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EVALUATION FOR THE CONVULSIVE LIABILITY OF VARIOUS QUINOLONE DERIVATIVES IN MICE

Kyung-Eob Choi, Young-Hoon Kim, Dal-Hyun Kim, Dong-Moon Lim and Min-Do Huh*

Department of Pharmacology/Toxicology, R&D Center, Cheil Foods & Chemicals, Co., Ltd, 467-810, Korea
**Department of Fish Pathology, College of Fisheries Science, National University of Pusan, Pusan 608-737, Korea*
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ABSTRACT: *The present study was performed to evaluate whether the application of Fenbufen is reasonable for predicting the convulsive liability of the quinolone derivatives and to examine whether pentylenetetrazole (PTZ) can be used as a screening tool for their Central Nervous System (CNS) toxic pontential. The convulsive activity of the quinolones was markedly potentiated by the pretreatment of Fenbufen. In combination with Fenbufen, enoxacin (ENX), norfloxacin (NFLX), and ciprofloxacin (CPFX) provoked convulsions and subsequent death at the intravenous doses of 5 mg/kg, 10 mg/kg, and 40 mg/kg, respectively, whereas ofloxacin (OFLX) and pefloxacin (PFLX) did not induce convulsions and death even at a relatively high dose of 100 mg/kg, iv. However, when given alone, OFLX and PFLX showed lower CD_{50} values than the other agents used. ICR and DBA strains were found to be highly sensitive to the ENX-induced convulsion, which occurred maximally within 30 to 60 minutes post treatment of oral Fenbufen. The PTZ-induced convulsive activity was potentiated not only by ENX, NFLX and CPFX, but also by OFLX. But, there was no significant difference in the proconvulsive activity between the quinolones when combined with PTZ. These findings suggest that the Fenbufen method should be meaningful to some extent in predicting the CNS toxic potential of the quinolones via interaction with nonsteroidal anti-inflammatory drugs (NSAIDs). Since there are still not enough data to support the Fenbufen method is a reasonable screening tool for the CNS toxic liability, further intensive studies should be conducted to elucidate the mechanisms of the quinolone-induced convulsions and to develop more reasonable and rapid screening methodology.*

Key words: *Quinolone Derivatives, Convulsion, Fenbufen, Pentylenetetrazole*

INTRODUCTION

The quinolone derivatives have been used for the treatment of a variety of infections because of their excellent tissue permeability and high level of activity against Gram positive and negative bacterial pathogens.

However, most of these quinolones have been reported to possess possible side effects on the central nervous system (Arcieri *et al.*, 1987). Such CNS-related signs as tremor, headache, dizziness and restlessness have been observed in patients treated with the quinolones. It has been known that the CNS effects of the quinolones are related to their competitive inhibition of γ-aminobutyric acid (GABA) receptors (Tsuiji *et al.*, 1988). In particular, severe signs such as seizures and hallucinations have been rarely observed in patients who received the quinolones alone, but more frequently in patients who received the quinolones in combination with nonsteroidal anti-inflammatory drugs (NSAIDs) (Janknet *et al.*, 1986; Segev *et al.*, 1988). The convulsive thresholds of various quinolones were significantly lowered by NSAIDs in experimental animals, as well (Akahane *et al.*, 1989, Dimpfel *et al.*, 1991). Thus, in the development of new quinolone derivatives, Fenbufen, an NSAID, is usually applied as a screening tool for the CNS toxic potential.

In addition, pentylenetetrazole NSAID (PTZ) has been also used as a laboratory tool for screening the anti-convulsant drugs. It is generally known that a major action of PTZ may reduce GABAergic inhibition in the CNS (Alfred *et al.*, 1985). Therefore, PTZ could be also utilized for evaluating the epileptogenic potentials of the quinolones.

The present study was performed to evaluate whether the application of Fenbufen is reasonable for predicting the convulsive liability of the quinolones and to consider whether PTZ can be used as a screening tool for their CNS toxic potential.

MATERIALS AND METHODS

1. Animals and Chemicals

Male ICR, BALB/c, C57BL/6 and DBA mice weighing 18 to 22g, were used to find an optimal animal model that is sensitive to the induction of convulsion. ICR mice were used throughout the experiment, in otherwise specified. All mice were bred in our Animal Facilities and received tap water and food (Cheil Foods and Chemicals Co.) *ad libitum*.

