of the NQs into mice in the presence or absence of PFA was shown in Fig. 1. Clonic convolution was induced by the administration of CPFX or ENX, and the time of onset of the occurrence of convulsions due to CPFX was shorter than that due to ENX. A decrease in the time before the onset of CPFX-induced convolution was observed after co-administration of PFA, while there was no significant decrease in the onset time in the case of ENX.

The concentrations of the NQs in the plasma and brain were measured at the onset of convulsion. There was no change in the concentration of CPFX or ENX in plasma after the concomitant use of PFA (Fig. 2A). On the other hand, the concentration of CPFX in the brain was significantly decreased after the concurrent injection of PFA at the onset of convolution, while there was no effect of PFA coadministration on the concentration of ENX in the brain (Fig. 2B).

As shown in Fig. 3A, the CPFX concentration in plasma increased in the presence of PFA, but there was no significant difference. Also, no significant change in the concentration of CPFX in the brain was observed in the presence of PFA (Fig. 3B).

The unbound fraction of CPFX in plasma, as measured by...
equilibrium dialysis, was 0.74. No change in the unbound fraction (0.78) of CPFX in plasma in the presence of PFA was observed.

In order to elucidate the mechanism of convulsion induced by the concurrent use of CPFX and PFA, the effect of CPFX or ENX on the specific binding of \([3H]GABA\) to dissociated mouse brain cells was investigated. Specific binding of \([3H]GABA\) to dissociated mouse brain cells was used as a control, and the inhibition ratio of GABA binding containing PFA or FLB alone was summarized in Table 1 as percent decrease of \([3H]GABA\) binding. As shown in Table 1, the binding between \([3H]GABA\) and the GABA receptor was inhibited by ENX or CPFX alone. However, no effect on GABA binding in the presence of PFA or FLB alone was observed. There was no change in the inhibitory effect of GABA binding by ENX in the presence of PFA, while binding was inclined to increase in the presence of FLB. On the other hand, the inhibitory effect of CPFX on GABA binding was significantly enhanced in the presence of PFA but not in the presence of FLB (Table 1).

Recently, it has been reported that serious convulsions were induced by the concomitant use of ENX-fenbufen, norfloxacin-fenbufen, and CPFX-ketoprofen (5, 10, 16). More recently, the occurrence of convulsions due to the concomitant use of CPFX and PFA was reported as a clinical case (6, 14). In this study, we tried to elucidate the mechanism of convulsion induced by the concomitant use of CPFX and PFA based on in vivo and in vitro experiments with mice.

To confirm whether or not the convulsion induced by PFA was specific for CPFX, the occurrence of convulsions due to CPFX was compared with that due to ENX. Based on the intravenous constant infusion experiments with CPFX and ENX, the time of onset of convulsion due to CPFX was shorter than that due to ENX (Fig. 1), suggesting that adverse reactions to CPFX in central nervous system were stronger than to ENX. These findings are consistent with the results reported by Kawakami et al. (11). Further, the time of onset of ENX-induced convulsions did not change with coadministration of PFA, whereas significant reduction of the time of onset of CPFX-induced convulsions was observed in the presence of PFA (Fig. 1). This finding indicates that the enhancement of convulsion in the presence of PFA was specifically related to CPFX. Both the plasma and brain concentrations of the NQs were examined at the onset of convulsion. There was no change in the concentration of either NQ in plasma in the presence of PFA (Fig. 2A). Both NQ concentrations in the brain, as an effect related to convulsions, were compared between two animal groups (Fig. 2B). The concentration of CPFX in the brain at the time of onset of convulsion was significantly decreased after concomitant injection of PFA, whereas there was no effect of PFA treatment on the concentration of ENX in the brain (Fig. 2B). These findings suggest specific and potentiated interaction between CPFX and PFA.

To determine whether this potentiated interaction is based on pharmacokinetic or toxicodynamic factors, we investigated the effects of PFA on the plasma and brain concentrations of CPFX. As shown in Fig. 3, significant differences in the CPFX concentrations in the brain and plasma were not observed at any time regardless of the presence or absence of PFA. Therefore, it is considered that PFA does not affect the distribution of CPFX in the brain. However, the distribution of CPFX in the brain was apparently decreased, because no difference in the concentration in plasma but a significant difference in concentration in the brain, as shown in Fig. 2, was observed. These results are in conflict with the results shown in Fig. 3. Although we calculated the drug brain-to-plasma concentration ratio

![Figure 2](image)

*FIG. 2. Concentrations of ENX and CPFX in plasma and brain at the onset of convulsion after intravenous infusion at a constant rate in mice in the presence or absence of PFA. ENX (144 nM) or CPFX (130 mM) was infused through the tail veins at a constant rate of 0.03 ml/min in the presence or absence of 67 mM PFA until the occurrence of clonic convulsion. At the occurrence of clonic convulsion, plasma (A) and brain (B) concentrations of the NQs were measured by HPLC as described in the text. Each value represents the mean ± SE (n = 6 to 8). White bars, ENX only; black bars, CPFX only; shaded bars, indicated drug plus PFA. *, significantly different from CPFX administered alone (P < 0.05).

