LB20304, a New Generation of Fluoroquinolone Antibacterial

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Introduction

A number of new quinolone antibacterials such as ciprofloxacin, ofloxacin, norfloxacin, sparfloxacin and tosufloxacin have been recently developed and commercialized. Compared to their predecessors such as nalidixic acid and oxolinic acid, these new fluorinated quinolones showed a broader antibacterial spectrum, including both gram-negative and gram-positive bacteria. However, they still possess limited activity against many gram-positive cocci including *Staphylococci* and *Streptococci*, which are the major causative pathogenic strains of respiratory tract infections. In addition, the emergence of resistant strains to quinolones is presently increasing probably due to their moderate activities against gram-positive bacteria.

Therefore, recent efforts have been directed toward the development of novel quinolone compounds that provide improved antibacterial activity against gram-positive organisms while retaining excellent antibacterial activity against gram-negative organisms. After years of research activities, LG Chemical Ltd. has recently determined to develop a new fluoroquinolone antibacterial, LB20304. It is a fluoronaphthyridone carboxylic acid with a novel pyrrolidine substituent.

The *in vitro* antibacterial activity of LB20304a has been studied against 1231 clinical isolates collected in Korea including ciprofloxacin-resistant strains, ceftazidime-resistant strains, erythromycin-resistant strains and anaerobes, and over 800 strains obtained in USA. Overall, LB20304 has enhanced *in vitro* antibacterial activities against clinically important gram-positive bacteria compared to other fluoroquinolones including ciprofloxacin, grepafloxacin and trovafloxacin, while retaining potent activities against gram-negative bacteria as of ciprofloxacin. The potent antibacterial activity of LB20304a has been confirmed in several experimental

animal models. With the advantages of the improved activity against gram-positive pathogens, and the favorable pharmacokinetic and safety profiles, LB20304a is presently under active clinical evaluation. The treatments have been well tolerated in humans and a once-a-day dosage regimen is expected for the full therapy.

LB20304a is anticipated to possess remarkable advantages over other fluoroquinolones for the treatment of respiratory tract infections including bronchitis and pneumonia.

In Vitro Antibacterial Activities

Initial antibacterial activity of LB20304 has been evaluated against clinical isolates obtained from Korea. In addition, its effect was also confirmed against American clinical isolates at the Department of Pathology, University of Iowa College of Medicine, USA.

In the study against Korean clinical isolates, ciprofloxacin, sparfloxacin, lomefloxacin, and ofloxacin were used as reference quinolones for the activity comparisons. For testing against gram-positive bacteria (Table 1), LB20304 was the most potent quinolone, compared to the reference compounds. The gram-positive strains that were clearly susceptible to LB20304 were *Streptococcus pneumoniae, S. pyogenes, S. aureus* (MSSA and MRSA), and *S. epidermidis.* LB20304 was also active against aerobic gram negative bacilli and its activity was comparable to ciprofloxacin (Table 2).

Table 1. Comparative *in vitro* activities of LB20304 against gram-positive clinical isolates collected in Korea

(MIC_{90}, μg/ml)

	LB20304	Ciprofloaxacin	Sparfloxacin	Lomefloxacin	Ofloxacin
S. *pneumoniae* (Pen-R)	0.031	2	0.5	16	
S. pyogenes	0.031	0.5	0.5	8	1
S. aureus (Met-S)	0.063	2	0.13	2	1
S. aureus (Met-R)	1	32	8	128	16
S. epidermidis (Met-S)	0.13	1	0.25	2	1
S. *epidermidis* (Met-R)	1	32	8	64	16

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For evaluating its activity against American strains, around 800 clinical isolates collected at the University of Iowa College of Medicine have been tested.