Enoxacin (ENX), norfloxacin (NFLX), ciprofloxacin (CPFX) and ofloxacin (OFLX) were synthesized in our Organic Chemistry Laboratory. And, pefloxacin (PFLX) was purchased from Rhone-Ploulenc Ro. These quinolones were greater than 99% in purity and dissolved in physiological saline solution containing 0.1 N NaOH,

except for CPFX, which was dissolved in physiological saline containing 10% lactic acid (final pH 3.5). Commercial Fenbufen (Yuhan Pharmaceutical Co., Ltd.) was suspended in 0.5% CMC solution, and pentylenetetrazole (PTZ, Sigma Chemial Co., St. Louis, Mo, U.S.A.) was dissolved in physiological saline. All solutions prepared as above were filtered with a microfilter (Sterile Acrodisc 0.45 μm, Gelman Sci. USA) prior to intravenous administration.

2. Determination of Convulsive Activity

2.1. Quinolone Derivatives Given Alone

All of the quinolone derivatives were intravenously administered at different doses in a fixed volume of 10 ml/kg of body weight. The outbreaks of convulsions represented as twitch and head oscillation were recorded for 30 minutes and the CD_{50} (50% Convulsive Dose) was calculated by using the Probit method.

2.2. Quinolone Derivatives Given in Combination with Fenbufen

To examine and compare the convulsive activities of ENX, NFLX, CPFX, OFLX and PFLX, each of them was intravenously injected 30 minutes after oral administration of Fenbufen (300 mg/kg). Thereafter, manifested signs and symptoms, particularly pertaining to convulsive parameters, were continuously and carefully observed for up to 4 hrs. The onset times of head oscillation (HO) and running/fit (R/F) and the subsequent death for each mouse were recorded.

To assess the strain differences of the convulsive liability to the quinolone derivatives, four strains of mouse, ICR, BALB/c, C57BL/6 and DBA were used. Doses of 2.5 mg/kg and 5 mg/kg of ENX were given intravenously 30 minutes after the oral administration of Fenbufen (300 mg/kg). The convulsive response of each mouse was observed and recorded as described above.

To determine the effect of dosing schedules on the convulsive activity, ENX (5 mg/kg) was intravenously injected 15, 30, 40, 60 and 90 minutes after the oral administration of Fenbufen (300 mg/kg) and then the convulsive parameters were observed and recorded as described previously.

2.3. Quinolone Derivatives Given in Combination with PTZ

PTZ was intraperitoneally administered at varing doses in the range of 30 to 51.2 mg/kg and the CD_{20} was then calculated. The CD_{20} of PTZ was intraperitoneally injected 5 minutes following the intravenous administration of each quinolone at doses of 50, 100 and 150 mg/kg.

RESULTS

1. CD_{50} Values of the Quinolone Derivatives Given Alone

The intravenous CD_{50} values of ENX, NFLX, OFLX and PFLX were 246.3, 263.2, 154.2 and 176.9 mg/kg, respectively when administered alone (Table 1). The CD_{50} of CPFX could not be determined because the typical convulsive signs were not developed by the drug.

2. Quinolones Given in Combination with Fenbufen

The convulsive activities of the 5 quinolone derivatives were compared when

combined with Fenbufen (Table 2). The convulsive signs included head oscillation, (HO), staggering gait, tremor, running and fit (R/F) and subsequent death. Incidence rates of each convulsive parameter increased in a time- and dose-dependent manner. The epileptogenecity of the quinolones was great in descending order of ENX, NFLX, CPFX, OFLX and PFLX. The clonic convulsion and the resultant death occurred in the mice treated with ENX (5 mg/kg, i.v.), NFLX (10 mg/kg, i.v.) and CPFX (40 mg/kg, i.v.). But, neither OFLX nor PFLX induced convulsion even at a high intravenous dose of 100 mg/kg.

The convulsive activity of ENX (2.5 mg/kg and 5 mg/kg, i.v.) was different among the mouse strains studied (Table 3). The DBA and ICR mouse were shown to be high responders to ENX, while the C57BL/6 mouse was less sensitive than the other strains used.

As shown in Table 4, the convulsive activity of ENX (5 mg/kg, i.v.) was dependent upon various injection times following the administration of oral Fenbufen. The peak of convulsive responsiveness was observed when ENX was injected 30-60 minutes following the oral treatment of Fenbufen.

Table 1. CD_{50} values of the quinolone derivatives given alone in ICR mice

Quinolone	CD_{50} (mg/kg, i.v.)	Confidence Limits (95%)
Enoxacin	246.3	206.5-279.6
Norfloxacin	263.2	244.0-287.9
Pefloxacin	176.9	160.2-193.5
Ofloxacin	154.2	140.1-169.7

*The CD_{50} value of Ciprofloxacin could not be determined because its typical convulsive signs were not shown.

