Lack of Potentiation with Felbinac Patch on the Convulsive Toxicity of Enoxacin in Rats

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We investigated the possible potentiation of the convulsive toxicity of enoxacin (ENX) by the concomitant topical application of a felbinac (FLB) patch in rats. A felbinac patch (Seltouch®; 0.5%, 3 cm × 4 cm) was attached on the back of rats where their hair has been removed. ENX was infused from the left jugular vein at 8 h after the application of FLB patch under an unanesthetized and unstrained condition. Blood, CSF and brain samples were collected at the occurrence of convulsion, and ENX concentrations of each part were determined.

As a result, no significant potentiation by FLB patch was found in the onset time of convulsion or in the ENX concentration of each part. Moreover, based on the assumption that there are no inter-species differences in ENX concentration in the brain at the occurrence of a convulsion (C_{br}), the predicted plasma ENX concentration required to elicit convulsions in humans, which was estimated from the C_{br} and K_p value of ENX in the brain of rats, was 20 times higher than the therapeutic plasma level.

Key words convulsion; enoxacin; felbinac; patch; NSAID; new quinolone

With a broad antimicrobial spectrum and high distributivity to organs, new quinolone antimicrobial agents (NQs) are widely used against infectious disease.^{2,3)} However, their clinical drawback is focused upon the occurrence of convulsions with concomitant systemic administration of non-steroidal antiinflammatory drugs (NSAIDs), even a with normal dosage regimen.⁴⁾ Among the NQs, most of which are not approved with the use of NSAIDs, enoxacin (ENX) possesses a high potency for the convulsion and is quite susceptible against potentiation with NSAIDs.⁵⁾ In addition, felbinac (FLB) is known to be a strong potentiator among NSAIDs.⁵⁾

FLB is used against inflammation not only orally but also topically as patch, and the latter is recommended to use with antimicrobial agents against infectious inflammation.⁶⁾ The interaction between the FLB patch and oral NQs was not noted, conceivably because of the low systemic distribution of topically applied FLB.⁷⁾

However, the possibility of an interaction between the NQs and the FLB patch was not thoroughly excluded because pharmacodynamic interactions between oral drugs and topically applied agents have been reported in some cases, such as between warfarin and methyl salicylate.⁸⁾ Since FLB was detected, even with a low plasma concentration (600–1300 ng/ml), after the topical application of FLB patch in humans,⁷⁾ the possibility of a convulsant interaction remains.

To quantitatively estimate the possible interaction of an ENX-induced convulsion with the concomitant use of the FLB patch, we investigated the convulsive toxicity of ENX in the presence or absence of the FLB patch in rats.

MATERIALS AND METHODS

Materials Male Wistar rats were purchased from Nihon Ikagaku Dōbutu Co., Ltd. (Tokyo, Japan) and caged for 7 d. Animals weighing from 200 to 250 g were used for the experiments. FLB patch (Seltouch®; containing 0.5% FLB) and ENX were kind gifts from Nihon Lederle and Dainippon Pharm. Co., Ltd., respectively.

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All other agents were of analytical grade.

Animal Preparation Animals were anesthetized with pentobarbital sodium (50 mg/kg, i.p.), and the hair on the back was denuded with the use of electric clipper (THRIVE, model 900, Daito Electric Co., Ltd., Osaka, Japan) and hair-removing cream (Kanebo, Tokyo, Japan). Left jugular veins were cannulated with polyethylene cannulae (SP-31, Natsume, Tokyo, Japan), the other end of which was subcutaneously lead to the back of the neck. At 16 h after surgical operation, a FLB patch (3 cm × 4 cm) was attached onto the back. Then, the animals were restrained in Ballman cages for 8 h to allow the plasma FLB concentration to stabilize.

At 8 h after the application of the FLB patch, ENX was intravenously infused at a rate of 50 mg/kg/min using an infusion pump (Compact infusion pump, model 975, Harvard apparatus, U.S.A.) until the occurrence of clonic convulsion. At this infusion rate, no acute peripheral toxicity was observed. After anesthesia with diethyl ether, cerebrospinal fluid (CSF) was drawn by cisternal puncture. The blood sample was collected from the aorta descendens. The animal was sacrificed by decapitation and the whole brain was dissected.

Determination of ENX The analytical procedure of ENX was according to Nakamura *et al.*,⁹⁾ with minor modification.

