응용약물학회지, The Journal of Applied Pharmacology 3, 322-326(1995)

Safety Evaluation of LB20304, a New Quinolone Antibiotic

Seong Il KIM, Hee Jin KIM, Jin Hwan KWAK, In Chull KIM and Chang Ho LEE*

Biotech Research Institute, LG Chem Research Park, LG Chemical Ltd., Taejon 305-380, Korea

(Received December 1, 1995; accepted December 15, 1995)

Abstract—General pharmacology of LB20304, a quinolone antibiotic, were examined in terms of general behaviour, cardiovascular, and central nervous system. LB20304 at oral dose of 2,000 mg/kg did not induce significant behavioural changes in mice. In contrast with ciprofloxacin, LB20304 at dose of 20 mg/kg, *i.v.* did not show any observable effects on the blood pressure in rats. Displacement of [^{3}H]muscimol binding to the rat brain synaptic membranes was measured. LB20304 was shown to be about five times less potent than ciprofloxacin in specific GABA receptor binding. Drug interaction between LB20304 and 4-biphenyl acetic acid, an active metabolite of fenbufen, was assessed in mice by measuring convulsion and/or subsequent death. A single oral pretreatment with 4-BPA at 400 mg/kg increased the incidence of convulsion and death after oral administration of ciprofloxacin at the doses of 25, 50, and 100 from zero of five to three of five, two of five, and four of five, respectively, whereas LB20304 alone or combination with 4-BPA caused neither convulsions nor death at the doses of 12.5, 25, 50, and 100 mg/kg, respectively. Quinolones-induced epileptogenic activities were assessed by a direct intracerebral injection of test articles. The CD_{50} values (nmole) are as follows; 169.47, 35.36, 105.29, and 88.67 for LB20304, ciprofloxacin, ofloxacin, and lomefloxacin, respectively. From these data, LB 20304 at therapeutic doses seems to be much more safe than any other quinolones tested.

Keywords □ quinolone, LB 20304, epileptogenecity, hemodynamics, GABA receptor

Safety profiles of fluoroquinolones have been reported. Possible targets of quinolone adverse effects include the juvenile joint, the kidney, the central nervous system, the eye, and the cardiovacular system. In immature animals all quinolones studied cause arthropathies of the major diarthrodial joints (Christ, W., and Lehnert, T., 1990; Christ, W. *et al.*, 1988). At high doses the quinolones exert effects on renal function such as crystalluria (Thorsteinsson, S. B. *et al.*, 1987). Some quinolone analogs exhibited notable central nervous system effects such as confusion, hallucination, anxiety, agitation, nightmares, convulsive seizures, and depression (Christ, 1990; Janknegt, 1989; Lucet *et al.*, 1988). Seizures have been reported in patients receiving norfloxacin (Anastasio, G. E., *et al.*, 1988), ciprofloxacin (Arcieri, G., *et al.*, 1987), and ofloxacin (Tack, K. J. and Smith, J. A. 1989). Fluoroquinolone therapy may have lowered the seizure threshold possibly by the abilities of quinolones to antagonize binding of inhibitory neurotransmitter γ-aminobutyric acid to its receptor in the central nervous system (Halliwell *et al.*, 1991; Yakushiji *et al.*, 1992). Pefloxacin causes cataracts in dogs after long term treatment (Christ, W. *et al.*, 1988). Dermatologic and photosensitivity reaction have been reported in patient receiving number of quinolones (Ferguson, J., *et al.*, 1988) although the mechanisms of these reaction are completely understood. Low-dose quinolones with *i.v.* infusion cause pronounced but transient systolic hypotension in dogs and cats which may be mediated by histamine release (Christ, W. *et al.*, 1988).

LB20304, [7-(3-aminomethyl-4-methoxyimino-pyrrolidin-1-yl)-1-cyclopropyl-6-fluoro-4-oxo-1,4-dihydro-%1,8 %-naphthyridine-3-carboxylic acid], is a new fluoroquinolone antibacterial agent synthesized at LG Chemical Ltd. (Kim *et al.*, 1995) (Fig. 1). This compound was found to be extremely effective in the treatment of infectious disease caused by gram-positive and gram-negative, including MRSA (Oh *et al.*, 1995).

The present study was carried out to evaluate the pharmacological properties of LB20304 and to find po-

* To whom correspondence should be addressed.

