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Effect of Bile Duct Ligation and Unilateral Nephrectomy on Brain Concentration and Convulsant Potential of the Quinolone Antibacterial Agent Levofloxacin in Rats

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To mimic the excretion route of the quinolone antibacterial agent levofloxacin (LVFX) in humans, we produced an excretion-limited (EL) model in male Sprague–Dawley rats by bile duct ligation and unilateral nephrectomy. We then examined the relationship between brain levels of LVFX and its convulsant effects in control and EL animals. Serum concentrations of LVFX in EL animals (EL + LVFX) were 2.38- and 1.59-fold and brain concentrations were 1.33- and 1.19-fold those of the controls (control + LVFX) at 30 min after a single intravenous injection of 10 and 100 mg/kg LVFX, respectively. Furthermore EL animals became more susceptible to the convulsant effect of LVFX with a 1.28-fold decrease in convulsion-inducing dose. In combination with oral pretreatment with 400 mg/kg 4-biphenylacetic acid (BPAA), convulsion-inducing doses in the control (control + LVFX + BPAA) and EL (EL + LVFX + BPAA) groups were markedly decreased by 2.25 and 9 times that of the control + LVFX group. EL operation and BPAA pretreatment slowed the elimination of LVFX in the serum and brain 4 hr later in the following order: EL + LVFX + BPAA, control + LVFX + BPAA, EL + LVFX, and control + LVFX groups. This order reflects that for the convulsion-inducing doses. These results suggest that EL rats may be a useful model for humans and that the convulsant effect of LVFX with or without BPAA arises not only from the attainment of maximum brain concentration but also from delayed disappearance from the brain. © 1996 Society of Toxicology

Quinolone antibacterial drugs have been reported to induce adverse effects on the central nervous system at an incidence of 1% or less. Among these, convulsion is a serious problem reported at a very low incidence in patients receiving enoxacin (ENX), norfloxacin (NFLX), and ciprofloxacin (CPFX) (Arcieri *et al.*, 1987) alone or in combination with nonsteroidal anti-inflammatory drugs (NSAIDs) (Anastasio *et al.*, 1988; Arcieri *et al.*, 1987; Simpson and Brodie, 1985; Takeo *et al.*, 1989; Wolfson and Hooper, 1988). NSAIDs have also been shown to exacerbate the convulsant potential of quinolones in laboratory animals (Akahane *et al.*, 1989; Murayama *et al.*, 1987). The convulsion induced by quinolone is ascribed to its inhibition of γ-aminobutyric acid (GABA) binding to the receptor in the postsynaptic membrane (Akahane *et al.*, 1989; Dodd *et al.*, 1989; Dodo *et al.*, 1991; Hori *et al.*, 1989; Roussel *et al.*, 1988; Segev *et al.*, 1988; Tsujii *et al.*, 1988a; Yamamoto *et al.*, 1988). NSAIDs have been shown to augment the interaction between quinolones and $GABA_A$ receptors (Akahane *et al.*, 1989; Halliwell *et al.*, 1991; Motomura *et al.*, 1991; Tsujii *et al.*, 1988b). Furthermore, we previously suggested that excitatory and inhibitory effects on glutamate and $GABA_B$ receptors, respectively, were also involved in levofloxacin (LVFX)- and CPFX-induced convulsions by intrathecal injection to mice (Akahane *et al.*, 1993).