Table 2. Comparative *in vitro* activities of LB20304 against gram-negative clinical isolates collected in Korea

(MIC_{90}, μg/ml)

	LB20304	Ciprofloaxacin	Sparfloxacin	Lomefloxacin	Ofloxacin
E. coli	0.13	0.031	0.13	0.5	0.25
E. cloacae	0.5	1	1	4	2
E. aerogenes	2	1	2	4	2
C. freundii	1	1	2	4	2
K. pneumoniae	0.25	0.25	0.5	2	1
P. vulgaris	0.063	0.016	0.13	0.25	0.13
P. mirabilis	0.25	0.13	0.5	0.5	0.25
M. morganii	0.5	0.25	2	1	1
P. aeruginosa	16	8	16	32	16
A. calcoaceticus	0.25	2	0.25	4	2

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As reference quinolones, they used ciprofloxacin, levofloxacin, ofloxacin, sparfloxacin, and trovafloxacin. Trovafloxacin is under development by Pfizer Central Research and is presently in the later stages of phase III clinical trials.

Against gram-positive species, LB20304 showed the similar patterns of effectiveness in the USA as those shown against Korean strains. LB20304 was the most potent quinolone of the compounds tested against penicillin-resistant *S. pneumoniae*, *S. aureus*, *S. epidermidis*, and Coagulase-negative *staphylococci* (Table 3).

Table 3. Comparative *in vitro* activities of LB20304 against gram-positive clinical isolates collected in USA.

(MIC_{90}, μg/ml)

	LB20304	Ciprofloaxacin	Levofloxacin	Sparfloxacin	Trovafloxacin
S. *pneumoniae* (Pen-S)	0.015	1	1	0.25	0.12
S. *pneumoniae* (Pen-R)	0.015	1	1	0.25	0.12
S. aureus (Oxa-S)	0.03	0.5	0.25	0.12	0.03
S. aureus (Oxa-R)	2	> 4	> 8	> 8	4
S. epidermidis (Oxa-S)	0.015	0.25	0.25	0.25	0.03
S. *epidermidis* (Oxa-R)	0.25	4	2	8	0.5
CNS	0.015	0.5	0.5	0.5	0.06

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When tested against non-fastidious aerobic gram-negative bacilli, LB20304 was the most potent against most species of Enterobacteriaceae. For the more fastidious gram-negative species such as *H. influenzae*, *Moraxella catarrhalis*, and *N. gonorrhoeae*, LB20304 was also the most potent (Table 4).

Table 4. Comparative *in vitro* activities of LB20304 against gram-negative clinical isolates collected in USA.

(MIC_{90}, μg/ml)

	LB20304	Ciprofloaxacin	Levofloxacin	Sparfloxacin	Trovafloxacin
E. coli	0.015	0.03	0.06	0.06	0.03
E. cloacae	0.25	0.12	0.25	1	0.5
E. aerogenes	0.12	1	0.5	1	0.25
C. freundii	2	1	2	8	2
K. pneumoniae	0.12	0.12	0.25	0.5	0.25
P. vulgaris	0.12	0.015	0.06	1	0.25
M. morganii	0.12	0.015	0.06	0.5	0.25
P. aeruginosa	2	0.5	2	8	2
Acinetobacter spp.	0.06	0.5	0.25	0.12	0.03
H. influenzae	≤0.004	0.008	0.015	0.015	0.015
M. catarrhalis	0.008	0.015	0.03	0.03	0.015
N. gonorrhoeae	0.008	ND	ND	ND	ND

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The unique characteristics of LB20304 based on its activities against various clinical isolates is coming from its effects against *S. pneumonieae, H. influenzae,* and *M. catarrhalis*. these results obviously suggest a potential role of LB20304 for the respiratory tract infection.

Antibacterial Activities against Resistant Strains

LB20304 showed potent activities against ofloxacin-resistant strains and β-lactamase-producing strains. *In vitro* activities of LB20304 against ciprofloxacin-resistant or ceftazidime-resistant strains were tested. LB20304 inhibited 50 % to 66 % of ciprofloxacin -resistant strains and 75 % to 85 % of ceftazidime-resistant strains at the ranges of 1 to 2 μg/ml (Table 5).