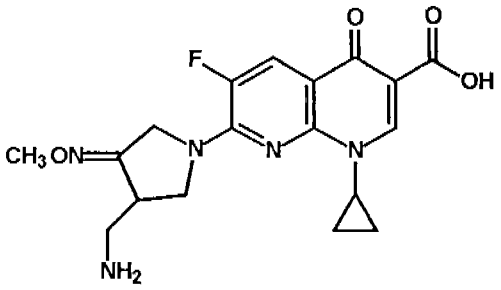

Fig. 1. Chemical structure of LB20304.

tential adverse effects in clinical use. For this purpose, the effects of LB20304 on the general behaviour, hemodynamics, and the central nervous system after intravenous or oral doses were assessed.

Materials and Methods

Drugs and Animals

In order to prepare test article for administration LB20304 (test article, m.w. 357.4) was dissolved in 0.9 % isotonic sterile saline (vehicle) at the highest concentration. Commercially available quinolones, such as ciprofloxacin (m.w. 367.8; Sigma, USA), ofloxacin (m.w. 351.3; synthesized by LG Chem), and lomefloxacin (m. w. 361.4; Shionogi Pharmaceutical, Ltd., Osaka, Japan) have been utilized as reference drugs in some experiments. Male ICR mice (23~26 g) and male Sprague Dawley rats (240~260 g) produced by LG Chem animal facility were used for the following experiments. All the animals were housed and feeded with a standard commercial food at th LG Chem animal facility of which environment was well controlled (temperature; 20~22℃, 12 hours light and 12 hours dark).

General Behaviour

Five male mice weighing 23~25 g were used in each group. Behavioural profile (spontaneous activity, motor-affective response, sensor-motor response), neurologic profile (posture, muscle tone, equilibrium and gait, CNS excitation), and autonomic profile (eye, secretion and excretion) were observed prior to and at 0.5, 1, 2, 6, and 24 h after oral administration of the test article according to the method of Irwin (Irwin, 1968). In another experiment, the individual clinical symptoms, such as skin rash, salvation, defecation, moist eye, vomitting, activity, tremor, heart rate, respiration, and vocalization of beagle dogs (12~14 kg, 2 male/group) were observed for 6 hours after a single bolus intravenous or oral dose.

Hemodynamics

The hemodynamics of rats (Sprague Dawley, 240~260g, 4~5 male rats/group) were assessed. On the day of treatment, rats were anesthetized by the inhalation of ether. Using surgical technique, one tenth m*l* of pentobarbital sodium (50 mg/m*l*) was injected into the left femoral vein through cannula. Catheter was inserted into the common carotid artery for the measurement of blood pressure by the polygraph (Grass Model 7 polygraphy, Grass Instrument Co., Quincy, Mass, U.S.A.). The test articles were administered by a single bolus intravenous injection into the femoral vein. The dose volumes of all animal were 1 m*l*/kg.

GABA receptor binding assay

GABA receptor binding assays were performed according to the method of Akahane *et al.*, 1989. Fifty mg of crude synaptic membranes prepared from rat cebral cortex were incubated with 10 nM [^{3}H]muscimol in the presence of various concentrations of test articles (LB20304 or ciprofloxacin) for 20 minutes at 4℃. The reaction was terminated by rapid filtration with GF/B filter followed by extensive washing. The residual radioactivity was counted. Non-specific binding was assessed in the presence of 1 mM GABA. Experiments were performed several times. Data shown are mean values of duplicate determinations in a representative experiment, and the variation is less than 10% of mean values.

Drug Interaction between LB20304 and 4-BPA in Mice

Five male mice weighing 23~25 g were used in each group. Various doses of test articles were treated *per os* thirty minutes after oral administration of 4-BPA (4-biphenylacetic acid, 400 mg/kg) and frequency of convulsion and mortality were measured to assess CNS toxicity.

Epileptogenecity by the direct intracerebral injection

Convulsions induced by the direct intracerebral injection of test articles were assessed as follows (Brittain and Handley, 1967; Haley and McCormic, 1957; Shimada, *et al.*, 1992). Five to seven male ICR mice weighing 24~26 g were used in each group. The needle (26-gauge, 3 mm in length) was inserted perpendicularly through the skull (within 1 mm of midline and on a line joining the anterior bases of the ears) into the brain and five microliters of test articles were injected. Immediately after injection, the symptoms such as convulsion, tremor, writhing, and death were observed for 2 h. The CD_{50} (dose eliciting convulsion in 50% of animals in a group) was calculated by Probit method (Bliss, C. I., 1985). Five microliter of crystal blue (1/10 diluent) was injected intracerebrally in separate animals and the third and forth ventricles were

shown to be stained through histological examination.