In rats, LVFX, an active optical isomer of ofloxacin (OFLX), is excreted into urine and feces in a cumulative ratio of 43.8 and 56.8% (mainly derived from biliary excretion), respectively, during 48 hr after single oral administration at 20 mg/kg (Aoki *et al.*, 1991). A similar excretion ratio (51.9 and 51.8%, respectively) was also seen after intravenous (iv) injection. In contrast, excretion in humans after an oral dose of 100 mg/kg LVFX was 86.7 and 3.9% in urine and feces, respectively (Daiichi Pharmaceutical, Co., Ltd., Tokyo, Japan, in-house data). Although many studies have reported quinolone-induced convulsions in mice and rats, the large difference in excretion routes of LVFX makes it difficult to extrapolate experimental data in rats to humans. Furthermore, the toxicokinetics of LVFX in relation to its convulsant effect has not been reported. Therefore, we produced excretion-limited (EL) rats with one main route (one kidney) of drug excretion and examined the relationship between brain concentration and the convulsant effect of LVFX in control and EL rats after iv injection with or without 4-biphenylacetic acid (BPAA). BPAA is an active metabolite of fenbufen which induced convulsion in humans by coadministration with quinolones and was shown to augment the

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interference of quinolones and $GABA_A$ receptors (Kohno *et al.*, 1994).

METHODS

Male Sprague–Dawley rats aged 6 or 7 weeks were purchased from Japan S.L.C. (Hamamatsu, Japan), housed two or three per wire-mesh cage in an air-conditioned room and acclimated to the environment (temperature, 23 ± 2°C; humidity, 55 ± 15%; light cycle, 12 hr/day) for several days. The animals were allowed free access to commercial laboratory chow (F-2, Funabashi Farm, Japan) and tap water. Body weight at use ranged from 144.8 to 232.4 g and from 198.3 to 273.2 g for 6- and 7-week-old rats, respectively. To limit drug excretion route mostly to one kidney in the EL model, the bile duct was ligated and the left kidney was removed under anesthesia by intraperitoneal injection of pentobarbital 4 days before use. Control animals underwent sham operation for the toxicokinetic study or no operation for the other experiments.

LVFX, (−)-(*S*)-9-fluoro-2,3-dihydro-3-methyl-10-(4-methyl-1-piperazinyl)-7-oxo-7H-pyrido[1,2,3-de][1,4]benzoxazine-6-carboxylic acid hemihydrate, CAS 100986-85-4, was synthesized at Daiichi Pharmaceutical Co., Ltd. and dissolved in saline for single iv injection. BPAA was purchased from Aldrich (Milwaukee, WI), suspended in 0.5% sodium carboxymethylcellulose, and orally administered once 30 min before quinolone injection.

Clinical examination. Blood was collected from the carotid artery of six control and six EL rats under ether anesthesia, and the serum was analyzed for concentrations of glutamic–oxaloacetic and –pyruvic transaminases (GOT and GPT, respectively), alkaline phosphatase (ALP), total bilirubin (T-BIL), creatinine (CRE), and urea nitrogen (UN) using an autoanalyzer (Type 736, Hitachi, Japan).

Convulsion-inducing dose. (1) A 2% solution of LVFX was infused at 1 ml/min into the tail vein of five control and five EL rats until the animals showed convulsion. The convulsion-inducing doses were calculated from the infusion time. (2) LVFX was injected (1 ml/min) into the tail vein at 350, 400, and 450 mg/kg to control and at 300, 350, 400, and 450 mg/kg to EL rats. In addition, after a single oral administration of 400 mg/kg BPAA, LVFX was injected at 150 and 200 mg/kg to other control and at 20, 50, and 100 mg/kg to other EL animals. Maximum drug solution concentration was 2% (450 mg/kg), and this solution was diluted with saline to maintain a fixed injection amount of 22.5 ml/kg for lower doses. Seven animals received quinolone alone and five quinolone with BPAA at each dose level. Clinical observation for convulsion and death was done for 1 day after treatment, and latency time from quinolone injection to the onset of convulsion was measured.

