Neurotoxicity and toxicokinetics of norfloxacin in conscious rats

ZHANG Li-Rong, WANG Yong-Ming, CHEN Bin-Yan, CHENG Neng-Neng[1]

Department of Pharmacology, School of Pharmacy, Fudan University, Shanghai 200032, China

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ABSTRACT

AIM: To study the neurotoxicity and toxicokinetics of norfloxacin (NFLX) in freely moving rats. **METHODS:** Rats were assigned randomly to four treatment groups that received a single iv dose of 50, 100, 200 mg/kg of NFLX and 0.9 % saline, respectively. Electroencephalogram (EEG) was continuously recorded with a computerized system in freely moving rats. Venous blood samples were collected for determination of the NFLX concentration by microbioassay method with *Escherichia coli* 441102 as the test strain. Toxicokinetic parameters were determined from serum concentration-time data with the 3p97 program. **RESULTS:** (1) The epileptiform discharges appeared in all NFLX groups with different latent periods, accompanied with limb twitching and clonic-tonic seizures. The relative total power of the EEG increased. (2) Drug serum concentration-time curves of different doses conformed to a two-compartmental model. The values of clearance, volume of distribution, and terminal half-life were dose-independent, while maximum serum concentrations (C_{max}) and the areas under the concentration-time curve ($AUC_{0\to\infty}$) of NFLX increased with dosage. (3) The relative total powers of EEG were closely correlated with the administered dose, C_{max} as well as $AUC_{0\to\infty}$. **CONCLUSION:** The present study established a suitable approach to quantitatively determine central nervous system (CNS) stimulant effect of NFLX. There is a significant correlation between $AUC_{0\to\infty}$ and the changes of relative total power, which may serve as the index for judgement and prediction of the CNS toxic effect induced by NFLX.

INTRODUCTION

Fluoroquinolones (FQ) were very effective for the treatment of various bacterial infections. Because they can spread into the central nervous system (CNS), they have been proposed as alternatives in the treatment of CNS infections[1]. Since their introduction to the clinical use, various adverse CNS effects, including headache, confusion, hallucination, anxiety, nervousness, nightmares, and seizures, have been reported for patients receiving FQ[2-4], of which convulsive seizures have mostly been reported for high-risk patients such as patients with a history of epilepsy[5]and patients who are cotreated with nonsteroidal anti-inflammatory drugs such as fenbufen[6,7]. Although the exact mechanism by which FQ exhibit epileptogenic activities remains unclear, it has been admitted for a long time that CNS excitation of FQ may result from inhibition of γ-aminobutyric acid (GABA) binding to its receptors[8]. As a consequence, the epileptogenic activities of FQ have often been assessed from *in vitro* GABA binding experiments[9,10]. However, FQ alone have no or only a

[1] Correspondence to Prof CHENG Neng-Neng.
Phn 86-21-5423-7465. Fax 86-21-6469-3495.
E-mail nncheng@shmu.edu.cn
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weak affinity for $GABA_A$ receptors, and in order to observe significant binding to these receptors, biphenyl acetic acid (BPAA), the active metabolite of fenbufen, is usually added to FQ[9-11]. Therefore it may be inappropriate to predict the convulsant risk associated with FQ administration in patients from *in vitro* GABA binding experiments in the presence of BPAA. Recently, the improved *in vitro* approaches have been proposed, in particular that using the *Xenopus* oocyte translation system of exogenous messenger RNA[12] and that of an optimized hippocampus slice model[13]. Although these approaches are very useful in characterizing the epileptogenic activities of FQ, their extrapolation onto the *in vivo* situation, in particular when FQ are administered without BPAA, remains questionable.

The individual FQ considerably differ in their physicochemical characteristics and pharmacokinetic properties. The pharmacokinetic behavior appears to play an important role in the induction of neurotoxicity in combination with their intrinsic potency[11].

