PHARMACOLOGICAL EVALUATION OF GARENOXACIN,  
A NOVEL DES-F(6)-QUINOLONE ANTIMICROBIAL AGENT:  
EFFECTS ON THE CENTRAL NERVOUS SYSTEM

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ABSTRACT — The effects of garenoxacin (formerly T-3811 or BMS-284756) on the central nervous  
system (CNS) were compared with various quinolones. Garenoxacin injected intracerebroventricularly  
into mice caused clonic convulsions at a higher dose (50 mg/kg) than norfloxacin, ciprofloxacin, sita-  
floxacins and trovafloxacin. Additionally, the convulsant activity of garenoxacin was not potentiated by  
biphenylacetic acid (BPAA). Garenoxacin did not induce any convulsions at intravenous doses up to 60  
mg/kg in combination with 200 mg/kg oral administration of fenbufen in mice, and its convulsant activity  
was weaker than those of enoxacin, norfloxacin, ciprofloxacin, alatrofloxacin and ofloxacin. In addition,  
convulsions were not induced by combination administration of garenoxacin (60 mg/kg, i.v.) and any of  
9 kinds of nonsteroidal anti-inflammatory drugs (NSAIDs) or BPAA. In a rotarod test, which was  
performed in order to evaluate the drug-induced dizziness, coordinated locomotor activity of mice was  
suppressed by alatrofloxacin at an intravenous dose of 60 mg/kg, but not by garenoxacin, ciprofloxacin  
and norfloxacin at up to 60 mg/kg. In an in vitro study using rat brain synaptic membrane, garenoxacin  
had no inhibitory effect on GABA binding in the presence or absence of NSAIDs. In conclusion, the effects  
of garenoxacin on CNS were weaker than those of other quinolones in experimental animals, so it might  
possess a low potential for CNS adverse reactions such as convulsion and dizziness in clinical use.

KEY WORDS: Garenoxacin, Central nervous system (CNS), Convulsion, Dizziness, Coordinated locomotion

INTRODUCTION

Garenoxacin (formerly T-3811 or BMS-284756) is a novel des-F(6)-quinolone, which characteristically  
differs from other quinolone antimicrobial agents by the lack of a fluorine at the C-6 position. Garenoxacin  
exhibits a broad spectrum of activity against most gram-positive and gram-negative aerobes and anaer-  
obes (Fung-Tomc et al., 2000; Hoellman et al., 2001; Takahata et al., 1999), and also has low potency of cyto-  
chrome P-450 inhibition, articular toxicity, phototoxicity and QTc prolongation (Ferguson et al., 2001;  
Furuhata et al., 2000; Gajjar et al., 2001b; Nagai et al., 2002; Rubinstein and Camm, 2002). It is under develop-  
ment for both oral and parenteral administration. It has been reported that the common adverse effects  
associated with quinolone treatment include gastrointestinal, skin, and central nervous system (CNS)  
reactions (Ball, 1986; Hooper and Wolfson, 1985). Among the newer quinolones, trovafloxacin  
appears to have an increased potential for CNS adverse reactions, notably dizziness (Ball, 2000; Blondeau,
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1999). Igarashi et al. (1995) previously investigated the relationship between pharmacological findings in experimental animals and clinical adverse reactions. Interestingly, the reduction of coordinated locomotion in animals is significantly associated with adverse reaction of dizziness observed in clinical investigations.

Convulsive seizures, which are the severe CNS reactions of quinolones, occur rarely (Christ, 1990), but are observed more frequently in patients who receive quinolones in combination with nonsteroidal anti-inflammatory drugs (NSAIDs) such as fenbufen (Japanese Ministry of Health and Welfare, 1986, 1989). Furthermore, inhibitory effects of quinolones on gamma-aminobutyric acid (GABA) binding are possibly related to their epileptogenic activities (Akahane et al., 1989; Tsuji et al., 1988; Tsutomi et al., 1994), and the inhibitory effects are potentiated in the presence of biphenylacetic acid (BPAA), an active metabolite of fenbufen (Akahane et al., 1989, 1994; Davey, 1988).