LVFX concentration in serum, brain, and urine. LVFX (10 or 100 mg/kg) was injected alone or with BPAA pretreatment to control and EL rats. Forty-eight rats were used. At 30 min after injection, about 0.4 ml of blood was sampled from the tail vein of conscious rats. The animals were then immediately killed by bleeding from the carotid artery under ether anesthesia for sampling of the whole brain. The time of 30 min was selected on the basis of preliminary study which showed that LVFX concentration reached maximum at this time point. In the second study, 42 animals were treated under the same regimens as described previously (excluding BPAA + 100 mg/kg LVFX in EL animals due to high mortality), and blood was collected at 1, 2, and 4 hr after injection. At 4 hr the animals were killed and the brain was removed. For urine collection, 10 mg/kg LVFX was injected into 10 control and 10 EL animals. Water (10 ml) was then orally administered to all animals, and they were kept in metabolic cages for 4 hr. Serum, brain after removal of dural and subarachnoidal vessels and weighing, and urine were frozen at −80°C until measurement of drug concentration.

Serum or urine (200 μl) was assayed undiluted or diluted 50-fold with 50 mM KH_2PO_4 (pH 4.5). To determine total levels including LVFX glucuronide, other urine samples were diluted 10-fold with KH_2PO_4. The samples were mixed with 1 N NaOH (25 μl) and treated at 100°C for 30 min. To this mixture was added 50 mM KH_2PO_4 (pH 2) (50 μl), and the volume was adjusted to 5 ml with KH_2PO_4. The internal standard (50 μg/ml) DL-8493 (50 μl) was added at an equivalent volume as to serum and urine sample. Brains were homogenized with IS (50 μl) and 10 ml physiological saline (PBS). Homogenate was adjusted to 12 ml with PBS after addition of 1 ml MeOH and then centrifuged at 3000 rpm for 15 min, and the supernatant was obtained. These samples were applied to solid phase extraction to obtain samples for the following HPLC procedures. Following passage through a Bond Elute C8 LRC column (Varian) activated with MeOH, H_2O, and 50 mM KH_2PO_4, the samples were washed with 50 mM KH_2PO_4 and THF/H_2O (20/80, v/v), then eluted with THF/0.15% H_3PO_4 (50/50, v/v). LVFX concentration in 0.05 ml of elute was measured with an HPLC system consisting of a constant-flow pump (C-6A, Shimadzu, Japan), an automatic injector (CR-4A, Shimadzu, Japan), a fluorescence detector (821-FP: excitation, 296 nm; emission, 504 nm; Nihonbunko, Japan), an autosampler (AS-8010, Tosoh, Japan), and an Inertsil ODS-2 column (150 × 4.6 mm i.d., GL Science, Japan). A mixture of THF/50 mM KH_2PO_4 (pH 2)/1 M ammonium acetate (8:92:1, v/v/v) was used as the mobile phase at the flow rate of 1.0 ml/min. The column temperature was kept at 35°C. LVFX glucuronide levels in urine were determined by subtraction of LVFX level from the total level.

Statistical analysis. For statistical analysis of values between two groups, Aspin–Welch or Student's *t* test was employed following the *F* test. For comparisons among three or more groups, Dunnett's or Tukey's multiple test was used following Bartlett's test and one-way analysis of variance.

TABLE 1
Hepatic and Renal Function in Excretion-Limited (EL) Rats 4 Days after Bile Duct Ligation and Unilateral Nephrectomy

Parameter	Control (n = 6)	EL (n = 6)
GOT (IU/liter)	132 ± 9	425 ± 128*
GPT (IU/liter)	42 ± 4	170 ± 46*
ALP (IU/liter)	987 ± 245	1788 ± 194*
T-BIL (mg/dl)	0.08 ± 0.02	13.81 ± 2.06*
CRE (mg/dl)	0.40 ± 0.03	0.50 ± 0.02*
UN (mg/dl)	12.4 ± 1.1	16.6 ± 3.8*

Note. GOT, glutamic–oxaloacetic transaminase; GPT, glutamic–pyruvic transaminase; ALP, alkaline phosphatase; T-BIL, total bilirubin; CRE, creatinine; UN, urea nitrogen.

* Significantly different from the control ($p < 0.05$).