Table 2. Convulsive acitivities of quinolones antibiotics given in combination with fenbufen in ICR mice

Quinolone	Dose (mg/kg, i.v.)	Incidence Rate and Time (min) of Convulsive Parameter*		
		HO	R/F	Death
Enoxacin	2	5/5(16.6±9.21)	0/5	0/5**
	5	5/5(13.4±12.8)	4/5(33.6±26.0)	4/5(57.5±9.45)
	10	5/5(4.05±1.19)	5/5(12.6±4.07)	5/5(20.8±3.34)
Norfloxacin	5	4/5(11.0±0.82)	0/5	0/5
	10	4/4(4.0±1.83)	4/4(34.0±6.88)	4/4(86.0±8.29)
	20	5/5(2.3±0.45)	5/5(12.8±6.5)	5/5(24.6±12.4)
Ciprofloxacin	10	4/5(9.38±2.84)	0/5	0/5
	20	5/5(11.26±6.03)	0/5	0/5
	40	5/5(8.20±4.4)	2/5(24.5±2.12)	1/5(99)
Ofloxacin	100	0/5	0/5	0/5
Pefloxacin	100	0/5	0/5	0/5

All quinolones were injected 30 min. after the administration of fenbufen (300 mg/kg, p.o.)
*: HO, Head Oscillation R/F, Running and Fit
**: Manifested number/used number

3. Quinolones Given in Combination wiht PTZ

Table 5 shows the PTZ-induced convulsion was increased in a dose-dependent manner in the ICR mouse. The CD_{20} of PTZ (37.5 mg/kg, i.p.) was selected and administered to evaluate the potentiating effect of the quinolones. The results were summarized in Table 6, which shows that PTZ given alone at the CD_{20} resulted in an incidence rate of 40%. However, it was found that all of the quinolones used at i.v doses of 50, 100 and 150 mg/kg. Consistently potentiated the PTZ-induced convulsions in a dose-dependent manner. But, there were no differences among the quinolones in potentiating the convulsive rates.

Table 3. Convulsive acitivities of enoxacin given in combination with fenbufen in different strains of mice

Strain of Mice	Dose (mg/kg, i.v.)	Incidence Rate and Time (min) of Convulsive Parameter*		
		HO	R/F	Death
ICR	2.5	5/5(6.1±3.5)	4/5(165±81)	0/5**
	5	5/5(2.26±1.85)	5/5(58±24.5)	4/5(105.3±7.9)
BALB/c	2.5	5/5(33.6±20.3)	1/5(127)	0/5
	5	5/5(20.2±12.6)	4/5(37±23.1)	2/5(218.5±20.3)
DBA	2.5	5/5(28.8±8.23)	5/5(76.6±38.1)	5/5(370±48.6)
	5	4/4(21.7±6.4)	4/4(54.5±25.9)	4/4(209±44.3)
C57BL/6	2.5	3/4(4.23)	0/4	0/4
	5	5/5(1.99)	0/5	0/5

Enoxacin was injected 30 min. after the administration of fenbufen (300 mg/kg, p.o.)
*: HO, Head Oscillation R/F, Running and Fit
**: Manifested number/used number

Table 4. Convulsive acitivities of enoxacin given at various times after the administration of fenbufen in ICR mice.

Injection Time(min) after Fenbufen	Incidence Rate and Time(min) of Convulsive Parameters*		
	HO	R/F	Death
15	4/4(10.5)	2/4(27)	2/4(50.5)**
30	4/4(4.3)	3/4(26.7)	3/4(68)
40	5/5(2.3)	3/5(14.3)	2/5(33.5)
60	5/5(2.4)	4/5(11.8)	3/5(58.3)
90	5/5(7.8)	1/5(15.0)	1/5(28.0)

Enoxacin (5 mg/kg, i.v.) was injected at various times after the administration of fenbufen (300 mg/kg, p.o.).
*: HO, Head Oscillation R/F, Running and Fit
**: Manifested number/used number

DISCUSSION

The present study was undertaken to examine the convulsive activity of the quinolone derivatives through the *in vivo* method using Fenbufen and then to evaluate whether this method is reasonable for screening the CNS toxic potential of the quinolone derivatives. In addition, it was also aimed to assess the feasibility to use PTZ, an agent affecting the excitability of CNS, as a tool for estimating the convulsive liability of newly synthesized quinolones in the primary screening stage. Since the oral absorption of each quinolone is highly variable, intravenous route was used for the administration of all quinolons investigated in this study.