The CNS effects of quinolones can be divided into two categories: those which arise from the direct action of the quinolones on CNS receptors and those which are mediated by co-administration of other drugs (Domagala, 1994). In this study, therefore, the direct CNS effects of garenoxacin (convulsant activity, GABA inhibition and dyscoordination effect) and drug interactions with NSAIDs (convulsant activity and GABA inhibition) were evaluated and compared to those of other quinolones.

MATERIALS AND METHODS

Chemicals

Garenoxacin mesylate was synthesized in Toyama Chemical Co., Ltd., Tokyo, Japan or manufactured in Sumika Fine Chemicals, Co., Ltd., Osaka, Japan. Ciprofloxacin was extracted from Ciproxan® (Bayer Yakuin Ltd., Osaka, Japan) in Toyama Chemical Co., Ltd. Trovafloxacin, alatrofloxacin, sitafloxacin, olamufloxacin and piroxicam were synthesized in Toyama Chemical Co., Ltd. The purity of these compounds was not less than 96.5%. Enoxacin, ofloxacin, diclofenac sodium (diclofenac), fenbufen, ketoprofen, indomethacin, ibuprofen, ketoprofen, naproxen and piroxicam were synthesized by Sigma Chemical Co., Ltd., USA. Norfloxacin and aspirin were purchased from Wako Pure Chemical Industries, Ltd., Osaka, Japan. 4-Amino-n-[2,3-3H]butyric acid ([3H]GABA, 3.29 TBq/mmol) was obtained from Amersham International Ltd., Buckinghamshire, United Kingdom. Biphenylacetic acid (BPAA) was obtained from Sigma Chemical Co. or Aldrich Chemical Co., Inc., Milwaukee, USA. All the purchased chemicals and other reagents used were of guaranteed grade.

Preparation of test solutions

1. in vivo experiments

Garenoxacin mesylate, trovafloxacin mesylate, alatrofloxacin mesylate and olamufloxacin mesylate were dissolved in 5% mannitol solution. Alatrofloxacin, the L-alanyl-L-alanyl prodrug of trovafloxacin, was used for intravenous administration. Ciprofloxacin, norfloxacin, ofloxacin and sitafloxacin were dissolved as hydrochloric acid salts in 5% mannitol solution. Enoxacin was dissolved as a sodium salt in 5% mannitol solution. NSAIDs (aspirin, mefenamic acid, diclofenac, fenbufen, indomethacin, ibuprofen, ketoprofen, naproxen and piroxicam) were suspended in 0.5% methylcellulose (MC) solution. BPAA was suspended in 0.5% MC solution for oral dose, or dissolved as a sodium salt in 5% mannitol solution for intracerebroventricular injection. The doses of quinolones represent as the amount of free base except alatrofloxacin, of which the dose represents as trovafloxacin.

2. in vitro experiments

Garenoxacin mesylate, trovafloxacin mesylate and GABA were dissolved in distilled water. Ciprofloxacin, norfloxacin and ofloxacin were dissolved as hydrochloric acid salts in distilled water. Enoxacin, NSAIDs and BPAA were dissolved as sodium salts in distilled water.

Animals

Male Wistar/ST rats (8 weeks old) and male ICR mice (3-4 weeks old) were purchased from Japan SLC Inc., Shizuoka, Japan. The animals were housed under a 12hr light/dark cycle at 22±2°C with a 60±10% humidity. Commercially available solid food (MF, Oriental Yeast Co., Ltd., Tokyo, Japan) and water were given ad libitum. They were acclimatized for at least 3 days before experiments.