RESULTS

Clinical Examination

Values for hepatic and renal function variables are shown in Table 1. GOT, GPT, ALP, T-BIL, CRE, and UN all significantly increased in EL rats compared to the controls, but CRE and UN remained within the normal range.

Convulsion-Inducing Dose

Continuous infusion. In control rats, group mean convulsion-inducing dose of LVFX on continuous infusion was

TABLE 2

Incidence of Convulsion and Death and Latency Time to Convulsion in Control and Excretion-Limited Rats Given a Single Intravenous Injection of Levofloxacin (LVFX) with or without Oral 4-Biphenylacetic Acid (BPAA)

LVFX dose (mg/kg)	Control rat		Excretion-limited rat	
	LVFX	LVFX + BPAA	LVFX	LVFX + BPAA
20				0/0/5[a]
50				1/0/5 (61)[b]
100				4/3/5 (85)
150		0/0/5		
200		4/4/5 (73)		
300			0/0/7	
350	0/0/7		1/0/7 (40)	
400	0/0/7		2/0/7 (6)	
450	1/0/7 (0)		7/3/7 (0)	

[a] Number of convulsions/deaths/animals used.
[b] Average latency time (min) from injection to convulsion.

518 mg/kg. In contrast, this value was significantly decreased to 316 mg/kg in EL rats.

Single iv. Convulsion incidence, mortality, and latency time to convulsion in control and EL animals given a single iv injection of LVFX alone or with BPAA are presented in Table 2. Minimum convulsion-inducing dose was 450 mg/kg in the control + LVFX group but only 200 mg/kg (2.25-fold decrease) in the control + LVFX + BPAA group. EL animals in the LVFX group showed convulsions at 350 mg/kg (1.28-fold decrease of the control + LVFX value) and in the LVFX + BPAA group at 50 mg/kg (9-fold decrease). Convulsions occurred at 0 (immediately after injection), 73, 0–40, and 61 or 85 min on average after treatment in the control + LVFX, control + LVFX + BPAA, EL + LVFX, and EL + LVFX + BPAA groups, respectively. Mortality in these groups (at respective LVFX doses of 450, 200, 450, and 100 mg/kg) was 0/7, 4/5, 3/7, and 3/5, respectively. In the EL + 100 mg/kg LVFX + BPAA group, the latency time to convulsion ranged from 64 to 112 min.

LVFX Concentration in Serum, Brain, and Urine

Time course of LVFX concentration in serum after a single iv injection is shown in Fig. 1. Thirty minutes after treatment with 10 mg/kg LVFX, concentrations in the control + LVFX and control + LVFX + BPAA groups were 6.44 and 9.10 μg/ml (1.41-fold increase), respectively. In EL animals the concentration was markedly increased to 15.3 μg/ml (2.38 times the respective control) in the LVFX group, but was similar (10.3 μg/ml) to the control in the LVFX + BPAA group. A similar tendency in concentration was seen after injection of 100 mg/kg LVFX: values were control + LVFX group, 65.2 μg/ml; control + LVFX + BPAA, 118.9 μg/ml (1.82-fold increase); EL + LVFX, 104.3 (1.59-fold increase); and EL + LVFX + BPAA, 119.9 (similar to the respective control). Thus, serum concentrations of LVFX in EL animals were 2.38 and 1.59 times the control values at 30 min after injection of 10 and 100 mg/kg, respectively, but were not markedly different on coadministration with BPAA. LVFX concentrations thereafter rapidly or slowly decreased in control animals receiving 10 or 100 mg/kg LVFX alone, respectively. Corresponding to the order in convulsion-inducing doses, the decrease in the 10 mg/kg LVFX groups slowed in the following order: EL + LVFX + BPAA, control + LVFX + BPAA, EL + LVFX, and control + LVFX, with concentrations of 2.6, 1.5, 0.5 μg/ml and undetectable, respectively, at 4 hr after injection. This order of decrease was also seen after injection of 100 mg/kg (no EL + LVFX + BPAA group), with concentrations at the same time point of 16.7, 8.5, and 3.0 μg/ml.

