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EFFECTS OF 8-FLUOROCIPROFLOXACIN AND CIPROFLOXACIN ON THE CENTRAL NERVOUS SYSTEM

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ABSTRACT: *The objectives of this study were to investigate the effects of 8-fluorociprofloxacin (8-FCP) on the central nervous system (CNS) and to compare with those of ciprofloxacin (CP). The LD_{50} values of intravenous 8-FCP were similar or slightly lower in rat (M; 203.6 mg/kg, F; 186.1 mg/kg) and markedly lower in mice (M; 126.5 mg/kg, F; 163.1 mg/kg), as compared to those of CP. However, no recognizable differences in the clinical signs and recovery were found between 8-FCP and CP in both species. In combination with fenbufen, the convulsive liability of 8-FCP was higher than that of CP. At an intravenous dose of 10 mg/kg, 8-FCP provoked convulsive signs and subsequent death in mice, whereas CP produced convulsion at a dose of 40 mg/kg. The hexobabital-induced sleeping time was markedly lengthened by the oral administration of 8-FCP, but slightly increased by CP. In addition, the two quinolone derivatives had analgesic effects. The analgesic activity of 8-FCP was approximately two times higher than that of CP. However, both 8-FCP and CP had little effects on pentylenetetrazole- or strychnine-induced convulsion and muscle relaxation. Our finding that 8-FCP had more remarkable CNS effects than CP strongly suggests that there should be differences in the pharmacokinetic characteristics and/or in the binding affinity for specific biologic targets, or receptors, in the CNS.*

Key Words: *Ciprofloxacin, 8-fluorociprofloxacin, CNS effects, convulsion, sleeping time, analgesic effects.*

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INTRODUCTION

There are a number of derivatives substituted at C-8 with halogen, alkyl, or alkoxy group. It has been already known that most of these quinolone derivatives have generally comparable or slightly inferior *in vitro* antibacterial activities, but comparable or superior *in vivo* efficacy to their respective non-substituted one. This overall improvement in the *in vivo* activity of the substituted derivatives has been explained by many factors including increased oral absorption (Chu, *et al.*, 1989; Culbertson, *et al.*, 1989).

Lomefloxacin, fleroxacin, and sparfloxacin, recently commercialized quinolone antibiotics, have a fluorine atom at C-8 (Fig. 1). The characteristics of these quinolones include a relatively longer serum half-life, better oral bioavailability, and higher tissue penetration. 8-Fluorociprofloxacin (8-FCP) used in this study is also a derivative of ciprofloxacin (CP) substituted at C-8 with fluorine (Fig. 1), and its *in vitro* antibacterial potency is similar to that of CP. To date, little information is available about the *in vivo* activity, pharmacokinetics, and toxicity of CP when fluorine is introduced at C-8.