Determination of ENX in Plasma and CSF One hundred microliter of plasma or CSF was pipetted into a 10-ml glass tube, and 1.0 ml of 0.1 M phosphate buffer was added. The sample was spiked with an internal standard (I. S.), 1 µg of ciprofloxacin (CPFX) dissolved in methanol. The compounds were extracted with 5 ml of chloroform containing 1.0% of ethyl chloroformate by shaking with a reciprocal shaker for 10 min. Four milliliters of the organic phase was transferred into another tube and dried with a rotary evaporator at 40 °C. The residue was dissolved into 100 µl of the mobile phase, and 20 µl were injected onto HPLC.

Determination of ENX in Brain The brain samples were homogenized (1:4, w/v) into 0.1 M phosphate buffer

(pH 7.0). One milliliter of homogenate was then spiked with the internal standard solution, and the compounds were extracted with 5 ml of dichloromethane by shaking with a reciprocal shaker for 10 min. Four milliliters of organic phase was transferred into another tube and back-extracted with 4 ml of 1 mM NaOH. The aqueous phase was submitted to the plasma extraction procedure.

Correlation of ENX Concentration in Brain The corrected brain concentration (C_{br}) was calculated from the observed concentration (C_{br-obs}) with Eq. 1 to exclude the components contained in the blood capillary.

$$C_{br} = \frac{C_{br-obs} - r \cdot R_b \cdot C_p}{1 - r} \quad (1)$$

where R_b , r and C_p are the blood-plasma ratio of ENX, the voluminal ratio of capillary in brain and plasma ENX concentration, respectively.

R_b and r were assumed to be 1.2 and 0.015, respectively, derived from the preliminary experiment.¹⁰⁾

Condition of HPLC HPLC systems consisted of an LC-6A liquid chromatograph (Shimadzu, Kyoto) and a SPD-6A spectrophotometer (Shimadzu, Kyoto).

The separations were performed on a column (6 i.d. $\times$ 250 mm) packed with Nucleosil 5C₁₈ at 50 °C. The samples were eluted with methanol–5.0 mM sodium lauryl sulfate (3 : 2, v/v) adjusted to pH 2.5 with phosphoric acid at a rate of 1.2 ml/min. The detection wavelength was adjusted to 280 nm. The limit of the quantification was 0.05 μ g/ml.

Analysis The predicted C_p , at which a convulsion may clinically occur in human, was calculated by dividing the C_{br} in rats by the K_p value ($= C_{br}/C_p$; 0.3) reported in human,¹¹⁾ based on the assumption that there are no significant inter-species difference in C_{br} which would elicit a clonic convulsion.

RESULTS

Table 1 shows the pharmacodynamic parameters; *i.e.* onset time for convulsion (t_s) and the ENX concentration at the occurrence of convulsion in plasma (C_p), CSF (C_{csf}) and brain (C_{br}).

No significant difference between the FLB patch group and the control group were shown in these parameters.

Figure 1 shows the predicted plasma concentration which may elicit a convulsion in human in the presence or absence of concomitant application of the FLB patch.

DISCUSSION

Although no one has referred to the interaction between NQs and topically applied NSAIDs, it is noteworthy to evaluate the risk of such interaction because these drugs are frequently used together, and the outcome of the interaction may be serious.

We focused upon the interaction between ENX and FLB, with which the interaction may occur when orally administered,⁴⁾ and of which the electrophysiological interaction was scrutinized *in vitro*.¹²⁾

We employed pharmacokinetic and pharmacodynamic

Table 1. The Onset Time for the Convulsion (t_s) and ENX Concentration at the Time of Convulsion in Plasma (C_p), Brain (C_{br}) and Cerebrospinal Fluid (C_{csf})

Parameters	Control	+ FLB patch
t_s (min)	21.0 $\pm$ 4.1	23.3 $\pm$ 7.6 ^{NS}
C_p of ENX (μ g/ml)	203 $\pm$ 60	261 $\pm$ 44 ^{NS}
C_{br} of ENX (μ g/ml)	15.3 $\pm$ 2.1	13.5 $\pm$ 2.6 ^{NS}
C_{csf} of ENX (μ g/ml)	14.9 $\pm$ 1.5	9.2 $\pm$ 1.7 ^{NS}

Each value represents the mean $\pm$ S.E.M. of three experiments. NS, not significantly different from control value by Students' *t*-test.

Fig. 1. Comparison of the Predicted Enoxacin Plasma Concentration of ENX in Humans Required to Induce Convulsion in the Presence or the Absence of FLB Patch

The plasma concentration required to induce the convulsion is quite higher than that observed after oral administration of the clinical dose of ENX.

data such as C_p , C_{csf} , C_{br} and t_s of ENX, at the event of convulsion, as the indices of convulsive toxicity. The t_s is parallel to the total dose of ENX required to elicit a convulsion, because ENX was infused at a constant rate. However, C_{csf} and C_{br} may be more appropriate indices than the others, since this interaction occurs in the central nervous system (CNS).