Brain concentration of LVFX and the brain/serum ratios are shown in Table 3. Concentrations obtained 30 min after injection were similar among groups with or without BPAA pretreatment in both control and EL animals, ranging from 0.33 to 0.54 μg/g and from 5.03 to 6.11 μg/g for 10 and 100 mg/kg injections, respectively. However, concentrations in the EL + 10 and 100 mg/kg LVFX groups were 1.33 and 1.19 times higher than those of the respective control groups, and these values were mostly in inverse proportion (−1.28) to the convulsion-inducing dose of the groups. At 4 hr after injection, in accordance with the serum concentrations and convulsion-inducing doses, brain concentrations decreased in the order EL + LVFX + BPAA (0.62 μg/ml), control + LVFX + BPAA (0.20 μg/ml), EL + LVFX (0.11 μg/ml), and control + LVFX group (0.07 μg/ml) for the 10 mg/kg injection, and control + LVFX + BPAA (3.60 μg/ml), EL + LVFX (1.97 μg/ml), and control + LVFX (0.90 μg/ml) for the 100 mg/kg injection. In the 10 mg/kg LVFX groups, brain concentration was increased by BPAA pretreatment to 2.85 and 8.85 times that of the control + LVFX group in control and EL animals, respectively. These values were in good inverse proportion to the 2.25- and 9-fold decreases in the convulsion-inducing dose of the groups. However, no such correspondence was seen among the 100 mg/kg LVFX groups. Brain/serum ratios of LVFX concentration were markedly higher at 4 hr (0.15–0.30) compared to those at 30 min (0.06–0.11), but showed no consistent tendency in BPAA pretreatment or EL operation among any group at either 30 min or 4 hr.

Cumulative drug levels in urine for 4 hr after a single iv injection of 10 mg/kg LVFX are presented in Fig. 2. LVFX was detected at 0.58 mg in the control + LVFX group, decreasing to 0.38 mg in the control + LVFX + BPAA group. By comparison, LVFX levels were somewhat lower (0.46 mg) in the EL + LVFX group but similar (0.37 mg) in the EL + LVFX + BPAA group. In addition, LVFX

