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Celecoxib does not induce convulsions nor does it affect $GABA_A$ receptor binding activity in the presence of new quinolones in mice

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Abstract

We sought to determine whether celecoxib would induce convulsions when coadministered with new quinolone antimicrobial agents in mice. The oral administration of celecoxib (500 mg/kg) alone or in combination with enoxacin (500 mg/kg), lomefloxacin (1000 mg/kg), ciprofloxacin (1000 mg/kg), or levofloxacin (1000 mg/kg) induced no convulsions in mice. In contrast, some nonsteroidal anti-inflammatory drugs (NSAIDs), fenbufen (200 mg/kg), indomethacin (500 mg/kg), and naproxen (500 mg/kg) induced convulsions in combination with the majority of the new quinolones tested. γ-Aminobutyric acid $(GABA)_A$ receptor blockade-mediated neuronal excitation is assumed to be involved in these toxic convulsions. Enoxacin (100 μM) and lomefloxacin (100 μM) only slightly reduced [^{3}H]muscimol binding to $GABA_A$ receptors in mouse whole brain membrane. However, these reductions were markedly enhanced by the addition of fenbufen (100 μM), indomethacin (100 μM), or naproxen (100 μM). Conversely, celecoxib (100 μM) had no apparent effect on [^{3}H]muscimol binding when applied alone or in combination with enoxacin or lomefloxacin. These results suggest that celecoxib may be a more desirable anti-inflammatory agent with respect to drug interactions with new quinolones compared with some conventional NSAIDs.

Keywords: New quinolone; Nonsteroidal anti-inflammatory drug; Drug interaction; Convulsion; $GABA_A$ receptor

1. Introduction

A series of fluorinated nalidixic acid derivatives, termed new quinolones, are among the most frequently used antimicrobial agents because of their wide spectra of antibacterial activities and excellent tissue permeability (Andriole, 1993). At present, they are prescribed for many conditions, including respiratory and urinary tract infections. They are also occasionally used together with anti-inflammatory agents such as nonsteroidal anti-inflammatory drugs (NSAIDs; Brouwers, 1992; Janknegt, 1990). However, new quinolones have been shown to possess excitatory side effects on central nervous system, such as headache, dizziness, and tremor (Simpson and Brodie, 1985; Anastasio et al., 1988; Slavich et al., 1989; Kushner et al., 2001). Although the incidence of their side effects on central nervous system is quite low, serious convulsions have been reported in patients taking certain combinations of new quinolones with NSAIDs, e.g., enoxacin and fenbufen, and ciprofloxacin and ketoprofen (Pharmaceuticals and Chemicals Safety Division, Pharmaceutical Affairs Bureau, Ministry of Health and Welfare of Japan, 1986, 1989). In practice, these combinations of new quinolones with NSAIDs are contraindicated in Japan. The mechanisms of action involved in the toxic convulsions are not clearly understood; however, the combined application of new quinolones and NSAIDs has been shown to attenuate ligand-binding affinity at the γ-aminobutyric acid $(GABA)_A$ receptor (Akahane et al., 1994a, 1989; Motomura et al., 1991), which mediates inhibitory neurotransmission in the

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mammalian central nervous system. In addition, some quinolone derivatives are demonstrated to act as antagonists at both central and peripheral $GABA_A$ receptors (Blandizzi et al., 1991). Furthermore, GABA-induced current response in Xenopus oocytes injected with mouse brain messenger RNA was demonstrated to be abolished by a combined application of enoxacin with 4-biphenylacetic acid, an active metabolite of fenbufen (Kawakami et al., 1997). These data suggest that blockade of the $GABA_A$ receptor by these compounds results in a functional reduction in GABA-ergic neuronal transmission. Therefore, the inhibition of GABA-ergic signals, which may lead to neuronal excitation, is assumed to be one of the mechanisms involved in the generation of new quinolone/NSAID-induced convulsions.

Celecoxib is one of the novel class of anti-inflammatory agents, the coxibs (Penning et al., 1997), that selectively inhibit the inducible enzyme, cyclooxygenase-2, while sparing cytoprotective prostanoids produced via the action of the cyclooxygenase-1 enzyme isoform. As a consequence of its cyclooxygenase-1 sparing properties, celecoxib has been shown to be associated with a lower incidence of gastrointestinal events than conventional NSAIDs (Silverstein et al., 2000). Celecoxib is classified as a diaryl-substituted pyrazole (diarylheterocyclic) and thus is structurally distinct from those NSAIDs reported to cause convulsions in combination with new quinolones (Fig. 1).