Thus, this study was performed to investigate the CNS effects of 8-FCP to com-

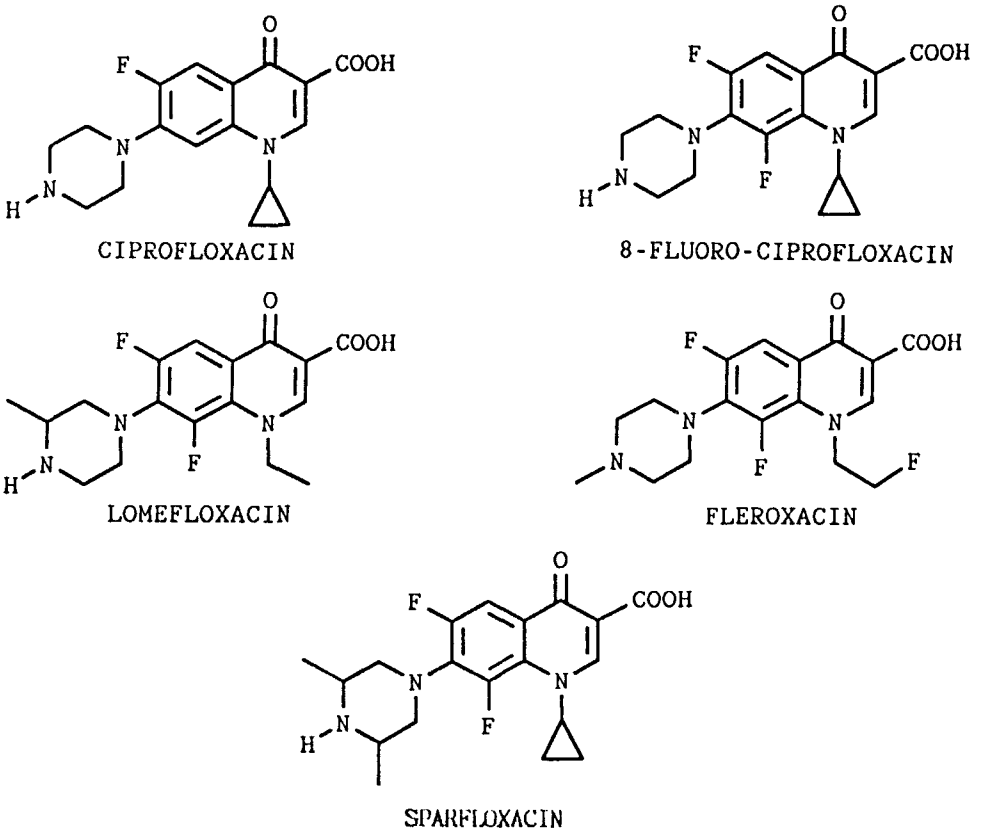

Figure 1. Chemical structures of 8-fluorociprofloxacin, ciprofloxacin, and other 8-fluorinated quinolones.

pare with those of CP, and then to provide information regarding the safety prediction of newly synthesized quinolone derivatives substituted at C-8 with fluorine.

MATERIALS AND METHODS

Animals and Chemicals

ICR mice, weighing 18 to 20 g, and SD rats, weighing 150 to 200 g, were used throughout these experiments. All animals were bred in our Animal Facility and received food (Cheil Foods and Chemicals Inc.) and tap water *ad libitum* in temperature- and humidity-controlled rooms. The animals were acclimatized for 10 days prior to treatment.

8-FCP and CP were synthesized and purified in our Organic Synthesis Laboratory. These quinolones were dissolved in physiological saline solution containing 1N NaOH. Pentylenetetrazole (PTZ), strychnine, hexobarbital sodium, acetic acid and pentobarbital sodium were all purchased from Sigma Chemical Co., (St. Louis, MO, USA). Fenbufen was kindly presented by Yuhan Pharmaceutical Co., (Kyungki-do, Korea).

Acute Toxicity Test

Five- to 6-week old male and female, ICR mice and SD rats, were used. 8-FCP and CP were dissolved in a solution containing 1N NaOH and final pH was adjusted in the range of 10 to 11. These drug solutions were filtered with a microfiter (Sterile Acordisc 0.45 μm, Gelman Sci.) just before intravenous (iv) administration. The iv dosing volume of each solution to the tail vein was fixed at 10 ml/kg. The clinical signs were observed frequently for the first 6 hrs, and then twice a day from Day 1 through Day 7 post administration. The LD_{50} values were calculated by the Probit method, as based on the death rate by Day 7. Simultaneously, the survivals given at a dose near LD_{50} were closely followed to record the body weight gains for 14 days.

Convulsive Liability Test

All ICR mice were fasted over night. Each group was composed of 5 mice. Fenbufen was suspended in 0.5% CMC solution and administered orally to mice at a dose of 300 mg/kg. Forty minutes after, 8-FCP and CP were intravenously injected at doses of 10, 20, and 40 mg/kg. The onset times of each convulsive parameter were recorded for 60 minutes following administration of the two quinolones. The convulsive parameters included head oscillation (HO), running and fit (R/F), and death.

Anticonvulsive Activity Test

8-FCP and CP were orally administered to mice at doses of 300 and 1,000 mg/kg. One hour later, each mouse was intraperitoneally treated with PTZ at a dose of 100 mg/kg or with strychnine at a dose of 1.2 mg/kg. In the negative- and positive-control groups, 0.5% CMC (dosing volume/10 ml/kg) was orally treated, and pentobarbital sodium was intraperitoneally treated at a dose of 50 mg/kg, respectively. The onset times of convulsion and death were recorded for 10 minutes following administration of PTZ or strychnine.

Determination of Hexobarbital-Induced Sleeping Time

8-FCP and CP were orally administered to mice at doses of 300 and 1,000 mg/kg. One hour later, hexobarbital sodium was intraperitoneally injected at a dose of 70 mg/kg. The individual sleeping time was recorded, based on the recovery of righting reflex.