Convulsion after intracerebroventricular injection

The convulsion test was performed using mice weighing 20-28 g (n = 10). An aliquot (10 μl/body) of a quinolone solution was injected intracerebroventricularly using Hamilton syringe type #702 with a 27-
gauge needle (Haley and McCormick, 1957; Watanabe et al., 1978). Briefly, the needle was inserted 2 mm lateral to the midline on a line drawn through the anterior base of the ears at a depth of 4 mm. Five-percent mannitol solution adjusted to pH 3.7 with 1 M methane-sulfonic acid was used as the vehicle. In the combination experiment with BPAA, 5 μl/body of quinolone solution was injected immediately after the intracerebroventricular injection of BPAA (1 μg/5 μl/body). Following the injection, each mouse was placed in an individual transparent plastic box (W100, D100, H130 mm) to observe the incidence of convulsions over a period of 1 hr. The mortality incidents were counted after overnight housing in a cage.

**GABA binding**

The preparation of rat brain synaptic membrane was performed by the method of Kuroda et al. (1984) with slight modification. Rats were decapitated and the whole brains immediately removed. The tissue was gently homogenized in 7 ml of ice-cold 0.32 M sucrose by a Potter-Elvehjem homogenizer with Teflon pestle. The homogenate was centrifuged at 900 g for 10 min at 4°C, and the supernatant was then centrifuged at 20,000 g for 20 min at 4°C. The pellet was suspended in 7 ml of 50 mM Tris-HCl buffer (pH 7.6), then centrifuged at 20,000 g for 20 min at 4°C. The pellet was frozen at -40°C for 48 hr and then treated for 30 min at 37°C with Tris-HCl buffer containing 0.05% Triton X-100. After centrifugation at 20,000 g for 20 min at 4°C, the pellet was suspended in 7 ml of Tris-HCl buffer without Triton X-100. This operation was repeated three more times. On the fourth operation, the pellets of synaptic membrane obtained from 3 rats were mixed and then suspended in 7 ml of Tris-HCl buffer for GABA binding assay. Protein concentration was determined by the Coomassie dye binding assay (Bio-Rad Labs., Hercules, CA) with bovine serum albumin as a standard (Bradford, 1976).

For specific [1H]GABA binding, aliquots of pooled synaptic membrane were incubated in duplicate at 0°C for 30 min in 500 μl of 50 mM Tris-HCl buffer (pH 7.6) containing 6 nM [1H]GABA alone (control) or in the presence of 1 mM GABA, or in the presence of test compounds. After incubation, membranes with bound [1H]GABA were trapped on glass fiber filters (GF/B, Whatman, Maidstone, USA). The filters were instantly washed three times with an additional 4 ml ice-cold Tris-HCl buffer and membrane-bound radioactivity was then measured by a liquid scintillation counter (LSC-3500, Aloka Co., Ltd., Tokyo, Japan). Specific GABA binding was calculated after subtraction of non-specific binding in the presence of 1 mM unlabelled GABA (Kuroda et al., 1984; Zukin et al., 1974).

**Convulsion after intravenous injection**

Quinolones were intravenously injected into the tail vein of mice (22-29 g of body weight, n = 7) at 30 min after the oral administration of NSAIDs or BPAA. The administration time of quinolones was decided in order to show the most potent convulsive activity (Tsutomi et al., 1994). The dose volume was 10 ml/kg of body weight. Following the injection, mice were placed in an individual transparent plastic box (W100, D100, H150 mm) to observe the incidence of convulsions over a period of 2 hr. The observation period was determined on the basis of the t1/2 (1.5 hr) of garenoxacin following intravenous administration to mice (Takahata et al., 1997). The mortality incidents were counted after overnight housing in a cage.

**Coordinated locomotor activity (rotarod test)**

Mice were preliminarily placed for 2 min on a 25-mm-diameter horizontal rod rotating at a rate of 13 rpm (rotarod; KN-75, Natsume, Tokyo, Japan) one or two days before the examination. This trial was performed twice. On the experiment day, mice were subjected to the trial before administration, and the animals that did not fall from the rotating rod in all three preliminary trials were selected for the test. The test drugs were injected intravenously in a volume of 10 ml/kg (n = 7). In the control group, 5% mannitol solution was injected instead of test drug. Five and 30 min after administration, at which quinolones were presumed to keep a high plasma concentration, mice were placed on a rotating rod and the number of dyscoordinated mice that fell off within 2 min was counted.