In the present study, we evaluated whether celecoxib would provoke convulsions when administered together with new quinolones in mice compared with conventional NSAIDs. In addition, the influence of the simultaneous application of celecoxib and new quinolones on $GABA_A$ receptor binding activity was also examined using mouse whole brain membranes.

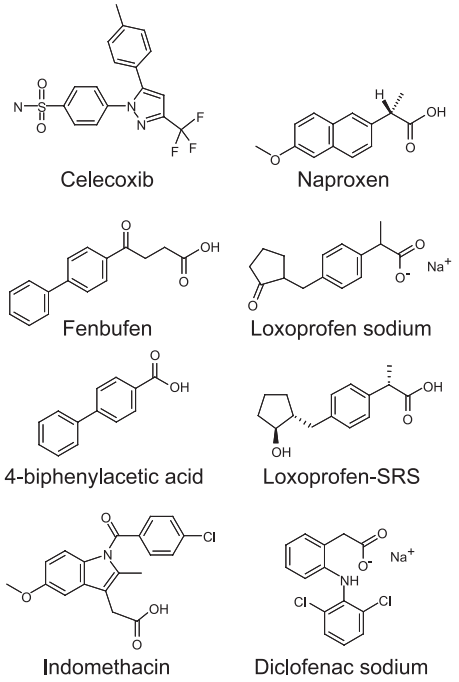

Fig. 1. Chemical structures of celecoxib and conventional NSAIDs.

2. Materials and methods

2.1. Animals

Five-week-old male ddy mice were obtained from Japan SLC (Shizuoka, Japan). Animals were maintained in cages with 12-h light/dark intervals with water and food available ad libitum. All animal experiments were performed in compliance with the regulations of the institutional Animal Ethics Committee of Yamanouchi Pharmaceutical.

2.2. Drugs and reagents

Celecoxib (4-[5-(4-methylphenyl)-3-(trifluoromethyl)-1H-pyrazol-1-yl]benzenesulfonamide) was obtained from Pfizer (New York, NY, USA). Loxoprofen sodium (monosodium 2-[4-(2-oxocyclopentylmethyl)phenyl]propanoate dihydrate) was obtained from Shiono Chemical (Tokyo, Japan). Loxoprofen-SRS (an active metabolite of loxoprofen, (2S)-2-{4-[(1R,2S)-2-hydroxycyclopentylmethyl]phenyl}-propanoic acid) was synthesized by Yamanouchi Pharmaceutical (Tokyo, Japan). Enoxacin, lomefloxacin hydrochloride, fenbufen, 4-biphenylacetic acid, indomethacin, naproxen, diclofenac sodium, and GABA were purchased from Sigma-Aldrich (St. Louis, MO, USA). [^{3}H]Muscimol was purchased from Perkin-Elmer Life Sciences (Boston, MA, USA). For in vivo experiments, new quinolone antimicrobial agents were purchased as the following commercial drugs: FLUMARK® (enoxacin, Dainippon Pharmaceutical, Osaka, Japan), Lomebact® (lomefloxacin hydrochloride, SHIONOGI, Osaka, Japan), Cravit® (levofloxacin, Daiichi Pharmaceutical, Tokyo, Japan), and Ciproxacin® (ciprofloxacin hydrochloride, Bayer Yakuhin, Osaka, Japan). Other reagents used were obtained from standard commercial suppliers. For in vitro [^{3}H]muscimol binding studies, test drugs were dissolved in dimethylsulfoxide, except that enoxacin and lomefloxacin hydrochloride were first dissolved with 40% volume of 0.1N NaOH and then added with 60% volume of dimethylsulfoxide. For in vivo experiments, test drugs were dissolved or suspended in a solution of 0.5% methylcellulose containing 0.025% Tween-20 to give 0.1 ml/10 g animal body weight.

2.3. Convulsion-inducing activity in mice

Mice were fasted for approximately 18 h prior to experiments. New quinolones, NSAIDs, celecoxib, or vehicle was administered orally, and mice were placed in observational cages. After drug administration, observations were continued for 8 h to determine the occurrence of clonic and tonic convulsions and death. Total numbers of deaths in the 24 h

following drug administration were also recorded. The overall lethality was calculated using total number of dead animals per group (n=6) at 24 h after drug administration.