Analgesic Activity Test

8-FCP and CP were orally administered to mice at doses of 300 and 1,000 mg/kg. Physiological saline and 100 mg of aspirin per kg were orally administered to the negative- and positive-control groups, respectively. Sixty minutes later, a solution containing 0.7% acetic acid was intraperitoneally injected for the induction of writhing. Five minutes later, the writhing frequency of each mouse was continuously recorded for 10 minutes.

Muscle Relaxation Activity Test (Rota-Rod Test)

After carefully selecting male SD rats which did not fall within 60 seconds from the rotating rod (Acceler. Rota-rod for Rat 7050, Ugo Bsile, Italy), each group containing 5 rats was treated with 8-FCP and CP at doses of 500 and 1,000 mg/kg. In a control group, saline was orally administered in a volume of 10 ml/kg. The enduring times on the rotating bar in an accelerated mode were recorded at the following times; 0.5, 1, 1.5, and 2 hours post administration of 8-FCP, CP and saline.

RESULTS

LD_{50} Values in Mice and Rats

The LD_{50} values of 8-FCP and CP in both mice and rats were shown in Table

Table 1. LD_{50} values of 8-FCP and CP given intravenously in mice and rats

Compound	Species	Sex	LD_{50} (mg/kg) [95% confidence limit]
8-FCP	Mouse	Male	126.5 [109.0~146.8]
		Female	163.1 [141.2~190.2]
CP		Male	219.7 [187.6~261.7]
		Female	121.6 [180.5~252.9]
8-FCP	Rat	Male	203.6 [171.1~219.4]
		Female	186.1 [163.5~210.2]
CP		Male	219.1 [182.3~263.1]
		Female	207.1 [152.2~225.7]

1. The clinical signs were not so different between mice treated with 8-FCP and CP. Main signs were depression, nervous-related signs and stagering gait, which were completely recovered or death within 30 minutes. No differences between two compounds were found in the body weight gains of animal surviving for 14 days following treatment (Fig. 2). However, intravenous 8-FCP showed similar or slightly lower LD_{50} values in rats, but markedly lower in mice, as compared to CP. The LD_{50} values of 8-FCP and CP were 203.6 mg/kg and 219.1 mg/kg in male rats, and were 126.5 mg/kg and 219.7 mg/kg in male mice, respectively.

Convulsive Liability and Anticonvulsive Activity

The convulsive signs and subsequent death occurred in the ICR mice treated with CP at an iv dose of 40 mg/kg. But, 8-FCP induced convulsion and death even at the lower dose of 10 mg/kg. The incidence rates of convulsion and death were dose-dependent, as shown in Table 2.

On the other hand, PTZ-induced or strychnine-induced convulsion was not potentiated by the oral administration of 8-FCP or CP, but the incidence rates of

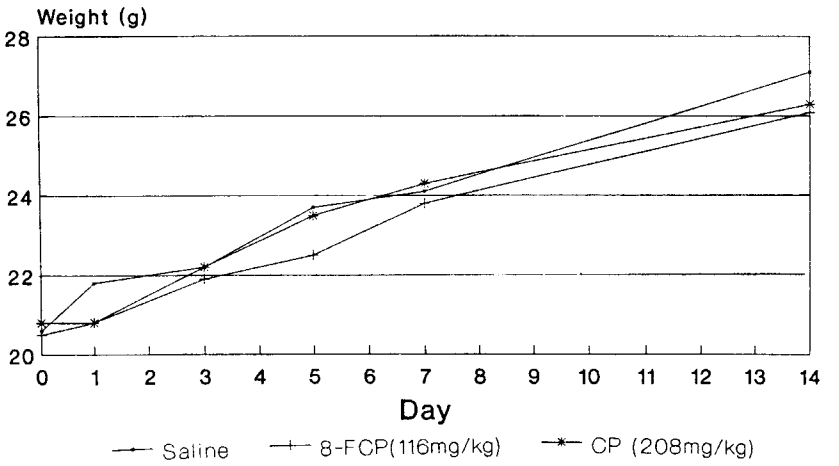

Figure 2. Body weight change in the male mice surviving for 14 days after single intravenous injection of 8-FCP and CP.