**Statistical analysis**

For the rotarod test, the number of dyscoordinated mice in the control group and that in the quinolone treatment group was compared statistically by one-tailed Fisher exact test (Fisher, 1955). The p values less than 0.05 were considered to be significant. Statistical analysis was performed with SAS, release 6.12 (SAS Institute Inc., Cary, NC, USA).

**RESULTS**

**Convulsant activity after intracerebroventricular injection**

The convulsant activities of quinolones after
intracerebroventricular injection in mice are shown in Fig. 1a. Garenoxacin caused clonic convulsion in 4 out of 10 mice at 50 µg/body and in 9 out of 10 mice at 100 µg/body. Subsequently, garenoxacin caused death in one mouse at 50 µg/body and in 4 mice at 100 µg/body (data not shown). Other quinolones also induced convulsions and subsequent death, and the minimum dose for induction of clonic convulsion was 3.13 µg/body for norfloxacin; 12.5 µg/body for ciprofloxacin; 25 µg/body for sitafloxacin and trovafloxacin; 50 µg/body for ofloxacin and olamufloxacin. In combination with 1 µg/body of BPAA, the minimum convulsive doses of garenoxacin (50 µg/body) and trovafloxacin (25 µg/body) did not change, but those of other quinolones were reduced to the following doses: 0.39 µg/body for norfloxacin; 3.13 µg/body for ciprofloxacin; 12.5 µg/body for sitafloxacin; 25 µg/body for ofloxacin and olamufloxacin (Fig. 1b). Vehicle did not cause any convulsions or death when it was administered with or without BPAA (data not shown).

**GABA binding**

The effects of various quinolones on GABA binding to its receptor in the absence or presence of 100 µM BPAA are shown in Fig. 2a and 2b. In the experiment of quinolone alone, garenoxacin, ofloxacin and trovafloxacin did not inhibit the GABA binding at concentrations up to 100 µM. Ciprofloxacin, enoxacin and norfloxacin at 100 µM decreased the GABA binding to approximately 65% of control. In the combination experiment with BPAA (100 µM), the inhibitory activities on the GABA binding were potentiated for ofloxacin, trovafloxacin, ciprofloxacin, enoxacin and norfloxacin, but not for garenoxacin. The rank order of the inhibitory activity with BPAA was norfloxacin > enoxacin > ciprofloxacin > trovafloxacin > ofloxacin > garenoxacin. The inhibitory activity of garenoxacin was not potentiated by concomitant 100 µM of NSAIDs (Fig. 2c).

**Convulsant activity after intravenous injection**

Each quinolone injected alone at an intravenous dose of 60 mg/kg to mice did not induce any convulsions or death (data not shown). Garenoxacin did not induce any convulsions or death at an intravenous dose of 60 mg/kg in combination with any of 9 kinds of NSAIDs and BPAA in mice (Table 1). In addition, garenoxacin caused no convulsions when the oral dose of fenbufen was increased to 200 mg/kg (Table 2). Alatrofloxacin, ofloxacin, ciprofloxacin, norfloxacin and enoxacin caused convulsions and subsequent death (except alatrofloxacin) in combination with 200 mg/kg of fenbufen. The rank order of the convulsant activity with fenbufen was enoxacin > norfloxacin > ciprofloxacin > ofloxacin = alatrofloxacin > garenoxacin.

**Coordinated locomotor activity**

Garenoxacin had no effect on coordinated locomotor activity in mice at intravenous doses up to 60 mg/kg (Table 3). In addition, ciprofloxacin and norfloxacin had no effect at doses up to 60 mg/kg, while alatrofloxacin at a dose of 60 mg/kg caused a significant increase in the number of dyscoordinated mice at 30 min after administration.

**DISCUSSION**

The CNS effects of quinolones can be divided into two categories, *i.e.* the direct CNS action and the drug interaction with other drugs (Domagala, 1994). Firstly, we evaluated the direct CNS effects of garenoxacin (convulsant activity, GABA inhibition and dysco-
CNS effects of garenoxacin.