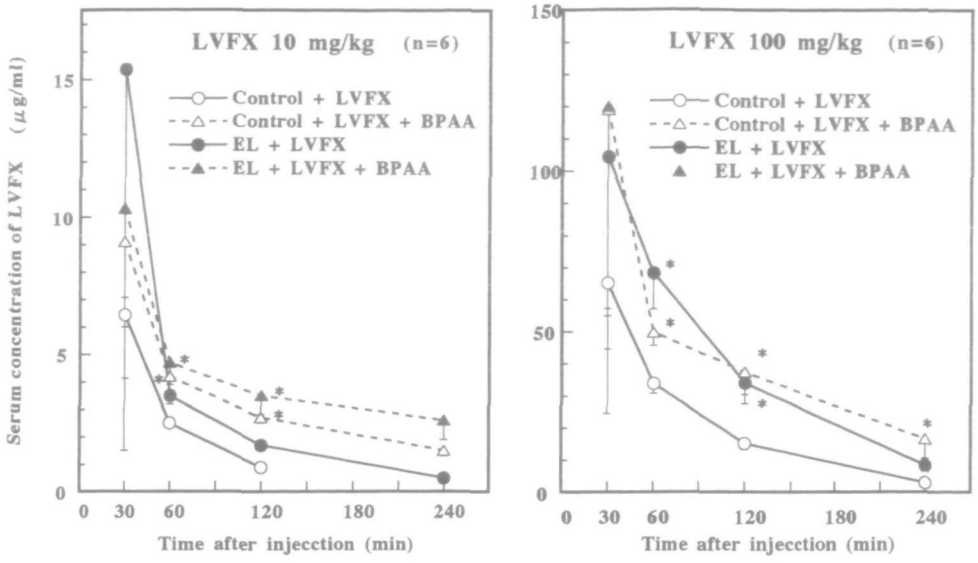

FIG. 1. Serum concentration of levofloxacin (LVFX) in control and excretion-limited (EL) rats after a single intravenous injection alone or with oral preadministration of 400 mg/kg 4-biphenylacetic acid (BPAA). Serum concentration in the EL + 100 mg/kg LVFX + BPAA group was determined only at 30 min after injection because of high mortality at later time points. Vertical bars, standard deviation; *significantly different from the control + LVFX ($p < 0.05$).

glucuronide was detected at closely similar levels (0.05 and 0.04 mg for the LVFX and LVFX + BPAA groups, respectively) in control animals, but was markedly increased to 0.59 and 0.60 mg in the respective EL groups. However, no correlation was seen between urinary LVFX levels and convulsion-inducing doses.

DISCUSSION

To compare the toxicokinetics and convulsant potential of LVFX between EL and control animals, we prepared EL rats by bile duct ligation and unilateral nephrectomy. EL animals were shown to have slightly higher LVFX concentrations in both serum (2.38 and 1.59 times increase) and brain (1.33 and 1.19 times increase) than those of the control group at 30 min after single iv injection at 10 and 100 mg/kg, respectively. Furthermore, the elimination of LVFX in the serum and brain was shown to be delayed in EL animals at 4 hr and was associated with markedly higher urinary excretion of LVFX glucuronide. These findings indicate that LVFX excretion in EL animals was successfully limited. After single iv injection of 20 mg/kg, elimination of LVFX in whole blood was shown to be faster in rats than in monkeys, with 43.8% and more than 85% of LVFX excreted into urine in rats and monkeys, respectively (Daiichi Pharmaceutical, Co., Ltd., in-house data). The elimination pattern and excretion route of LVFX in monkeys are similar to those in humans and also to those in EL rats. EL rats are therefore thought to be a useful model for examination of LVFX toxicity in humans.

BPAA was reported to have no affect on CPFX and OFLX levels in the serum and brain of mice 30 min after single iv injection (Tsutomi *et al.*, 1994). In the present control animals, however, pretreatment with BPAA increased serum LVFX concentration to 1.41 and 1.82 times those of LVFX alone at 30 min after iv injection of 10 and 100 mg/kg LVFX, respectively, and delayed elimination of the drug, with brain levels increasing 2.85 and 4 times at 4 hr. Coadministration of fenbufen with CPFX was reported to delay the disappearance of CPFX from serum, CSF, and the brain after single iv injection of the quinolone to rats (Naora *et al.*, 1991). This was due to an increase in the apparent diffusion clearance of CPFX across the blood–brain and blood–CSF barriers. In contrast, fenbufen has also been reported to increase the serum, CSF, and brain concentrations of OFLX and NFLX injected into rats at 10 mg/kg without any effect on permeability through the blood–brain barrier, but with an increase in the apparent diffusion clearance between blood and CSF (Ichikawa *et al.*, 1992). Increased CSF levels of these quinolones is also thought to be due to the elevation in serum drug concentrations. The binding of OFLX, NFLX, and CPFX to serum protein was not shown to be altered by coadministration with fenbufen (Ichikawa *et al.*, 1992; Naora *et al.*, 1991), despite the fact that fenbufen and BPAA were shown to bind strongly to human serum protein (Chiccarelli *et al.*, 1980). On the presumption that BPAA had no effect on LVFX binding to serum protein, the fact in the present study that there was no consistent tendency for BPAA to change brain/serum ratios of LVFX levels suggests the lack of any contribution of permeability of the drug through the

TABLE 3
Levofloxacin (LVFX) Concentrations in the Brain and Brain/Serum Ratios in Control and Excretion-Limited Rats after a Single Intravenous Injection of LVFX with or without 4-Biphenylacetic Acid (BPAA)