Table 2. Convulsive liability of 8-FCP and CP given in combination with fenbufen in mice

Compound	Dose (mg/kg)	Incidence Rate and Time of Convulsive Parameters		
		H/O[a]	R/F[b]	Death
8-FCP	10	1/5 (5)[c]	3/5 (27.7± 10.6)	1/5 (48)
	20	4/4 (1.6± 1.1)	4/4 (10.6± 3.2)	4/4 (16.4± 2.5)
	40	4/4 (1.0± 1.2)	4/4 (4.3± 2.3)	4/4 (8.1± 2.7)
CP	10	0/5	0/5	0/5
	20	0/5	0/5	0/5
	40	3/5 (18.5± 12.1)	3/5 (19.7± 10.2)	3/5 (30.8± 6.8)

a) Head oscillation, b) Running and fit, c) Mean onset time (min)± S.D. Compounds were intravenously injected 40 minutes after the oral administration of fenbufen (300 mg/kg).

both convulsion and death were slightly inhibited by the administration of CP (Tables 3 and 4).

Effect on the Hexobarbital-Induced Sleeping Time

Table 5 demonstrates the effects of 8-FCP and CP on hexobarbital-induced sleeping time in mice. The mean (± SD) sleeping time was 51.0± 7.3 min in the control group treated with a solution containing 0.5% of CMC. 8-FCP significantly lengthened the hexobarbital-induced sleeping time in mice with mean (± SD) sleeping times of 71.6± 10.5 min and 88.0± 8.6 min at the oral doses of 300 mg/kg and 1,000 mg/kg, respectively. However, CP had a slight effect on the sleeping time with means (± SD) of 54.6± 6.6 min and 64.7± 9.4 min at the two corresponding doses.

Analgesic Activity

Both the quinolones tested in this study had some analgesic effects as depicted in Table 6. The analgesic activity of 8-FCP was much higher than that of CP. The

Table 3. Effects of 8-FCP and CP on the strychnine-induced convulsion in mice

Group	Dose (mg/kg)	Incidence Rate	
		Convulsion	Death
Control (0.5% CMC)	–	4/5 (6.48)[a]	2/5
8-FCP	300	3/3 (5.50)	3/3
	1,000	2/2 (6.54)	2/2
CP	300	4/5 (6.96)	3/5
	1,000	4/5 (5.70)	3/5
PBS[b]	50	2/2 (4.28)	0.2

a) Mean onset time (min), b) Pentobarbital sodium. Strychnine (1.2 mg/kg) was intraperitoneally injected 60 minutes after the oral administration of each compound.

Table 4. Effects of 8-FCP and CP on the pentylenetetrazole-induced convulsion in mice

Group	Dose (mg/kg)	Death Rate
Control (0.5% CMC)	–	5/5
8-FCP	300	5/5
	1,000	5/5
CP	300	3/5
	1,000	3/5
PBS[a]	50	1/5

a) Pentobarbital sodium. Pentylenetetrazole (100 mg/kg) was intraperitoneally injected 60 minutes after the oral administration of each compound.

frequency of writhing was decreased in a dose-dependent manner. In the control group treated with physiological saline, the mean (± SD) writhing frequency was 39.6± 1.6 times/10 min., which was considered as a 0% inhibition rate. At oral doses of 300 mg/kg and 1,000 mg/kg, the inhibition rates of 8-FCP were 85.6% and 99.0%, while those of CP were 43.5% and 48.9%, respectively.

Muscle Relaxation Activity

Table 7 shows the enduring times on the rotating rod at various times after

Table 5. Effects of 8-FCP and CP on the pentylenetetrazole-induced sleeping in mice

Group	Dose (mg/kg)	Sleeping Time (min)
Control (0.5% CMC)	–	51.0± 7.3
8-FCP	300	71.6± 10.5*
	1,000	88.0± 8.6*
CP	300	54.6± 6.6
	1,000	64.7± 9.4

* Significantly different from control group ($P<0.05$). Hexobarbital sodium (70 mg/kg) was intraperitoneally injected 60 minutes after the oral administration of each compound.

Table 6. Effects of CP and 8-FCP on acetic acid-induced writhing in mice

Group	Dose (mg/kg) (mg/kg)	Writhing Frequency	% Inhibition
Control (Saline)	–	39.6± 1.6	100
8-FCP	300	5.7± 4.3	85.6
	1,000	0.4± 0.8	99.0
CP	300	22.4± 6.8	43.5
	1,000	20.8± 8.1	48.9
Aspirin	100	1.0± 1.1	97.5

Acetic acid (0.7%, 0.2 ml/mouse) was injected intraperitoneally 60 minutes after the oral administration of each compound.