2.4. Preparation of brain synaptic plasma membranes

Crude synaptic membranes were prepared from the whole brains of male ddy mice according to the method reported previously (Zukin et al., 1974) with slight modifications. Mice were decapitated, and whole brains were isolated. Tissues were homogenized in 10 volumes of ice-cold 0.32 M sucrose solution and centrifuged at 1600 $\times g$ for 15 min. The supernatant was further centrifuged at 15,000 $\times g$ for 20 min. The resultant pellet was suspended in 50 mM Tris hydrochloride buffer (pH 7.4) containing 0.08% Triton X-100, placed on ice for 30 min to remove endogenous GABA, and washed three times with 5 mM Tris hydrochloride buffer (pH 7.4). Finally, the membranes were suspended in 50 mM Tris hydrochloride buffer and stored at −80 °C.

2.5. [^{3}H]Muscimol binding studies

The $GABA_A$ receptor-binding assay was performed as previously described (Akahane et al., 1989) with slight modifications. Aliquots (0.5 ml) of crude synaptic membranes (0.2 mg protein/tube) were incubated on ice for 1 h in 50 mM Tris citrate buffer (pH 7.4) containing [^{3}H]muscimol (20 nM; specific activity=29.5 Ci/mmol) plus test drugs or the vehicle. After incubation, the reaction was terminated by filtration of the mixture through a glass fiber filter (GF/B; Whatman, Clifton, NJ, USA) using a cell harvester (BRANDEL, Gaithersburg, MD, USA). Filters were washed three times with 3 ml of ice-cold 50 mM Tris citrate buffer, dried, and the radioactivity (total binding) was determined in 5 ml of a scintillation cocktail (AQUASOL-2; Perkin-Elmer), using a liquid scintillation counter (2200CA; Packard, Meriden, CT, USA). Non-specific binding was determined in the presence of 0.5 mM of unlabeled GABA in the reaction mixture, and specific binding was obtained by subtracting the nonspecific from total binding. The protein content of the membrane preparation was determined by the Bradford method (Bradford, 1976). Under the conditions used, the membrane preparation showed a binding affinity (K_d) of 5.6±0.94 nM for [^{3}H]muscimol and a receptor-protein density of 0.66±0.070 pmol/mg (n=11).

2.6. Statistical analysis

Convulsion-inducing activity is expressed as the number of animals with convulsive events per the total number of tested animals in each treatment group. For analysis of $GABA_A$ receptor binding, data are expressed as the mean±S.E.M. and statistical significances were determined by using either Student's t test or Dunnett's multiple range test. All data analyses were performed using the SAS statistical software (SAS Institute, Cary, NC, USA). A P value of less than 0.05 was considered statistically significant.

3. Results

3.1. Convulsions induced by NSAIDs and celecoxib alone or in combination with new quinolones in mice

Based on the preliminary results, the dose for each drug examined was determined as follows: fenbufen 200 mg/kg, conventional NSAIDs and celecoxib 500 mg/kg, enoxacin 500 mg/kg, and other new quinolones 1000 mg/kg. At these doses, none of the tested drugs induced convulsions or deaths in mice within 24 h after administration (n=6 in each group, data not shown).

Combined-administration experiments are summarized in Table 1. Celecoxib did not produce convulsions when coadministered with any of the new quinolones tested. In contrast, fenbufen, indomethacin, and naproxen induced convulsions in combination with the majority of new quinolones tested.

Fenbufen in combination with enoxacin, lomefloxacin, or ciprofloxacin induced clonic convulsions in all mice in each treatment group, which led to tonic convulsions in the majority of cases; all mice died immediately following the convulsions (Table 1). Only one out of six mice administered with fenbufen in combination with levofloxacin exhibited clonic and tonic convulsions followed by death.

Clonic convulsions were observed in all mice treated with indomethacin in combination with enoxacin; five out of six mice died within 24 h after drug administration, and four of these exhibited tonic convulsions before death. A combination of indomethacin with lomefloxacin induced clonic and tonic convulsions in five out of six mice, which then died. For the combination of indomethacin with ciprofloxacin, five out of six mice displayed clonic convulsions and died, one of which went through a tonic convulsion. No convulsions were observed for the combination of indomethacin with levofloxacin.