The objective of this study was to explore the correlation of the induction of neurotoxicity with the concentrations of norfloxacin (NFLX) in serum in conscious rats. It might provide a better basis for a reasonable risk assessment. NFLX was selected as a probe drug because of its presumably high convulsant activity due to the absence of methyl group[2].

MATERIALS AND METHODS

Animals Male Sprague-Dawley rats (10-14 weeks old, 200-250 g, Grade II, Certificate No 003) were provided by Experiment Animal Center of Fudan University and housed under controlled conditions (temperature: 21 ° C±1 ° C and lighting: 8:00–20:00) with food and water *ad libitum*.

Surgical procedure For the measurement of electroencephalogram (EEG) signals, 4 permanently stainless steel screw electrodes were implanted bilaterally into the frontoparietal area under anesthesia with sodium pentobarbital (50 mg/kg, ip) 1 week before the kinetic-dynamic experiments. Briefly, the electrodes were placed at the locations 2 mm anterior and 2.5 mm lateral, 1.5 mm posterior and 3 mm lateral to bregma. The ground electrode was implanted epidurally over the nasal bone. The electrodes were fixed to the skull with dental acrylic cement. Every day in the post-operative period (7 d) each rat was habituated for 1 h to the recording box (Plexiglas, 40 cm×40 cm×30 cm). One day before the experiment, indwelling cannula was implanted into the right jugular vein for drug administration.

Drug dosage and blood sampling Rats were assigned randomly to four treatment groups that received NFLX 50, 100, 200 mg/kg, and 0.9 % saline iv, respectively, by an infusion pump for over 2 min. NFLX was dissolved in 0.1 mol/L NaOH and sterile saline. Solution was prepared immediately before injection (volume 1 mL per 100 g of body weight). Venous blood was collected just before and at 1, 5, 15, 30, 45 min and 1, 1.5, 2, 3, 4, 6, 8 h after drug administration from an incised tail vein. Serum containing norfloxacin was separated and stored at –20 ° C until assayed.

EEG measurements The output from bipolar EEG leads was continuously recorded using a Nihon-Kohden AB-621G Bioelectric Amplifier and concurrently digitized at a rate of 210 Hz using a data acquisition system (Gould Electronics, DASA system). The digitized signal was fed into an IBM PC/286 compatible personal computer and stored on hard-disk for off-line analysis. The EEG was recorded 15 min before drug administration for base-line determination. After injection of the drug, the EEG recordings were continued until the signals had returned to base-line values. For each 4-s epoch, quantitative EEG parameters were obtained off-line by fast Fourier analysis with a user-defined program. The EEG parameters were calculated by averaging at least 120 s of consecutive EEG data. To account for large interindividual differences in the level of absolute total power, total power was expressed as a percentage of the value measured before the administration of NFLX in that individual.

Microbioassay method NFLX concentrations in serum were determined by a validated agar diffusion microbioassay method using *Escherichia coli* 44102[14]. The standard curve was prepared in rat normal serum. The linear relation was determined at the concentration of 1, 2, 4, 8, 32, 64, and 128 mg/L. The intra- and inter-day coefficients of variation were less than 6 %. The limit of sensitivity of the assay was about 0.5 mg/L.

Data analysis Pharmacokinetic analysis was performed with the 3p97 program. Values were expressed as mean±SD. One-way analysis of variance with Dunnett's test was used for comparisons of the mean values. Incidence of clonic and tonic convulsions and death in the different groups were compared with Fisher's exact probability test. A probability level of $P<0.05$ was chosen as statistical significance for all tests.

RESULTS

General epileptic behavior We observed the intensity of seizures after a single injection of different doses of NFLX in rats (Tab 1). After administration of NFLX 50, 100 mg/kg, rats showed an increase in episodes of wild running or escape response, ear and facial twitching, and clonus of the forelimbs and hindlimbs. With NFLX 200 mg/kg tonus of forelimbs and hindlimbs was also observed, and in 2 of 5 animals the tonic extension of the hindlimbs was followed by respiratory arrest and death.