	Control rat		Excretion-limited rat	
	LVFX	LVFX + BPAA	LVFX	LVFX + BPAA
Convulsion-inducing dose (mg/kg)	450	200 (−2.25)	350 (−1.28)	50 (−9.00)
Brain concentration (μg/g)				
30 min				
10 mg/kg LVFX	0.33[a]	0.51* (1.54)[c]	0.44* (1.33)	0.54*† (1.63)
(*n* = 6)	0.03[b]	0.05	0.04	0.03
100 mg/kg LVFX	5.03	5.36 (1.06)	5.99 (1.19)	6.11 (1.21)
(*n* = 6)	0.86	1.39	0.60	0.69
4 hr				
10 mg/kg LVFX	0 07	0.20* (2.85)	0.11 (1.57)	0.62*† (8.85)
(*n* = 6)	0.03	0.03	0.01	0.06
100 mg/kg LVFX	0.90	3.60* (4.00)	1.97* (2.18)	N.D.
(*n* = 6)	0.07	1.20	0.25	
Brain/serum ratio				
30 min				
10 mg/kg LVFX	0.09	0.09 (1.00)	0.06 (−1.50)	0.08 (−1.12)
(*n* = 6)	0.03	0.03	0.03	0.02
100 mg/kg LVFX	0.11	0.09 (−1.22)	0 10 (−1.10)	0.10 (−1.10)
(*n* = 6)	0.02	0.05	0.05	0.04
4 hr				
10 mg/kg LVFX	—	0.15	0.20	0.25
(*n* = 6)		0.01	0.06	0.07
100 mg/kg LVFX	0.30	0.22* (−1.36)	0.24 (−1.25)	ND
(*n* = 6)	0.05	0.04	0.03	

Note. ND, not determined; —, no value because serum concentration undetected.
[a] Mean.
[b] Standard deviation.
[c] Control + LVFX group value.
* Significantly different from the control + LVFX group ($p < 0.05$).
† Significantly different from the EL + LVFX group ($p < 0.05$); analysis was performed between the EL + LVFX and EL + LVFX + BPAA groups.

blood–brain barrier. Rather, the higher LVFX level in brain is speculated to be at least partially due to the higher serum level. Furthermore, cumulative excretion of LVFX into urine for 4 hr after administration was slightly reduced by BPAA pretreatment in control animals in the present study, a finding supported by a previous report that renal clearance and cumulative renal excretion of CPFX were decreased by coadministration with fenbufen (Naora *et al.*, 1990). This decreased renal clearance of LVFX is thought to play a role in the delayed disappearance of the drug from serum and brain. However, it is unclear why such decreased renal clearance of LVFX was not seen in EL animals with markedly high excretion of LVFX glucuronide in urine.

On continuous iv infusion, EL animals became highly susceptible to the convulsant effect of LVFX compared to control animals. Moreover, increased susceptibility, but of a lesser degree, was also seen on single iv injection. Pretreatment with BPAA markedly increased susceptibility of control animals to the effect of LVFX, with the convulsion-inducing dose decreasing 2.25-fold. Furthermore, EL animals were more susceptible to the convulsion-enhancing effect of BPAA than control animals, with the convulsion-inducing dose decreasing 9-fold. This effect of BPAA, as well as the increasing effect on mortality which was shown in the present study, has been reported for many quinolones *in vivo* (Akahane *et al.*, 1993; Dodo *et al.*, 1991; Nozaki *et al.*, 1991; Suzuki *et al.*, 1992). The greater susceptibility of EL rats to the convulsant effect of LVFX alone and especially with BPAA may be extrapolated to humans, who have a markedly higher rate of drug excretion into urine than feces.