Naproxen induced clonic and tonic convulsions in all mice in combination with enoxacin or lomefloxacin, and all mice died immediately after the convulsions. A combination of naproxen with ciprofloxacin or levofloxacin caused clonic convulsions and death in five out of six mice, and two of the animals also displayed a tonic convulsion.

Loxoprofen sodium induced clonic convulsions in two out of six mice when coadministered with lomefloxacin; one animal displayed tonic convulsion before death. Combinations of loxoprofen sodium with other new quinolones tested produced no convulsions or death.

Diclofenac sodium induced no convulsions when coadministered with any of the new quinolones tested, although one mouse in each group died within 24 h after admin-

Table 1
Incidence of clonic convulsions (CL), tonic convulsions (TN), and death (L) after the combined administration of celecoxib or conventional NSAIDs, with various new quinolones in mice

Drug	New quinolone antimicrobial agents											
	Enoxacin 500 mg/kg			Lomefloxacin 1000 mg/kg			Ciprofloxacin 1000 mg/kg			Levofloxacin 1000 mg/kg		
	CL	TN	L	CL	TN	L	CL	TN	L	CL	TN	L
Celecoxib 500 mg/kg	0	0	0	0	0	0	0	0	0	0	0	0
Fenbufen 200 mg/kg	6	5	6	6	6	6	6	3	6	1	1	1
Indomethacin 500 mg/kg	6	4	5	5	5	5	5	1	5	0	0	0
Naproxen 500 mg/kg	6	6	6	6	6	6	5	2	5	5	2	5
Loxoprofen Na 500 mg/kg	0	0	0	2	1	1	0	0	0	0	0	0
Diclofenac Na 500 mg/kg	0	0	1	0	0	1	0	0	0	0	0	0

Celecoxib or a conventional NSAID was orally administered together with a new quinolone to fasted mice at the indicated dose. Observations were continued for 8 h after drug administration, and occurrence of clonic (CL) and tonic convulsions (TN) were recorded. Overall frequency of deaths (L) in the 24 h after drug administration was also counted. Data represent the number of animals within each group having a positive response (n=6).

istration of diclofenac in combination with enoxacin or lomefloxacin.

3.2. $GABA_A$ receptor binding studies

[^{3}H]Muscimol (20 nM) binding to $GABA_A$ receptors in mouse whole brain membrane was not affected by celecoxib or any of the conventional NSAIDs tested (fenbufen, 4-biphenylacetic acid, indomethacin, naproxen, loxoprofen sodium, loxoprofen-SRS, and diclofenac sodium) at concentrations of 100 μM (Fig. 2). Enoxacin (Fig. 3) and lomefloxacin (Fig. 4), at concentrations of 100 μM, demonstrated a trend toward reduced [^{3}H]muscimol specific binding at $GABA_A$ receptors [18±2.0% inhibition (n=8) and 11±0.94% inhibition (n=5), respectively].

In the combined application experiments, we investigated whether celecoxib or the conventional NSAIDs at 100 μM would produce an additional reduction in the [^{3}H]muscimol binding activity in the presence of 100 μM of enoxacin or lomefloxacin. As shown in Fig. 3, celecoxib and diclofenac sodium did not produce any additional reduction in [^{3}H]muscimol binding to $GABA_A$ receptors in the presence of enoxacin. In contrast, the combination of enoxacin with fenbufen, 4-biphenylacetic acid, indomethacin, or naproxen further reduced the amount of [^{3}H]muscimol binding to $GABA_A$ receptors compared with enoxacin treatment alone (61%, 95%, 67%, and 43% inhibition compared with enoxacin alone, respectively). Loxoprofen sodium reduced [^{3}H]muscimol binding in the presence of enoxacin by 36% compared with enoxacin-alone group, but the effect of loxoprofen-SRS was only marginal (13% inhibition compared with enoxacin-alone group).