![Figure 3](image)

*FIG. 3. Concentration-time profiles of CPFX in plasma and brain after concomitant intravenous infusion at a constant rate in mice in the presence or absence of PFA. CPFX (65 mM) was infused through the tail veins at a constant rate of 0.03 ml/min with or without of 67 mM PFA for 5, 10, and 20 min. Concentrations of CPFX in plasma (A) and brain (B) were measured by HPLC as described in the text. Each value represents the mean ± SE (n = 3). Filled symbols, CPFX alone; open symbols, CPFX plus PFA.*

<table>
<thead>
<tr>
<th>Drug(s)</th>
<th>% Decrease in [3H]GABA binding &lt;sup&gt;b&lt;/sup&gt;</th>
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<tbody>
<tr>
<td>ENX</td>
<td>80.60 ± 10.45</td>
</tr>
<tr>
<td>ENX + FLB</td>
<td>60.36 ± 0.83</td>
</tr>
<tr>
<td>ENX + PFA</td>
<td>71.28 ± 6.52</td>
</tr>
<tr>
<td>CPFX</td>
<td>74.00 ± 7.91</td>
</tr>
<tr>
<td>CPFX + FLB</td>
<td>72.74 ± 3.94</td>
</tr>
<tr>
<td>CPFX + PFA</td>
<td>54.06 ± 2.35&lt;sup&gt;*&lt;/sup&gt;</td>
</tr>
<tr>
<td>FLB</td>
<td>109.59 ± 7.97</td>
</tr>
<tr>
<td>PFA</td>
<td>112.81 ± 4.60</td>
</tr>
</tbody>
</table>

*Concentrations of ENX and CPFX were 1 mM (each); those of FLB and PFA were 100 μM (each).

<sup>b</sup> Specific binding of 0.5 nM [3H]GABA to dissociated mouse brain cells in the presence or absence of drug(s) was measured as described in the text. Specific binding of 0.5 nM [3H]GABA alone to dissociated mouse brain cells was used as a control. Results, expressed as percentages of control values, are means ± SE (n = 3 to 6). *, significantly different from CPFX administered alone (P < 0.05).
transmission based on a blockade of GABA receptors by
vulsion and the enhanced effect of FLB on ENX-induced con-

Based on these findings, the mechanism of ENX-induced con-
ceptors, to synaptoneurosomes in the presence of FLB (9, 22). Therefore, we investigated the effects of PFA and NQs on GABA receptor binding by using dissociated mouse brain cells (8). The dissociated mouse brain cells isolated from the minced mouse brain tissue corre-
responded to postsynaptic membranes on the cell bodies of brain parenchymal cells. Thus, these cells possessed advantages— such as the lack of need to aspirate the medium, great safety in the operation, and ease of preparation and maintenance of the cell body as a closed membrane system presenting the receptors for neurotransmitters in the right direction—not available with the synaptic plasma membranes generally used in receptor binding assays.

The inhibitory effect of ENX on GABA binding was inclined to increase in the presence of FLB (Table 1). Recently, Tsuji et al. reported an enhancement of the inhibitory effects of ENX and FLB on the binding of muscimol, an agonist of GABA re-
ceptors, to synaptoneurosomes in the presence of FLB (9, 22). Based on these findings, the mechanism of ENX-induced con-
vulsion and the enhanced effect of FLB on ENX-induced con-
vulsion may be due to the suppression of GABA-mediated neu-
rotransmission based on a blockade of GABA receptors by ENX and enhanced blockading in the presence of FLB. However, there was no change in the inhibitory effect of GABA binding by ENX in the presence of PFA (Table 1). This in vitro finding was consistent with the in vivo situation (Fig. 1). The inhibitory effect of CPFX on GABA binding was significantly enhanced in the presence of PFA but not in the presence of FLB (Table 1). Previously, we reported that the inhibitory effect of ENX on GABA receptor response was approximately fivefold higher than that of CPFX in the presence of FLB in mouse brain mRNA-injected Xenopus oocytes (11). These findings indicated that the enhanced convolution activity resulting from coadministration of PFA and NQs was due to the enhanced convolution of GABA-mediated neurotransmission caused by the inhibition of GABA binding. Moreover, these phenomena were specific for CPFX with NQs. The mechanism of CPFX-induced GABA binding inhibition in the presence of PFA is still unclear. We hypothesize that alteration of the structure or sensitivity of the GABA receptor by PFA might be a mechanism of CPFX-induced GABA binding inhibition in the presence of PFA.

In conclusion, convolution induced by concurrent use of
CPFX and PFA is not due to alteration of the CPFX concen-
tration in the brain but to the potentiated inhibition of GABA binding to GABA receptors in the presence of both CPFX and PFA.

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