Results and Discussions

General Behaviour

As for test with mice (data not shown), LB20304 and ciprofloxacin, after oral administration of 1,000 mg/kg, induced respiratory depression, slight depression of activity, reduction of limb tone, and wire maneuver. At oral dose of 2,000 mg/kg, LB20304 caused the depression of activity, respiratory rate, muscle tone, touch escape, and positional struggle which lasted upto one hour. Even more depression of activity and respiration were also observed with treatment of 2,000 mg/kg of ciprofloxacin. As for test with dogs (data not shown), LB20304 and ciprofloxacin at an approximately 15 times the anticipated clinical oral dose was virtually devoid of clinical symptoms. These agents at the dose of 10 mg/kg, *i.v.* caused skin rash and severe pruritus which lasted up to half hour. In addition, one out of two animals given ciprofloxacin, 10 mg/kg, *i.v.* showed sign of convulsion immediately after injection, but not with LB20304.

Hemodynamics

As shown in Table I, LB20304 at doses of 20 mg/kg and 100 mg/kg, *i.v.* exhibited transient rise of mean arterial blood pressure upon injection (data not shown) followed by drops (8% and 35% of baseline at 20 mg/kg and 100 mg/kg, respectively). Values thereafter gradually returned to those of baseline within 5 minutes. This remained within normal range and LB20304 did not show any observable effects on the blood pressure for this species under the experimental conditions of the protocol.

Central nervous system

Quinolones are known to exhibit a low incidence of CNS adverse reactions including hallucination, depression, nightmare, confusion, and manic reaction in human (Christ, 1990; Janknegt, 1989; Lucet *et al.*, 1988). Fenbufen is a nonsteroidal anti-inflammatory drug which has been routinely coadministered with quinolone for therapy of infectious disease. Recently, some interactions, mainly convulsive seizure, of quinolones and fenbufen were reported in both human and experimental animals (Hori *et al.*, 1989; Tsuji *et al.*, 1988; Yakushiji *et al.*, 1992). It has been previously reported that all the quinolones have substantially CNS stimulant activity although there are some inter-quinolone variations in the intensity of CNS stimulant activity (Akahane *et al.*, 1989). The incidences of CNS side effects caused by quinolones with a piperazine or aminopyrrolidine moiety at their 7 positions have been observed more frequently in patients receiving quinolones in combination with non-steroidal anti-inflammatory drugs such as fenbufen (Akahane *et al.*, 1989; Hori *et al.*, 1989). The occupation by quinolones of the GABA receptors is supposed to cause general CNS excitation (Halliwell *et al.*, 1991; Yakushiji *et al.*, 1992). To test these possibilities several experiments were performed. Displacement of [^{3}H]muscimol binding to the rat brain synaptic membranes in the presence of LB20304 and ciprofloxacin was measured. As shown in Fig. 2, LB20304 was observed to be about five times less potent than ciprofloxacin in specific GABA receptor binding. The incidences of convulsion and death after oral administration of LB20304 and ciprofloxacin (12.5, 25, 50, and 100 mg/kg) to mice are shown in Table II. A single oral pretreatment with 4-BPA at 400 mg/kg increased the incidence of convulsion and death after oral administration of ciprofloxacin at the doses of 25, 50, and 100 from zero of five to three of five, two of five, and four of five, respectively, whereas LB20304 alone or combination with 4-BPA caused neither convulsions nor death at the doses of 12.5, 25, 50, and 100 mg/kg, respectively. Supposedly, when co-administered with 4-BPA, GABA receptor inhibition

Table I. Effects of LB200 and ciprofloxacin on the mean arterial blood pressure (MBP), diastolic pressure (DP), and systolic pressure (SP) after single bolus intravenous injection (20 and 100 mg/kg) in rats. Experiments were performed in 4~5 animals. Data shown are measurements in a representative experiment.