Since loxoprofen sodium caused convulsions in mice only when coadministered with lomefloxacin, its effect on [^{3}H]muscimol binding was investigated in the presence of lomefloxacin. As shown in Fig. 4, loxoprofen sodium reduced the amount of [^{3}H]muscimol binding to $GABA_A$ receptors in the presence of lomefloxacin (43% inhibition compared with lomefloxacin-alone group). Loxoprofen-

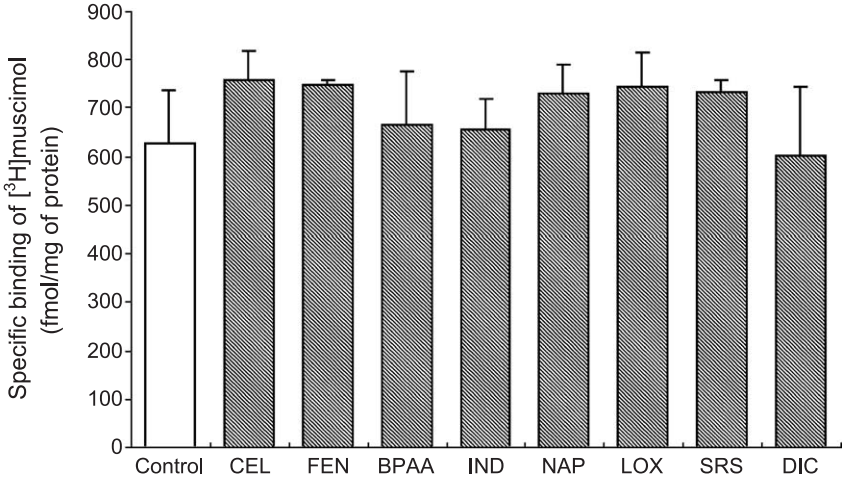

Fig. 2. Effects of celecoxib and conventional NSAIDs on the specific binding of [^{3}H]muscimol to $GABA_A$ receptors in mouse whole brain membranes. Membrane preparations (0.2 mg protein/tube) were treated with each drug (100 μM) or vehicle and incubated with 20 nM [^{3}H]muscimol for 1 h. Values of specific binding were then counted. Data are expressed as fmol/mg protein (mean±S.E.M. of three experiments). CEL—celecoxib; FEN—fenbufen; BPAA—4-biphenyl acetic acid; IND—indomethacin; NAP—naproxen; LOX—loxoprofen sodium; SRS—loxoprofen-SRS; DIC—diclofenac sodium.

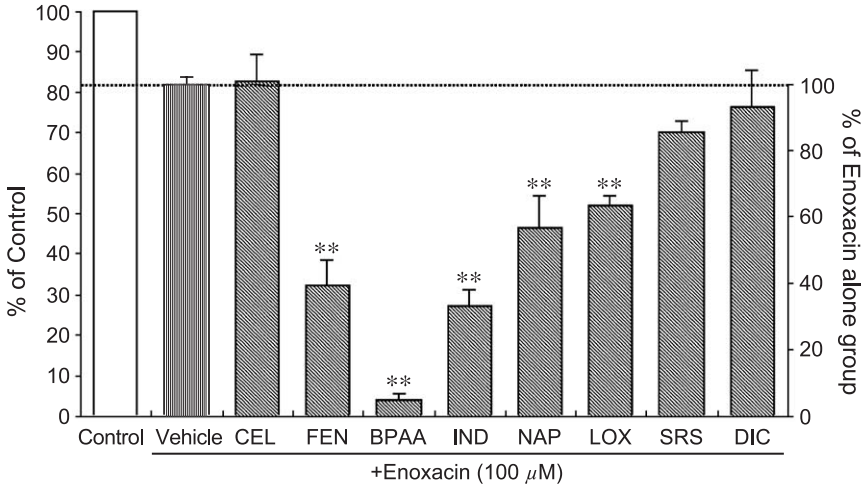

Fig. 3. Effects of celecoxib and conventional NSAIDs on the specific binding of [^{3}H]muscimol to $GABA_A$ receptors in the presence of enoxacin. Membrane preparations (0.2 mg protein/tube) were treated with each drug (100 μM) or vehicle and incubated with 20 nM [^{3}H]muscimol in the presence of 100 μM enoxacin for 1 h. Values of specific binding were then counted. Data are shown as % of control (left vertical axis) or % of enoxacin-alone group (right vertical axis) and expressed as the mean±S.E.M. (n=3–8). **P<0.01 significant deference from enoxacin-alone group (Dunnett's multiple range test). CEL—celecoxib; FEN—fenbufen; BPAA—4-biphenyl acetic acid; IND—indomethacin; NAP—naproxen; LOX—loxoprofen sodium; SRS—loxoprofen-SRS; DIC—diclofenac sodium.