![Graph showing receptor binding of garenoxacin and other quinolones on GABA receptor binding in rat synaptic membrane.](image)

**Fig. 2.** Effects of garenoxacin and other quinolones on GABA receptor binding in rat synaptic membrane. GABA binding ratio is designated as percent of control, in which distilled water was added instead of test article, and represents as the mean \( \pm \) S.E. of 3 separate experiments. BPAA and NSAIDs were used at the concentration of 100 \( \mu \)M. †: Not tested.

**Table 1.** Induction of convulsions after intravenous administration of garenoxacin pretreated with NSAIDs in mice.

<table>
<thead>
<tr>
<th>Drug (mg/kg)a)</th>
<th>NSAIDs (mg/kg)b)</th>
<th>Incidencec)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Clonic</td>
</tr>
<tr>
<td>Garenoxacin (60)</td>
<td>-</td>
<td>0/7</td>
</tr>
<tr>
<td>Aspirin (300)</td>
<td>0/7</td>
<td>0/7</td>
</tr>
<tr>
<td>Mefenamic acid (200)</td>
<td>0/7</td>
<td>0/7</td>
</tr>
<tr>
<td>Diclofenac (17.5)</td>
<td>0/7</td>
<td>0/7</td>
</tr>
<tr>
<td>Fenbufen (100)</td>
<td>0/7</td>
<td>0/7</td>
</tr>
<tr>
<td>Indomethacin (12.5)</td>
<td>0/7</td>
<td>0/7</td>
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<tr>
<td>Ibuprofen (100)</td>
<td>0/7</td>
<td>0/7</td>
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<tr>
<td>Ketoprofen (25)</td>
<td>0/7</td>
<td>0/7</td>
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<tr>
<td>Naproxen (100)</td>
<td>0/7</td>
<td>0/7</td>
</tr>
<tr>
<td>Piroxicam (3.5)</td>
<td>0/7</td>
<td>0/7</td>
</tr>
<tr>
<td>BPAA (50)</td>
<td>0/7</td>
<td>0/7</td>
</tr>
</tbody>
</table>

a) Dose of garenoxacin represents as the amount of free base.
b) Ten times higher dose of each therapeutic use, except BPAA.
c) Each value represents the number of mice which exhibit convulsions or death / number of mice tested.
-: 0.5% methylcellulose solution.
Garenoxacin was injected intravenously at 30 min after the oral administration of NSAIDs.
ordination effect) using the in vivo and the in vitro animal models and compared to those of other quinolones. The intrinsic convulsant activity and the CNS penetration ability of quinolones are the major determinant of their neurotoxicity (Delon et al., 1997). The brain to plasma (or serum) concentration ratios of quinolones in experimental animals ranged from 0.06 to 0.37 (Nagatsu et al., 1981; Nakamura et al., 1984; Okazaki et al., 1984; Siefert et al., 1986), and those of garenoxacin ranged from 0.03 to 0.05 (unpublished data). In order to determine the intrinsic convulsant activity of garenoxacin, therefore, garenoxacin and other quinolones were administered intracerebroventricularly to mice, even though the concentrations of quinolones in the brain were presumed to be higher than clinical cerebrospinal fluid concentrations (Bergan, 1998; Hasbun and Quagliarello, 1998). After the intracerebroventricular injection of quinolone alone, garenoxacin induced convulsive seizure at higher dose than norfloxacin, ciprofloxacin, sitafloxacin and trovafloxacin. In an in vitro experiment, garenoxacin had no inhibitory effect on GABA binding, which might be involved in the convulsant activities of quinolones (Akahane et al., 1989; Tsuji et al., 1988; Tsutom et

### Table 2. Induction of convulsions after intravenous administration of quinolones pretreated with 200 mg/kg oral dose of fenbufen in mice.