Tab 1. Incidence of seizure phases induced by norfloxacin (NFLX) in rats. *n*=5-10. $^{c}P<0.01$ *vs* saline group.

Dose/mg· kg^{-1}	Clonic seizures	Tonic seizures	Death
Saline	0/5	0/5	0/5
NFLX 50	2 /6	0/6	0/6
100	8/10^{c}	2/10	0/10
200	5/5^{c}	5/5^{c}	2/5

Groups of rats were injected iv with 0.9 % saline, 50, 100, and 200 mg/kg of NFLX, respectively. Animals were observed for 8 h after drug administration.

EEG effects We observed the electrocorticographic epileptic discharges after a single injection of different doses of NFLX in rats. The EEG during seizures were characterized by sharp- or spike-waves, followed by polyspikes in NFLX 200 mg/kg group (Fig 1). The epileptic discharges appeared more rapidly in NFLX 200 mg/kg group than in NFLX 50 and 100 mg/kg groups.

The characteristic EEG parameters were calculated from the raw EEG data. Relative total power increased after a single injection of different doses of NFLX in rats. It reached the maximum at 1.5 h after the adiministration of NFLX and returned gradually to baseline in about 8 h. Compared with pretreatment and control group, a significant increase in relative total power was observed from 15 min to 8 h in NFLX 200 mg/kg group ($P<0.05$). In NFLX 100 mg/kg group, the relative total power increased obviously from 1 h to 2 h too ($P<0.05$). NFLX 50 mg/kg group also showed a trend of elevated relative total power (Fig 2). In addition, the power percentage of α and β band increased, while that of δ band decreased in NFLX 200 mg/kg group

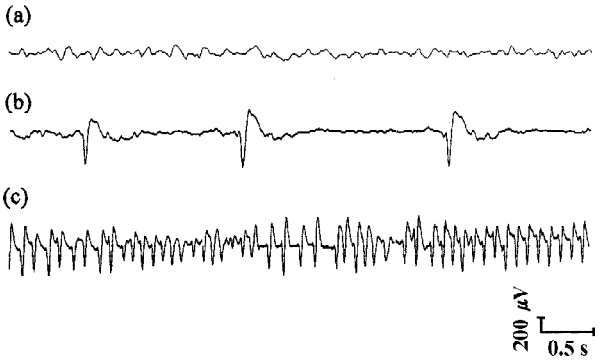

Fig 1. Electrocorticograms, before and during seizures induced by a single iv injection of NFLX 200 mg/kg, in rats. (a) Baseline activity. (b) Electrocorticogram 10 min after the adiministration of NFLX. (c) Electrocorticogram 60 min after the adiministration of NFLX.

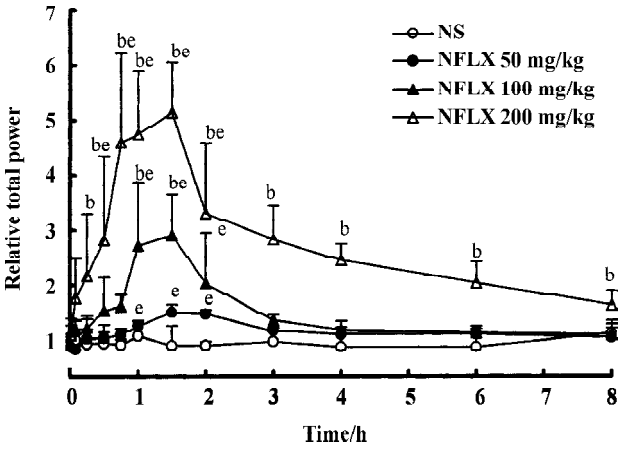

Fig 2. Relative total power of electrocorticogram versus time profile in different treated groups. *n*=4-6. Mean±SD. $^{b}P<0.05$ *vs* normal saline treated group. $^{e}P<0.05$ *vs* baseline.

($P<0.05$ *vs* baseline, data not shown).