SRS had no apparent effect on [^{3}H]muscimol binding to $GABA_A$ receptors in the presence of lomefloxacin. Celecoxib had no effect on [^{3}H]muscimol binding in the presence of lomefloxacin, but 4-biphenylacetic acid significantly reduced it (7.8% and 91% inhibition, respectively, compared with lomefloxacin-alone group).

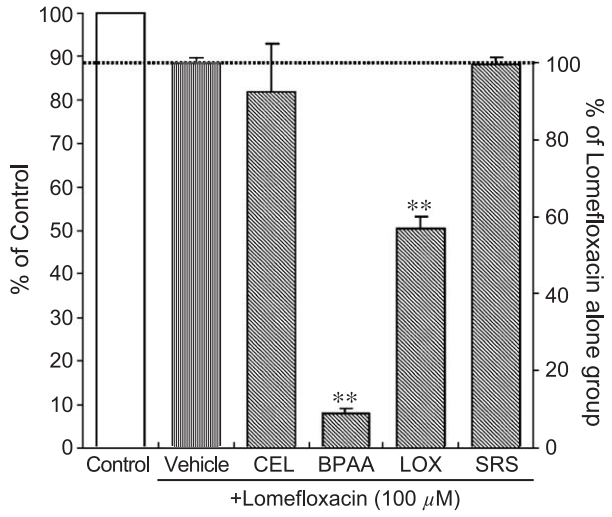

Fig. 4. Effects of celecoxib, 4-biphenylacetic acid, loxoprofen sodium, and loxoprofen-SRS on the specific binding of [^{3}H]muscimol to $GABA_A$ receptors in the presence of lomefloxacin. Membrane preparations (0.2 mg protein/tube) were treated with each drug (100 μM) or vehicle and incubated with 20 nM [^{3}H]muscimol in the presence of 100 μM lomefloxacin for 1 h. Values of specific binding were then counted. Data are shown as % of control (left vertical axis) or % of lomefloxacin-alone group (right vertical axis) and expressed as the mean±S.E.M. (n=4–5). **P<0.01 significant deference from lomefloxacin-alone group (Dunnett's multiple range test). CEL—celecoxib; BPAA—4-biphenyl acetic acid; LOX—loxoprofen sodium; SRS—loxoprofen-SRS.

4. Discussion

In 1986, seven patients were reported as having experienced serious convulsions after taking fenbufen together with enoxacin (Pharmaceuticals and Chemicals Safety Division, Pharmaceutical Affairs Bureau, Ministry of Health and Welfare of Japan, 1986). In addition, some other cases of central nervous system excitation or toxic convulsions associated with the combined application of new quinolones with NSAIDs were also reported (Pharmaceuticals and Chemicals Safety Division, Pharmaceutical Affairs Bureau, Ministry of Health and Welfare of Japan, 1989; Kamali et al., 1998). Since then, certain combinations of new quinolones with NSAIDs (such as the combination of enoxacin with fenbufen) have been contraindicated in Japan. The convulsions induced by the combination of new quinolones with NSAIDs are reproducible in animals, and the convulsant potential of new quinolones or NSAIDs can be roughly estimated using mice in vivo (Akahane et al., 1989; Tsutomi et al., 1994; Taniguchi et al., 1996). In this study, we evaluated whether celecoxib, a specific cyclooxygenase-2 inhibitor, would provoke convulsions when administered together with new quinolones in mice compared with other NSAIDs. Fenbufen, which is reported to be associated with toxic convulsions when coadministered with new quinolones in clinical use, induced convulsions in combination with the majority of new quinolones tested. Furthermore, indomethacin and naproxen showed strong convulsant activities in combination with many of the new quinolones tested. The results obtained in this study are similar to those from previous reports of convulsions produced by combined application of new quinolones and NSAIDs in mice (Kohno et al., 1994a,b). Conversely, celecoxib at 500 mg/kg, which is much higher than its

clinical dose (200–400 mg/day), did not induce any convulsions when administered either alone or in combination with any of the new quinolones tested, suggesting that celecoxib is unlikely to induce convulsions in humans when administered in combination with new quinolones.