<table>
<thead>
<tr>
<th>Drug</th>
<th>Dose(^a) (mg/kg)</th>
<th>Clonic convolution</th>
<th>Tonic convolution</th>
<th>Death</th>
</tr>
</thead>
<tbody>
<tr>
<td>Garenoxacin</td>
<td>15</td>
<td>0/7</td>
<td>0/7</td>
<td>0/7</td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>0/7</td>
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<tr>
<td></td>
<td>60</td>
<td>0/7</td>
<td>0/7</td>
<td>0/7</td>
</tr>
<tr>
<td>Alatrofloxacin</td>
<td>15</td>
<td>0/7</td>
<td>0/7</td>
<td>0/7</td>
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<td></td>
<td>60</td>
<td>1/7</td>
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<tr>
<td>Ofloxacin</td>
<td>15</td>
<td>0/7</td>
<td>0/7</td>
<td>0/7</td>
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<tr>
<td></td>
<td>60</td>
<td>1/7</td>
<td>0/7</td>
<td>1/7</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>7.5</td>
<td>0/7</td>
<td>0/7</td>
<td>0/7</td>
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<td></td>
<td>60</td>
<td>7/7</td>
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<tr>
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<td>7.5</td>
<td>0/7</td>
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<td></td>
<td>30</td>
<td>7/7</td>
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</tr>
<tr>
<td>Enoxacin</td>
<td>0.94</td>
<td>0/7</td>
<td>0/7</td>
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</tr>
<tr>
<td></td>
<td>1.88</td>
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<td>3.75</td>
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<td>30</td>
<td>7/7</td>
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\(^a\) The doses of quinolones represent as the amount of free base except alatrofloxacin, of which the dose represents as trovafloxacin.

\(^b\) Each value represents the number of mice which exhibit convulsions or death / number of mice tested.

Quinolones were injected intravenously at 30 min after the oral administration of fenbufen.
al., 1994). Additionally, we tried to evaluate the dizziness-inducing potential of garenoxacin by the rotarod test. The motor coordination activity of mice was suppressed by alatrofloxicin (prodrug of trovafloxacin) but not by garenoxacin, ciprofloxacin and norfloxacin. In our preliminary study, abnormalities were not observed in body tone and grip strength as evaluated by the Irwin’s modified method (Irwin, 1968) following intravenous administration of alatrofloxicin to mice (data not shown). Therefore, it is likely that the suppression of motor coordination by alatrofloxicin can be attributed to the decrease in ability to balance rather than relaxation of muscle tone. As reported previously (Igarashi et al., 1995), such dyscoordination in mice is consistent with the incidence of dizziness in clinical study: dizziness was observed in 0.4% of patients receiving norfloxacin (Wang et al., 1986); 0.6% receiving ciprofloxacin (Ball, 1986); 2% receiving garenoxacin (Arnow et al., 2001); and 11% receiving trovafloxacin (Blondeau, 1999). These results suggest that garenoxacin has a lower potential for direct CNS action in the quinolone antimicrobial agents. In addition, it was shown that the rotarod test could be applicable to evaluate the potential for dizziness of quinolones in clinical use.

Secondly, we evaluated the drug interactions of garenoxacin and NSAIDs (convulsant activity and GABA inhibition) and compared to those of other quinolones. In the combination experiment of intracerebroventricular injection, concurrent BPAA potentiated the convulsant activity for norfloxacin, ciprofloxacin, sitafloxacin, ofloxacin and olamufloxacin by 2-8 fold, but not for garenoxacin and trovafloxacin. The convulsant activity for norfloxacin, which induced convulsion when it was co-administered with fenbufen in clinical use (Japanese Ministry of Health and Welfare, 1986), was potentiated with BPAA the most among the quinolones tested. Additionally, garenoxacin had no inhibitory effect on in vitro GABA binding in the presence of BPAA or NSAIDs. In combination of intravenous injection of quinolones and oral administration of fenbufen, garenoxacin induced no clonic convulsions at doses up to 60 mg/kg and its convulsant activity was weaker than those of other quinolones. The quinolone-induced convulsions are more potentiated by propionic acid and arylactic acid derivatives in NSAIDs (Hori, 2000). Garenoxacin injected intravenously at 60 mg/kg did not cause clonic convulsion in the presence of propionic acid-derivatives (ibuprofen, ketoprofen and naproxen), arylactic acid-derivatives (diclofenac, fenbufen, indomethacin and BPAA) or others (aspirin, mefenamic acid and piroxicam). These results suggest that garenoxacin has a low potential for drug interactions with NSAIDs.