Table 5. *In vitro* efficacy of LB20304 against ciprofloxacin resistant (MIC ≥ 2 μg/ml) and ceftazidime-resistant (MIC ≥ 16 μg/ml) strains

Resistance group	LB20304 MIC (μg/ml)					
	≤0.25	0.5	1	2	4	≥8
Ciprofloxacin [a]	24	10	33	22	25	21
Ceftazidime[b]	118	11	31	20	21	12

a. Includes Enterobactriaceae (18 strains), *S. maltophilia* (6), Methicillin resistant *Staphylococci* (61), *enterococci* (38), *streptococci* (3), *C. jeikeium* (9)

b. Includes Enterobactriaceae (32 strains), *S. maltophilia* (1), Methicillin resistant *Staphylococci* (98), *enterococci* (59), *streptococci* (4), *C. jeikeium* (9), *P. aeruginosa* (3), *Bacillus* spp. (7)

In Vivo Activity

The primary *in vivo* activity of LB20304 was evaluated against systemic infections in mice. The *in vivo* effect of LB20304 was well correlated with its *in vitro* MIC values (Table 6). Systemic infections initiated by *S. aureus* and *S. pneuomoniae* were consistently protected by LB20304 as shown in its *in vitro* activities.

Table 6. Comparative *in vivo* activities of LB20304 against systemic infection in mice

(ED_{50}, mg/kg/dose)

Microorganism	LB20304	Ciprofloxacin	Sparfloxacin
S. aureus giorgio	1.68	14.0	2.27
S. pyogenes 77A	10.4	>140	25.9
S. pneumoniae III	7.64	>200	32.6
E. coli 851E	0.47	0.2	0.3
P. aeruginosa 1912E	2.19	3.11	0.98
P. mirabilis 1315E	4.33	0.47	3.91
S. marcescens 1826E	7.96	1.45	10.3

a. Antimicrobial agents were orally administrated twice at 1 and 4hr after infection

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Pharmacokinetic Evaluations

Pharmacokinetic parameters of LB20304 in rats and dogs are summarized in Table 7. Following IV administration, the plasma concentrations of LB20304 disappeared biexponentially with a mean terminal elimination half-life of 94 min for rats and 363 min for dogs.

Table 7. Pharmacokinetic parameters of LB20304 in the blood following IV and oral administration of 20mg/kg to rats and 10mg/kg to dogs (mean ± SD, n=5~10)

	Parameters	Rat	Dog	P[a]
IV	$t_{1/2\beta}$ [min]	93.6±11.6	363±64.4	0.0001
	AUC [μg min/ml]	778.4±196.8	1090±322.4	
	CL [ml/min/kg]	21.8±6.57	7.95±2.67	0.0034
	Vd_{ss} [ml/kg]	2265±959.5	4144±1834	0.1082
Oral	AUC [μg min/ml]	239.6±48.24	884.0±162.4	
	t_{max} [min]	18.3±11.7	53.3±32.0	0.0059
	C_{max} [μg/ml]	2.23±0.640	1.72±0.225	
BA[%]		30.8	81.1	

a. Statistical significance of difference at $P<0.05$ by one-way analysis of variance (ANOVA) between rats and dogs. *Arch. Pharm. Res.* 19, 400-405 (1996)

The steady-state volume of distribution of LB20304 was 2265 ml/kg and 4144 ml/kg for rats and dogs, respectively, indicating extensive tissue distribution of LB20304 in these species. Following oral administration, LB20304 was rapidly absorbed in both species. The absolute oral bioavailability of LB20304 was 31% in rats and 81 % in dogs. In rats, approximately 44% and 6.4% of the dose were excreted unchanged in urine and bile, respectively, following IV administration, and approximately 14% and 4.5%, respectively, following oral administration.

Conclusion

From *in vitro* and *in vivo* primary screenings of the compounds synthesized, we have chosen LB20304 as a development candidate for a potential antibacterial agent. By testing its antibacterial activities more intensively, we have confirmed that LB20304 would be a competitive quinolone antibacterial for the treatment of respiratory tract infections. Subsequently, we have evaluated its pharmaceutical characteristics to identify whether the molecule could satisfy the minimum criteria needed for commercial requirement. The compound, in addition, has been in the preclinical studies including scale up and process research with the quality assurance of GLP and GMP compliance. LB20304 is presently under the initial clinical evaluations and its more detailed clinical usefulness will be demonstrated and presented in the near future.