With regard to the relationship between raised drug concentration and convulsant effect, the group order of LVFX level in the brain 4 hr after injection was EL + LVFX + BPAA > control + LVFX + BPAA > EL + LVFX > control + LVFX. This was the same as that for the convulsion-inducing doses of LVFX, suggesting a close correlation between LVFX level in the brain and the drug's convulsant potential. Maximum LVFX concentration in the brain was obtained 30 min after single injection (data not shown), and

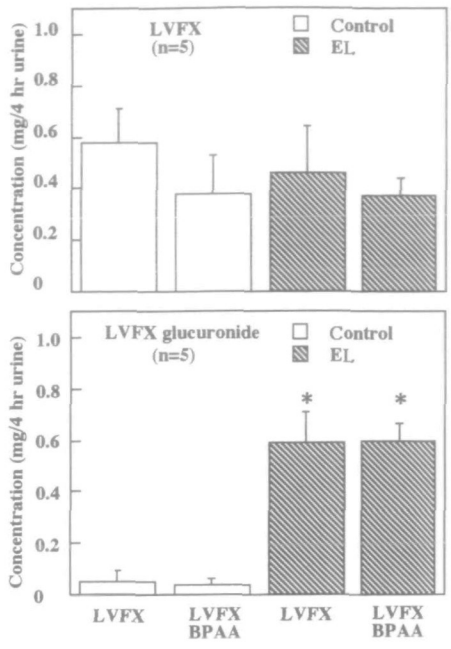

FIG. 2. Cumulative concentration of levofloxacin (LVFX) in urine collected for 4 hr after a single intravenous injection at 10 mg/kg to control and excretion-limited (EL) rats Vertical bars, standard deviation; *significantly different from the respective control ($p < 0.05$).

this was also shown for OFLX (Tsutomi *et al.,* 1994). At this time point, a close correlation was found between convulsion-inducing dose and brain level of LVFX on injection of LVFX alone to EL animals. In contrast, differences were observed after coadministration of LVFX with BPAA: the convulsion-inducing dose in the control + LVFX + BPAA group was 2.25 times less than that in the control + LVFX group, while LVFX levels in the brain after 10 and 100 mg/kg injections were 1.54 and 1.06 times greater; the dose was 9 times less in the EL group, while the levels were 1.63 and 1.21 times greater. These discrepancies are thought to arise from the difference in doses of LVFX and in latency times to the onset of convulsions; in other words, to induce convulsions, higher doses were needed for the control and EL animals receiving LVFX alone with shorter latency times (0 and 0–40 min, respectively), while lower doses were required for both animals receiving LVFX + BPAA with longer times (73 and 61 or 85 min). This suggests that the convulsant effect of LVFX derives from its maximum concentration in the brain after injection at higher doses and from its delayed disappearance from the tissue at lower doses. On the other hand, NSAIDs have been shown to enhance the inhibitory effect of quinolones on GABA binding to its receptor *in vitro* (Akahane *et al., 1989*; Halliwell *et al.,* 1991; Motomura *et al.,* 1991; Tsujii *et al.,* 1988b). However, LVFX and OFLX, even at the high concentration of 1 mM, have been shown to have no inhibitory effect on [^{3}H]muscimol binding to $GABA_A$ receptor in the presence of BPAA, while 50% inhibition of binding was induced by 100 μM ENX with BPAA (Kohno *et al.,* 1994). Furthermore, we previously showed that the excitatory neurotransmitters and $GABA_B$ contributed to convulsions induced by LVFX or CPFX with BPAA (Akahane *et al.,* 1993). These mechanisms may have been involved in the enhancing effect of BPAA on LVFX-induced convulsions observed in the present study.

In conclusion, clinical and 10 times clinical doses of LVFX, namely 10 and 100 mg/kg, respectively, were shown to be safe in terms of convulsion induction in both control and EL (hepatic dysfunction and delayed drug excretion) rats on intravenous injection alone or in combination with a very high dose of BPAA, except for the combination of 100 mg/kg LVFX + BPAA, in EL animals.

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