Garenoxacin did not cause any convulsions at an intravenous dose of 60 mg/kg regardless of whether NSAID was co-administered or not. Ciprofloxacin, clinically used as an intravenous formulation, caused convulsions at an intravenous dose of 15 mg/kg co-administered with fenbufen in mice. The plasma concentrations of garenoxacin and ciprofloxacin at doses described above were estimated to be 44 μg/ml and 5.8 μg/ml, respectively, which were extrapolated from Cmax values of garenoxacin (3.65 μg/ml) and ciprofloxacin (1.92 μg/ml) following intravenous administration at 5 mg/kg to mice (Takahata et al., 1997). The peak plasma concentrations of garenoxacin and ciprofloxacin after a single 400 mg intravenous administration in humans were 7.7 μg/ml and 4.0 μg/ml.

### Table 3. Effects of garenoxacin and other quinolones on coordinated locomotor activities in mice.

<table>
<thead>
<tr>
<th>Drug</th>
<th>Dose (mg/kg)</th>
<th>Incidence of dyscoordination&lt;sup&gt;a&lt;/sup&gt;</th>
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</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>5 min&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Control</td>
<td>–</td>
<td>1/7</td>
</tr>
<tr>
<td>Garenoxacin</td>
<td>15</td>
<td>0/7</td>
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<tr>
<td></td>
<td>30</td>
<td>2/7</td>
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<tr>
<td></td>
<td>60</td>
<td>0/7</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>15</td>
<td>0/7</td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>0/7</td>
</tr>
<tr>
<td></td>
<td>60</td>
<td>0/7</td>
</tr>
<tr>
<td>Norfloxacin</td>
<td>15</td>
<td>0/7</td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>1/7</td>
</tr>
<tr>
<td></td>
<td>60</td>
<td>3/7</td>
</tr>
<tr>
<td>Alatrofloxin</td>
<td>15</td>
<td>0/7</td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>0/7</td>
</tr>
<tr>
<td></td>
<td>60</td>
<td>4/7</td>
</tr>
</tbody>
</table>

<sup>a</sup>: p < 0.05, significant difference from control analyzed by one-tailed Fisher exact test.

<sup>b</sup> Each value represents the number of mice with dyscoordination / number of mice tested.

<sup>c</sup> Time after the intravenous injection of test drug.

The doses of quinolones represent as the amount of free base except alatrofloxacin, of which the dose represents as trovafloxacin. In the control group, 5% mannitol solution was injected instead of test drug.
respectively (Gajjar et al., 2001a; Lettieri et al., 1992). Garenoxacin had no effects at 5.7 times higher concentration than clinical C_max while ciprofloxacin induced convulsions at 1.5 times higher concentration than clinical C_max. Therefore, it is possible that garenoxacin does not cause convulsive seizures in clinical use.

It was suggested that the C-7 side chain substituent had the strongest influence on the degree of GABA binding inhibition, with the order of inhibitory activity as piperazine > pyrrolidine > C-alkylated piperazine and pyrrolidine (Domagala, 1994). The structural analogy between GABA and the piperazinyl and pyrrolidinyl side chains also may play an important role in epileptogenic activities of quinolones (Akahane et al., 1989). The structure-convulsant-activity relationships for quinolone antimicrobial agents used in the present study are shown in Table 4. The convulsant activities and the GABA binding inhibition of piperazinyl quinolones.