Table 7. Effects of CP and 8-FCP on Acetic Acid-Induced Writhing in Mice

Compound	Dose (mg/kg)	Time (hr) post Administration			
		0.5	1	1.5	2
Control (D.W)	–	126.2± 27.1	139.7± 47.6	138.0± 44.6	163.0± 40.8[a]
8-FCP	500	124.6± 44.2	154.6± 43.3	168.8± 46.2	154.2± 58.7
	1,000	112.4± 15.3	146.2± 57.9	154.6± 50.9	139.6± 59.3
CP	500	90.6± 15.8	143.6± 37.4	129.4± 40.8	129.2± 19.8
	1,000	123.0± 52.7	135.7± 39.5	143.7± 52.1	136.7± 40.9

a) Mean (min)± S.D. Each compound was orally administered.

the oral administration of 8-FCP and CP. Both 8-FCP and CP had no effect on the muscle relaxation activity, although oral doses were increased up to 1,000 mg/kg.

DISCUSSION

It has been generally known that the fluorinated quinolone derivatives have a variety of side efects in experimental animals and in humans as well (Christ *et al.*, 1988). A number of investigators reported that the substitution of halogen atoms at C-8 seems to potentiate the phototoxicity of the quinolones (Sekiguchi *et al.*, 1989; Sesnie *et al.*, 1990; Sanchez *et al.*, 1990; Robertson *et al.*, 1991). However, little information is currently available about the structure-toxicity relationships of the quinolones, particularly pertaining to the CNS toxicological profile due to the introduction of fluorine at C-6, and/or C-8. Therefore, in this study, we were mainly focusing on the effects of 8-FCP and CP on the CNS, by utilizing various screening tools for the toxicological evaluation. It has been reported that the quinolone antimicrobial agents become favorable in *in vitro* and *in vivo* efficacy through the introduction at C-8 with fluorine (Chu *et al.*, 1989). Although the quinolones substituted at C-5 and C-8 with fluorine are generally slightly lower in the *in vitro* activity, they show similar or better *in vivo* efficacy, when compared to their respective non-substituted compounds (Culbertson *et al.*, 1989; Remuzon *et al.*, 1991). There is evidence that this may be due to the improvement of pharmacokinetic properties such as oral bioavailability and tissue penetration. From our pharmacokinetic studies, 8-FCP showed better bioavailability as compared to CP (unpublished data). Supposing that the inherent toxic properties are nearly the same for all quinolone derivatives, the improved pharmacokinetic characteristics would not be necessarily advantageous in terms of toxicity. The toxicity of the quinolones might be a combined effect of substituents at C-7 and C-8 (Rustige *et al.* 1990). Thus, the fluorine atom of 8-FCP might modulate the inherent toxicity of CP.

There is also a report of interest that the quinolones with high potency against Gram positive bacterial might have relatively high cytotoxic effects on the mammalian cells (Suto *et al.* 1991). However, 8-FCP is similar in the *in vitro* activity against Gram positive bacteria. The present study showed 8-FCP had much lower LD_{50} values than CP in mice, but did not in rats. Both 8-FCP and CP produced, however, similar clinical signs at the doses near to their respective LD_{50} values. It is, therfore, considered that these findings may be a reflection of toxic property of 8-FCP similar to CP. When combined with fenbufen, the convulsive liability of 8-FCP was higher than that of CP. In combination with fenbufen, CP is still considered as a quinolone with relatively high convulsive liability (Akahane *et al.*, 1989). The high convulsive liability of 8-FCP compared to CP might be attributable to the differences in the affinity for GABA receptor or in the penetration into the CNS (Kitzes-Cohen, 1989). Since the convulsive mechanisms of the quinolones and of the interaction with fenbufen remain unclear, significance of our findings needs to be further evaluated. However, it is important to note that there is a drug interaction between the quinolones and fenbufen.

8-FCP and CP significantly lengthened the hexobarbital-induced sleeping time. However, the analgesic effect of 8-FCP was about 2-fold higher than that of CP. No remarkable effects on the PTZ- or strychnine-induced convulsion and the muscle relaxation activity were observed by the oral administration of both 8-FCP and CP, which was similar to the findings from other commercialized quinolones (Ohkubo *et al.*, 1981; Kojima *et al.*, 1984; Momo *et al.*, 1990).

In conclusion, the CNS effects of 8-FCP were found to be more intense than those of CP based upon the parameters tested. Our findings suggest that this discrepancy between 8-FCP and CP shoud be due to the differences in the pharmacokinetic characteristics and/or in the binding affinity for some specific biochemical targets, which were possibly altered by the introduction of fluorine at C-8.

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