The convulsive activity of the quinolones was significantly potentiated by the pretreatment of Fenbufen, which is consistent with the results reported by other

Table 5. Pentylenetetrazole (PTZ)-induced convulsion in ICR mice

Dose (mg/kg, i.v.)	Rate of Incidence	CD_{50} (mg/kg, i.v.)
30	0/10*	
35	1/10	
38.5	5/10	39.88 mg/kg
42.3	6/10	(37.39-42.09)
46.6	9/10	
51.2	10/10	

Each mouse was observed for 20 min. after the administration of PTZ. CD_{50} of PTZ was calculated by the Probit method.
*: Manifested number/used number

Table 6. Effects of the quinolone derivatives on PTZ-induced convulsion in ICR mice

Treatment	Dose (mg/kg, i.p.)	Convulsive Rate (%)
PTZ alone	37.5	40
PTZ plus:	50	60
Enoxacin	100	70
	150	70
Norfloxacin	50	50
	100	70
	150	90
Ciprofloxacin	50	40
	100	60
	150	80
Ofloxacin	50	40
	100	60
	150	100

PTZ was injected at a dose of 37.5 mg of PTZ/kg/10 m*l* alone and in combination with quinolones. Each mouse was observed for 30 minutes.

investigators (Akahane *et al.*, 1989). It was found that ENX induced convulsion and subsequent death occurred at an intravenous dose of 5 mg/kg, whereas both OFLX and PFLX provoked neither convulsion nor death at intravenous dose of 100 mg/kg. Thus, it would be reasonable to propose some guidelines as follows; when a newly synthesized quinolone provokes convulsion or death at an intravenous dose of 5 mg/kg, it would be considered as a high-risk compound in terms of producing CNS toxicity. The precise role of Fenbufen in the quinolone-related CNS effect is still unclear. However, it has been postulated that Fenbufen may enhance the binding of the quinolones to the GABA receptor sites in the central nervous system (Schluter *et al.*, 1985). Kohgi *et al.* (1991) reported that Fenbufen increases the permeability of CPFX across blood-brain barrier. From our results, it is interesting that there was no correlation in convulsive potencies of the quinolones given between in combination with Fenbufen and alone (Table 1). However, this discrepancy remained unclear. It is, therefore, suspected that more complex interaction of the quinolones with Fenbufen should exist in the CNS. Christ *et al.* (1988) and Nozaki *et al.* (1989) suggested that the dopaminergic, opioidergic or glutaminergic receptor in addition to GABAergic one should be possibly involved in CNS effects of the quinolones. It is still doubtful that the method using Fenbufen can predict the CNS toxic potential of the quinolones. The convulsive activity of ENX given in combination with Fenbufen was slightly different among the strains of mice. The ddY mouse has been used to screen the CNS side effect in many other reports (Akahane *et al.*, 1989). The ICR mice have been used for screening the toxic effect of newly synthesized quinolones because of its easy availability in most laboratories. Among the murine strains tested, the ICR mice which are commonly used in our laboratory turned out to be an optimum animal model for the screening of CNS toxic potential of the quinolones. The administration time of the quinolones following Fenbufen treatment was found to be an important factor of modulating the incidence rates of convulsion. This observation might be associated with the pharmacokinetic characteristics of Fenbufen in the brain tissue.

The usefulness of PTZ in assessing the convulsive liability of the quinolones was also evaluated in this study. Although no difference in the incidence rate was observed among the quinolones, as seen in the Fenbufen method, all of them consistently augmented the convulsive activity of PTZ. This potentiating effect has been also reported with β-lactam antibiotics as well (Williams *et al.*, 1988). It is of interest that OFLX showed a similar effect on the potentiation of PTZ-induced convulsion to that of the other derivatives, while OFLX showed a weak response in the Fenbufen method. There are some clinical reports on the CNS adverse effect of OFLX (Janknet *et al.*, 1986). Our results demonstrate that OFLX given alone had lower CD_{50} value compared to the other quinolones used. Until now, a few data are available to support the *in vivo* test using Fenbufen is a reasonable screening tool for the CNS toxic liability. However, it is obvious that the Fenbufen method should be meaningful to some extent in predicting the CNS toxic potential of the quinolones *via* interaction with NSAIDs. In addition, PTZ should be also further elucidated for its usefulness as a screening tool in the development of new drugs.

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