### Table 4. Convulsant activities of garenoxacin and other quinolones: Structure-activity relationships.

<table>
<thead>
<tr>
<th>Quinolone</th>
<th>R7</th>
<th>R6</th>
<th>X8</th>
<th>R1</th>
<th>R5</th>
<th>Minimum dose for clonic convulsion&lt;sup&gt;a&lt;/sup&gt; (mg/kg)</th>
<th>Degree of GABA inhibition with fenbufen (p.o.) alone with BPAA (i.c.) with BPAA&lt;sup&gt;b&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Enoxacin</td>
<td>H</td>
<td>N</td>
<td>N</td>
<td>CH3CH2-</td>
<td>H-</td>
<td>1.88 N.T.&lt;sup&gt;c&lt;/sup&gt;</td>
<td>high</td>
</tr>
<tr>
<td>Norfloxacin</td>
<td>H</td>
<td>N</td>
<td>F</td>
<td>CH</td>
<td>CH3CH2-</td>
<td>H-</td>
<td>7.5 3.13 0.39</td>
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<tr>
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<td>H</td>
<td>N</td>
<td>F</td>
<td>CH</td>
<td></td>
<td>H-</td>
<td>12.5 3.13</td>
</tr>
<tr>
<td>Sitaflaxcin</td>
<td>H</td>
<td>N</td>
<td>F</td>
<td>CCl</td>
<td></td>
<td>H-</td>
<td>N.T. 25 12.5</td>
</tr>
<tr>
<td>Trovaflaxcin</td>
<td>H</td>
<td>N</td>
<td>F</td>
<td>CH</td>
<td></td>
<td>H-</td>
<td>60&lt;sup&gt;b&lt;/sup&gt; 25 25</td>
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<tr>
<td>Olamufloxacin</td>
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<td>N</td>
<td>F</td>
<td>CCH3</td>
<td></td>
<td>NH2-</td>
<td>N.T. 50 25</td>
</tr>
<tr>
<td>Ofloxacin</td>
<td>H</td>
<td>N</td>
<td>F</td>
<td>CH2OH</td>
<td></td>
<td>H-</td>
<td>60 50 25</td>
</tr>
<tr>
<td>Garenoxacin</td>
<td>H</td>
<td>H</td>
<td>H- COCHF2</td>
<td></td>
<td>H-</td>
<td>&gt; 60 50 50</td>
<td>low</td>
</tr>
</tbody>
</table>

<sup>a</sup> Minimum dose for induction of clonic convulsion in this study: i.v., intravenous injection of quinolones pretreated with 200 mg/kg oral dose of fenbufen; i.c., intracerebroventricular injection of quinolones pretreated with or without 1 μg/body intracerebroventricular dose of 4-biphenylacetic acid (BPAA).

<sup>b</sup> Relative level of inhibitory effect on GABA binding at 10 μM of quinolones in combination with 100 μM of BPAA.

<sup>c</sup> Not tested.

<sup>d</sup> Alatrofloxacin was used.
CNS effects of garenoxacin.

Quinolones (enoxacin, norfloxacin and ciprofloxacin) were higher than those of alkylated piperazinyl (ofloxacin), alkylated pyrrolidinyl (trovafloxacin, sitafloxacin and olamufloxacin) and isoindolyl quinolones (garenoxacin). Garenoxacin had the lowest convulsant potential in the quinolones examined in this study, while it has improved antibacterial activity (Fung-Tomc et al., 2000; Hoellman et al., 2001; Takahata et al., 1999). Therefore, the isoindolyl substituent at C-7 position might be responsible for low CNS effects, without decreasing the antibacterial activity. Garenoxacin does not have fluorine at position 6, and the relationships between the fluorine at C-6 position and CNS effects of quinolones have not been reported previously. Thus, we preliminarily carried out the convulsion test of garenoxacin and its 6-fluoronated derivative. As a result, there was no significant difference in convulsant activity between these two compounds (data not shown). The relationship between the fluorine at C-6 position and CNS effects of quinolone is still unclear, and further work is needed to clarify the role of C-6 fluorine.

In conclusion, the effects of garenoxacin on CNS were weaker than other quinolones in experimental animals. Therefore, garenoxacin might possess a low potential for CNS adverse reactions such as convulsion and dizziness in clinical use.

REFERENCES


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CNS effects of garenoxacin.