The mechanisms of action involved in these toxic convulsions are not clearly understood; however, a number of radioligand binding experiments indicated that some new quinolones inhibit the binding of [^{3}H]GABA or [^{3}H]muscimol, a selective $GABA_A$ receptor agonist, to crude synaptic membranes prepared from mouse, rat, or human tissue (Akahane et al., 1994a, 1989; Motomura et al., 1991). Furthermore, the inhibitory effect of new quinolones on $GABA_A$ receptor binding is markedly enhanced by the addition of fenbufen or its active metabolite 4-biphenylacetic acid. Since the $GABA_A$ receptor mediates major inhibitory neurotransmission in the central nervous system, the blockade of GABA-ergic neurotransmission may inversely lead to neuronal excitation. Although the concentrations of new quinolones or NSAIDs evaluated in these studies are relatively high, Kawakami et al. (1997) demonstrated that, among various agonist-induced membrane currents evoked in Xenopus oocytes injected with mouse brain messenger RNA, only the GABA-induced response was inhibited by new quinolones, and the effect was markedly potentiated with 4-biphenylacetic acid. Thus, $GABA_A$ receptor blockade is assumed to be involved in toxic convulsions, and in vitro GABA binding experiments have been utilized to predict the convulsant potential of new quinolones or NSAIDs (Akahane et al., 1994a, 1989; Tsutomi et al., 1994). Therefore, we evaluated the effect of celecoxib on $GABA_A$ receptor binding activity in the presence of new quinolones and compared the results with those obtained from NSAIDs. The amount of [^{3}H]muscimol binding to mouse whole brain membrane was slightly reduced by enoxacin and lomefloxacin at a concentration of 100 μM. Their effects were markedly enhanced, and the binding activity was nearly abolished by the addition of 4-biphenylacetic acid. Similarly, indomethacin and naproxen, which showed convulsant effects in combination with enoxacin, further reduced the amount of [^{3}H]muscimol binding to $GABA_A$ receptors when compared with enoxacin alone. In contrast, celecoxib at 100 μM did not reduce the amount of [^{3}H]muscimol binding either by itself or in the presence of enoxacin or lomefloxacin. These results suggest that celecoxib up to 100 μM is unlikely to affect the $GABA_A$ receptor binding activity alone or enhance the action of new quinolones at the receptor, unlike NSAIDs. In addition, it has been previously reported that the combined application of 4-biphenylacetic acid and ciprofloxacin or levofloxacin inhibited ligand-binding activity of $GABA_A$ receptor in rat synaptic plasma membrane (Akahane et al., 1989, 1994b; Nakamura et al., 2003). However, the intensity of the effect of levofloxacin is significantly weaker than that of its optical isomers, ofloxacin, or norfloxacin, therefore, they concluded that levofloxacin may have lower neurotoxicity than other new quinolones. The results in these previous reports and those obtained in our in vitro [^{3}H]muscimol binding study well correlate with convulsion-inducing activity by corresponding combination of new quinolones and NSAIDs. Therefore, it is assumed that celecoxib did not induce convulsions in mice because it dose not affect ligand-binding activity of $GABA_A$ receptor in the presence of NSAIDs. On the other hand, one mouse in each group died within 24 h after administration of diclofenac sodium (500 mg/kg) in combination with enoxacin (500 mg/kg), or lomefloxacin (1000 mg/kg); however, no convulsions were observed at least 8 h after drug administration. Although the reason for the deaths is unclear, it might not be attributable to the neuronal excitation related to GABA-ergic transmission since diclofenac sodium at a concentration of 100 μM did not affect the amount of [^{3}H]muscimol binding either by itself or in the presence of enoxacin or lomefloxacin.

Celecoxib demonstrates anti-inflammatory activity in various animal models at doses of approximately 1–10 mg/kg. For example, in a rat model of acute inflammation, oral administration of celecoxib effectively prevented carrageenan injection-induced inflammatory paw edema, with an ED_{50} of 7.1 mg/kg (Penning et al., 1997). Compared with this anti-inflammatory dose, much greater concentrations of celecoxib (500 mg/kg) failed to induce convulsions in mice in our study. Furthermore, in a human study after administration of supratherapeutic doses of celecoxib (600 mg BID for 7 days), plasma concentrations of protein-unbound celecoxib did not reach more than 122 ng/ml (about 0.32 μM; Paulson et al., 1999). In our study, celecoxib did not affect $GABA_A$ receptor binding at 100 μM, and previous investigations have shown that the maximum concentration of celecoxib in the brain is approximately equal to that in plasma in rats after oral dosing with [^{14}C]-celecoxib at 2 mg/kg (Paulson et al., 2000). Taken together, the present study suggests that clinical doses of celecoxib are unlikely to inhibit $GABA_A$ receptor binding activity and therefore may not induce convulsions when administered concomitantly with new quinolones in practice.