In any case, the FLB patch did not affect any parameters in our present study.

The plasma FLB concentration in rats at 8 h after the application of FLB patch (3 $\times$ 4 cm) was reported to be 0.8 μ g/ml,¹³⁾ which agreed well with a result of our preliminary experiment (1.08 μ g/ml). This is substantially equivalent to the clinical concentration, 0.83 μ g/ml, which appears after a single dosage regimen (10 $\times$ 14 cm) of FLB patch in humans.⁷⁾ Thus the FLB concentration, at least in plasma, in our present study may reflect the clinical condition where the FLB patch is used.

We assumed that C_{br} in humans at the occurrence of convulsion is identical to that in rats in our present analysis. The potentiation of the convulsive toxicity of NQs is the result of the potentiation of inhibitory effects of NQs on GABA (γ -aminobutyric acid)-Cl⁻ current.¹²⁾ It is reported that there are no significant inter-species differences in the inhibitory effects and the potentiation rate among mouse, rat, rabbit, dog and monkey.¹⁴⁾ This

may confirm the above mentioned assumption.

A normal dosage of FLB patch did not seem to potentiate the convulsive toxicity of ENX in our present study. Moreover, as shown in Fig. 1, the estimated plasma concentration of ENX to induce the convulsion in human is 20 times or more higher than the clinical range, even in the case of the concomitant use of the FLB patch. Thus, there may be little possibility of the potentiation of ENX-induced convulsion by the FLB patch. However, these results did not exclude the risk of the concomitant use of the FLB patch under hazardous conditions such as overdose, hepatic or renal dysfunction, change in the blood-brain-barrier permeability or change in the response of CNS against ENX. Moreover, overdose of the FLB patch or the application of the FLB patch on damaged skin may also result in an increase in plasma FLB concentration and be hazardous.

In conclusion, a normal dosage of FLB patch does not potentiate the convulsive toxicity of ENX.

REFERENCES AND NOTE

- 1) Present address: *Faculty of Pharmaceutical Sciences, Kyushu University, Maidashi, Fukuoka 812, Japan.*
- 2) Fernandes P. B., *J. Clin. Pharmacol.*, **28**, 156—168 (1988).
- 3) Sogel F., Jaehde U., Naber K., Stephan U., *Clin. Pharmacokin.*, **16** (suppl.1), 5—24 (1989).
- 4) Paton J. H., Reeves D. S., *Drug Safety*, **6**, 8—27 (1991).
- 5) Hirai S., Tanaka K., Makino S., Narita H., *Yakugaku Zasshi*, **109**, 119—126 (1989).
- 6) Nihon Lederle, "The Interview form of 'SELTOUCH'," 1993, p. 18.
- 7) Oonishi A., Fujiwara H., Kitamura M., Sasaki T., *Jpn. Pharmacol. Ther.*, **20**, 115—138 (1992).
- 8) Chow W. H., Cheung K. L., Ling H. M., See T., *J. Roy. Soc. Med.*, **82**, 501—502 (1989).
- 9) Nakamura R., Yamaguchi T., Sekine Y., Hashimoto M., *J. Chromatogr.*, **278**, 321—328 (1983).
- 10) Nakada Y., Yamamoto K., Kawakami J., Sawada Y., Iga T., *Pharm. Res.*, **12**, 1953—1957 (1995).
- 11) Kawahara K., Kawahara M., Goto T., Ohi Y., *Chemotherapy* (Tokyo), **39** (suppl. 4), 149—157 (1991).
- 12) Kawakami J., Shimokawa M., Yamamoto K., Sawada Y., Asanuma A., Yanagisawa K., Iga T., *Biol. Pharm. Bull.*, **16**, 726—728 (1993).
- 13) Yamashita N., Kawashima K., Nomura K., Nakazawa T., Watanabe T., Takeuchi H., Naruke T., Nishiki K., Inomata N., Oji A., *Jpn. Pharmacol. Ther.*, **20**, 3957—3971 (1992).
- 14) Kawakami J., Yamamoto K., Sawada Y., Iga T., Asanuma A., Yanagisawa K., Abstract of Papers, The 114th Annual Meeting of the Pharmaceutical Society of Japan, Tokyo, March 1994, Part 4, p. 119.