Toxicokinetics Toxicokinetic parameters were calculated with 3p97 program, and maximum serum concentration (C_{max}) was taken directly from the observed data . Drug serum concentration-time curves of NFLX 50, 100, and 200 mg/kg groups conformed to a two-compartmental model (Fig 3). Main toxicokinetic parameters of three groups were shown in Tab 2. The C_{max} and the areas under the concentration-time curve ($AUC_{0\to\infty}$) of NFLX increasd with dosage in rats. The values of clearance (CL), volume of distribution (V_c) and terminal half-life ($T_{1/2\beta}$) were independent of the administered dose.

Relation of exposures and EEG changes The relative total power of EEG was closely correlated with the dose, C_{max}, and $AUC_{0\to\infty}$ of NFLX (Fig 4).

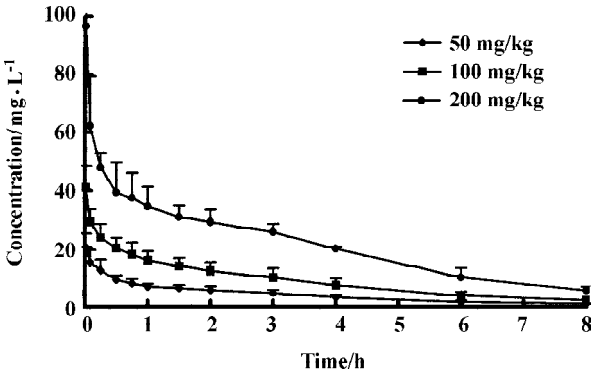

Fig 3. Serum concentration-time profile after a single intravenous dose of NFLX 50, 100, and 200 mg/kg in rats.

Tab 2. Main pharmacokinetic parameters after a single iv injection of NFLX in rats. *n*=5. Mean±SD.

Parameters	50 mg·kg^{-1}	100 mg·kg^{-1}	200 mg·kg^{-1}
C_{max}/mg·L^{-1}	20±5	41±7	97±22
$T_{1/2\alpha}$/h	0.10±0.05	0.11±0.13	0.04±0.02
$T_{1/2\beta}$/h	2.37±0.16	2.36±0.17	2.53±0.12
V_c/L·kg^{-1}	2.4±0.6	2.3±0.5	1.8±0.6
$AUC_{0\to\infty}$/mg·h·L^{-1}	35±8	79±16	185±29
CL/L·h^{-1}	1.5±0.3	1.34±0.28	1.14±0.20

DISCUSSION

Only a limited number of studies are presently available on the relationship between plasma concentration and neurotoxic effect of FQ[11,15]. These studies have measured the drug effect with behaviorial responses which are wholly or partly subjective[11,15,16]. Quantitative EEG measures have been proposed as more objective measures of central action of benzodiazepines and other classes of drugs[17]. Therefore, computerized quantitative analysis of the EEG was used in the present study. Here we present data combining information on the neurotoxic effect of NFLX as well as the kinetics of the drug in the serum of rats.

Our observed EEG-changes revealed a clear CNS stimulant effect of NFLX, identified mainly by an increase in relative total power and power percentage of α and β band and a parallel decrease of δ band in rats. Additionally, general epileptic behaviors and epileptic discharges occurred in a dose-dependent manner in the groups treated with NFLX. However, the effect on the power percentage of α, β, and δ bands was less consistent and showed more interindividual variation. The