Fenbufen induced convulsions in mice when administered together with many of the new quinolones tested. In addition, the in vitro experiments demonstrated that its active metabolite, 4-biphenylacetic acid, enhanced the inhibitory effect of enoxacin on [^{3}H]muscimol binding to mouse whole brain membranes more effectively than the parent compound. Similar results have been shown in many other studies (Akahane et al., 1994a, 1989; Motomura et al., 1991; Kawakami et al., 1997; Kita et al., 1999). Therefore, fenbufen is believed to exert its convulsion-inducing effect in the presence of new quinolones mainly by the action of its metabolite rather than itself. In the present study, the combined application of loxoprofen sodium with lomefloxacin induced convulsions in two out of six mice tested. Loxoprofen sodium is a prodrug, and its pharmacological

effect is principally produced by the active metabolite, loxoprofen-SRS, via inhibition of cyclooxygenases (Matsuda et al., 1984). Therefore, we examined loxoprofen-SRS as well as loxoprofen sodium in the [^{3}H]muscimol binding assay to determine which compound contributes mainly to the convulsions observed in combination with new quinolones. Loxoprofen-SRS did not show any apparent effect on [^{3}H]muscimol binding to mouse whole brain membranes in the presence of either lomefloxacin or enoxacin. Interestingly, loxoprofen sodium in itself significantly reduced [^{3}H]muscimol binding in combination with both new quinolones. The pharmacokinetic profile of loxoprofen sodium in mice has not been reported; however, the maximum plasma concentration of the unchanged form of loxoprofen is reported to be much higher than its metabolite in rats (Nagashima et al., 1984) and humans (Naganuma et al., 1986) after oral dosing. Thus, it is conceivable that the unchanged form of loxoprofen may interact with the $GABA_A$ receptor in vivo in combination with new quinolones after oral administration. Loxoprofen sodium produced a significant reduction in [^{3}H]muscimol binding even in combination with enoxacin, although convulsions occurred only when coadministered with lomefloxacin. However, Kohno et al. (1994a,b) reported that the combined application of enoxacin and loxoprofen sodium caused convulsion in 30% of tested mice. Therefore, when administered together with these new quinolones, loxoprofen sodium itself rather than its metabolite may have induced convulsions via inhibition of $GABA_A$ receptor binding. These results further indicate that $GABA_A$ receptor blockade by these compounds does not correlate with their cyclooxygenase inhibitory activities.

The structure–activity relationships of NSAIDs for the synergic effect on $GABA_A$ receptor antagonism by new quinolones are not fully understood. However, Akahane et al. (1994a) suggested that a planar biphenyl ring (which occurs in 4-biphenylacetic acid and naproxen) or similar structures (possibly provided by indomethacin) with a carboxyl group are critical to form the inhibitory complex with new quinolones at $GABA_A$ receptor sites. Celecoxib is classified as a diaryl-substituted pyrazole and is structurally distinct from those NSAIDs that showed convulsion-inducing effects, such as fenbufen (a phenylacetic acid derivative), naproxen (a propionic acid derivative), and indomethacin (an indoleacetic acid derivative). In addition, we have previously reported that rofecoxib, a furanone derivative cyclooxygenase-2 inhibitor, does not induce convulsions nor does it affect [^{3}H]muscimol binding to the mouse brain membrane in the presence of enoxacin or lomefloxacin in mice (Okutsu et al., 2004). Therefore, the absence of a convulsion-inducing activity in celecoxib may be partly attributable to the difference in structure between celecoxib and those conventional NSAIDs.

In conclusion, these results suggest that celecoxib, a specific cyclooxygenase-2 inhibitor, confers a pharmacological advantage for avoiding serious drug interactions with new quinolones compared with some conventional NSAIDs, which exert an inhibitory effect on GABA-ergic transmission in combination with new quinolones. Thus, combined administration of celecoxib with new quinolones is unlikely to be associated with toxic convulsions.

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