	Ciprofloxacin (20 mg/kg)			LB20324 (20 mg/kg)			LB20324 (100 mg/kg)		
	MBP	DP	SP	MBP	DP	SP	MBP	DP	SP
Pre-treatment (mmHg)	120	110	135	120	110	130	115	100	125
Max. pressure (mmHg)	150	135	150	125	115	135	135	115	150
Min. pressure (mmHg)	55	45	72	110	105	125	75	55	90
5 min after injection (mmHg)	120	115	130	110	100	125	125	105	140
10 min after injection (mmHg)	–	–	–	120	110	135	115	100	125
Max. change (%)	−54.2	−59	−44.6	−8.3	−9.1	−3.8	−34.8	−45	−28

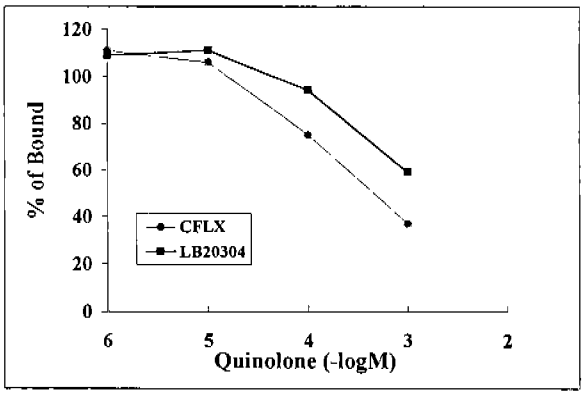

Fig. 2. Displacement of [^{3}H]muscimol binding to rat brain synaptic membrane in the presence of LB20304 and ciprofloxacin. Experiments were performed several times. Data shown are mean values of duplicate determinations in a representative experiment, and the variation is less than 10% of mean values.

Table II. Induction of convulsions by quinolones with 4-biphenyl actic acid (BPA)

Quinolone	Dose (mg/kg, po)	Frequency of Convulsion	Mortality
LB20304	100 (Quinolone only)	0/5	0/5
	12.5+BPA (400 mg/kg, po)	0/5	0/5
	25+BPA (400 mg/kg, po)	0/5	0/5
	50+BPA (400 mg/kg, po)	0/5	0/5
	100+BPA (400 mg/kg, po)	0/5	0/5
Ciprofloxacin	100 (Quinolone only)	0/5	0/5
	12.5+BPA (400 mg/kg, po)	0/5	0/5
	25+BPA (400 mg/kg, po)	3/5	3/5
	50+BPA (400 mg/kg, po)	2/5	2/5
	100+BPA (400 mg/kg, po)	4/5	4/5

*BPA: Active metabolite of Fenbufen, Quinilones were administered 30 min after oral administration of BPA, Five male ICR mice were used per each group.

induced by ciprofloxacin was enhanced more synergistically than that of LB20304.

The central action of test articles can be evaluated more properly by direct intracerebral injection into the brain, which eliminates the penetration process through the blood-brain barrier (BBB). As shown in Table III, the epileptogenic activities of various quinolones were assessed by a direct intracerebral injection of 5 μl of appropriate concentration of test articles. The CD_{50} values (nmole) obtained from the analysis of the dose-response data are as follows; 169.47, 35.36, 105.29, and 88.67 for LB20304, ciprofloxacin, ofloxacin, and lomefloxacin, respectively. Taken together, LB20 304 seems to be significantly less potent among reference quinolones tested, ciprofloxacin, ofloxacin, and lomefloxacin in causing adverse CNS stimulation.

Table III. CNS toxicity induced by direct intracerebral injection of quinolones

Quinolones	CD_{50} (nmol) (95% Confidence Limit)
LB20304	169.47 (95.89~295.91)
Ciprofloxacin	35.36 (24.51~ 50.99)
Ofloxacin	105.29 (36.55~239.90)
Lomefloxacin	88.67 (13.75~179.45)

*Animal: Five to seven male ICR mice were used per each group. Administration route: ICV (5 μl), CD_{50}: The dose which induced convulsion of 50% animals in a group.

References

Akahane, K, Sekiguchi, M., Une, T. and Osada, Y. (1989). Structure-epileptogenecity relationship of quinolones with special reference to their interaction with γ-aminobutyric acid receptor sites. *Antimicrob. Agents Chemother.* **33**, 1704-1708.

Anastasio, G. E., Menscer, D. and Little, J. M. (1988). Norfloxacin and seizures. *Ann. Intern. Med.* **109**, 169-170.

Arcieri, G., Griffith, E., Gruenwaldt, G., Heyd, A., OBrien, B., Becker, N. and August, R. (1987). Ciprofloxacin: an update on clinical experience. *Am. J. Med.* **82**(Suppl. 4A), 381-394.

Bliss, C. I., (1985). *The statistics in bioassay.* Academic Press, Inc., New York

Brittain, R. T. and Handley, S. L. (1967). Temperature changes produced by the injection of catecholamines and 5-hydroxytryptamine into the cerebralventricles of the conscious mouse. *J. Physiol.* **192**, 805-813.