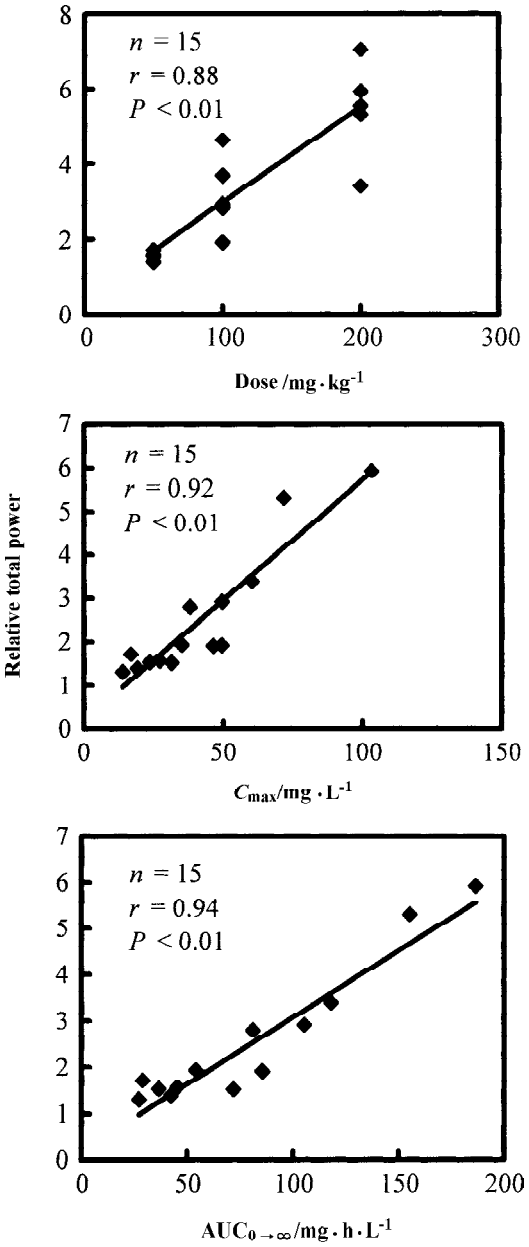

Fig 4. Correlations between the relative total power and the dose, maximum serum concentration (C_{max}), the area under the concentration-time curve ($AUC_{0\to\infty}$) of NFLX.

increase in the level of relative total power, as determined by EEG analysis, was finally used as a measure of the EEG effects of NFLX. In our study, the experiments were conducted in freely moving rats, which resulted in a more stable EEG effect parameter when compared with previous studies performed in anesthetized animals[18,19]. It is well known that anesthesia affects the function of CNS.

Usually, the data from toxicological studies can be interpreted only on the basis of the kinetics of the compound in the species studied. For most FQ kinet-

ics in various species differ considerably[15], therefore, we also assessed the serum levels of NFLX in rats. Our results indicated that drug serum concentration-time curves of 50, 100, and 200 mg/kg groups conformed to a two-compartmental model with a rapid distribution phase. The C_{max} and $AUC_{0\to\infty}$ of NFLX increased proportionally with doses ranged from 50 to 200 mg/kg, and $T_{1/2\beta}$ were dose-independent, exhibiting the characteristic of linear pharmacokinetics over the dose range.

It is notable that although we used doses that were up to 8-, 16-, and 32-fold clinical therapeutic doses (approximately 6 mg/kg) in our experiments, the mean peak concentrations after a single injection of 50, 100, and 200 mg/kg of NFLX were only 3, 6, and 14 times of those in human volunteers after administration of a single intravenous dose of 400 mg[20]. In view of the fact that the relative total power of EEG was better correlated with $AUC_{0\to\infty}$ of NFLX than with the dose, it is very important to include the toxicokinetic investigations as a part of toxicological studies in order to provide a reasonable basis for risk assessment.

Despite additional studies are needed to investigate the mechanism that causes the CNS adverse effects of quinolones, physicians should take into consideration the possible epileptogenic activity of FQ when treating patients with predisposing epileptic factors or when the penetration of FQ into the brain via a damaged blood-brain barrier is enhanced.

In conclusion, the present study established a suitable approach to quantitatively determine CNS stimulant effect of NFLX. There is a significant correlation between $AUC_{0\to\infty}$ and the changes of relative total power of EEG, which is chosen to be the index for judgement and prediction of the CNS toxic effect induced by NFLX. Meanwhile, toxicokinetics have to be taken into account.

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