Christ, W. (1990). Central nervous system toxicity of quinolones: human and animal findings. *J. Antimicrob. Chemother.* **26**(Suppl. B), 219-225.

Christ, W. and Lehnert, T. (1990). Toxicity of the quinolones, 165-187. In Siporin, C. Heifetz, C. L. and Domagala, J. M. (ed.), *The New Generation of Quinolones.* Marcel Dekker, Inc., New York.

Christ, W., Lehnert, T. and Ulbrich, B. (1988). Specific toxicologic aspects of the quinolones. *Rev. Infect. Dis.* **10**(Suppl. 1), S141-S146.

Ferguson, J., McIntosh, J. and Walker, E. M. (1988). Ciprofloxacin-induced photosensitivity: *in vitro* and *in vivo* studies. *J. Invest. Dermatol.* **91**, 385-388.

Haley, T. J. and McCormic, W. G. (1957). Pharmacological effects produced by intracerebral injection of drugs in the conscious mouse. *Brit. J. Pharmacol.* **12**, 12-15.

Halliwell, R. F., Davey, P. G. and Lambert, J. J. (1991). The effects of quinolones and NSAIDs upon GABA-evoked currents recorded from rat dorsal root ganglion neurons. *J. Antimicrob. Chemother.* **27**, 209-218.

Hori, S., Shimada, J., Sato, A., Matsuda, M. and Miyahara, T. (1989). Comparision of the inhibitory effects of new quinolones on γ-aminobutyric acid receptor binding in the presence of anti-inflammatory drugs. *Rev. Infect. Dis.* **11** (Suppl. 5), S1397-S1398.

Irwin, S. (1968). Comprehensive observational assessment:

Ia. A systematic quantitative procedure for assessing the behavioral physiologic state of the mouse. *Psychopharmacologia* **12**, 222-257.

Janknegt, R. (1989). Fluoroquinolones. Adverse reactions during clinical trials and postmarketing surveillance. *Pharm. Weekbl. (Sci.).* **11**, 124-127.

Kim, Y. K., Choi, H., Kim, S. H., Chang, J. H., Nam, D. H., Kim, Y. Z., Kwak, J. H. and Hong, C. Y. (1995). Synthesis and antibacterial activities of LB20304: a new fluoronaphthyridone antibiotic containing novel oxime functionalized pyrrolidine. In *Abstracts of the 35th Interscience Conference on Antimicrobial agents and Chemotherapy, San Francisco, CA, 1995.* Abstract F204, p. 148. American Society for Microbiology, Washington, D.C.

Lucet, J. C., Tilly, H., Lerebours, G., Gres, J. J. and Piguet, H. (1988). Neurological toxicity related to pefloxacin. *J. Antimicrob. Chemother.* **21**, 811-812.

Oh, J. I., Paek, K. S., Kim, M. Y., Seo, M. K., Lee, Y. H., Hong, C. Y., Nam, D. H., Kim, Y. Z., Kim, I. C. and Kwak, J. H. (1995). *In vitro* and *in vivo* antibacterial activities of LB 20304, a new fluoronaphthyridone. In *Abstracts of the 35th Interscience Conference on Antimicrobial agents and Chemotherapy, San Francisco, CA, 1995.* Abstract F205, p. 148. American Society for Microbiology, Washington, D.C.

Shimada, J., Hori, S., Kanemitsu, K., Shoji, Y., Nakashino, S. and Yanaguwa, A. (1992). A comparative study on the convulsant activity of carbapenems and beta-lactams. *Drug Exptl. Clin. Res.* **18**(9), 377-381.

Tack, K. J. and Smith, J. A. (1989). The safety profile of ofloxacin. *Am. J. Med.* **87**(Suppl. 6C), 78S-81S.

Thorsteinsson, S. B., Bergan, T., Oddsdottir, S., Rohwedder, R. and Holm, R. (1986). Crystalluria and ciprofloxacin, influence of urinary pH and hydration. *Chemotherapy* **32**, 408-417.

Tsuji, A. Sato, H., Okezaki, E. Nagata, O. and Kato. H. (1988). Effect of anti-inflammatory agent fenbufen on the quinolone-induced inhibition of γ-aminobutyric acid receptor binding to rat brain membranes *in vitro*. *Biochem. Pharmacol.* **37**, 4408-4411.

Yakushiji, T., Shiraski, T. and Akaike, N. (1992). Noncompetitive inhibition of $GABA_A$ response by-a new class of quinolones and nonsteroidal anti-inflammatories in dissociated frog sensory neurons. *Br. J. Pharmacol.* **105**, 13-18.