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Antimicrobial Activities of LB20304a, a New Quinolone Antibiotic

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Abstract – *In vitro* activities of LB20304a were compared with those of grepafloxacin (OPC-17116), Q-35, ciprofloxacin, and sparfloxacin against 380 clinical isolates collected from general hospitals in 1996. LB 20304a was the most active agent against gram-positive strains including staphylococci, streptococci and enterococci. LB20304a was also very active against gram-negative bacteria and its activity was comparable to that of ciprofloxacin but better than those of grepafloxacin, Q-35 and sparfloxacin. The therapeutic effect of LB20304a was superior to those of sparfloxacin and ciprofloxacin against systemic infection by methicillin-resistant *Staphylococcus aureus* K283 (MRSA) in neutropenic mice. Against urinary tract infection induced by *Escherichia coli* 851E in mice, LB20304a was more active than sparfloxacin and ciprofloxacin. However, LB 20304a was slightly less active than that of ciprofloxacin against urinary tract infection by *Pseudomonas aeruginosa* 1912E, but better than that of sparfloxacin.

Keywords □ LB20304a, quinolone, MIC, systemic infection, urinary tract infection, neutropenic mice

A number of new quinolone antibiotics including ciprofloxacin, ofloxacin, lomefloxacin and fleroxacin have been recently commercialized. Compared with their predecessor, nalidixic acid, these new fluorinated quinolones showed a broader antibacterial spectrum, including both gram-negative and gram-positive bacteria (Wolfson *et al.*, 1992). However, they still possess only moderate activity against many gram-positive cocci such as staphylococci and streptococci, which are the major causative pathogenic strains of respiratory tract infections (Raviglione *et al.*, 1990; Thys *et al.*, 1989). Therefore, recent efforts have been directed toward the development of novel quinolone compounds that provide improved activity against gram-positive organisms while retaining the spectrum of ciprofloxacin (against gram-negative bacteria) (Fuchs *et al.*, 1991; Piddock, 1994; Sato *et al.*, 1992).

LG Chemical Ltd. has developed a new quinolone compound, LB20304a, a mesylate salt form of LB20304. It is a fluoronaphthyridone carboxylic acid with a novel oxime functionalized aminomethyl pyrrolidine (Fig. 1). This compound has shown a broad-spectrum antibacterial activity. Especially, It demonstrated potent antibacterial activities against gram-positive bacteria both *in vitro* and *in vivo* efficacy studies (Kim *et al.*, 1996a; Oh *et al.*, 1996; Ahn *et al.*, 1996).

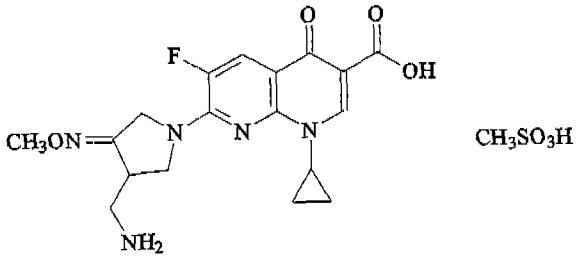

Fig. 1. Chemical structure of LB20304a.

In this study, *in vitro* activities of LB20304a against recent clinical isolates collected in 1996 were compared with those of grepafloxacin, Q-35, sparfloxacin and ciprofloxacin. We also studied *in vivo* efficacy of LB20304a against systemic infection caused by MRSA in neutropenic mice, and urinary tract infections by *E. coli* and *P. aeruginosa* in normal mice.

MATERIALS AND METHODS

Antimicrobial agents

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LB20304a, grepafloxacin and Q-35 were synthesized at Biotech Research Institute, LG Chem Research Park. The comparative quinolones, such as ciprofloxacin and sparfloxacin were obtained directly from their manufacturers.

Test organisms

The bacterial strains used in this study were originally isolated from human clinical specimens. These were collected at several hospitals in Korea in 1996. All isolates were stored frozen at -70 ℃.

Susceptibility tests

In vitro efficacy of LB20304a was determined by minimal inhibitory concentration (MIC) values expressed in μg/ml. MICs of LB20304a against clinical isolates were determined by the agar dilution method as described by the National Committee for Clinical Laboratory Standards M7-A3 (NCCLS, 1993). Mueller-Hinton medium (Difco Laboratories, Detroit, MI) was used for testing aerobic and facultative organisms. For *Streptococcus pneumoniae*, Mueller-Hinton Broth (MHB) was supplemented with 5% defibrinated sheep blood. Test strains were grown for 18 h in MHB. These overnight cultures were diluted with the same fresh medium to a density of approximately 10^7 CFU/ml and applied to Mueller-Hinton Agar (MHA) plates which contain serially diluted antimicrobial agents by use of an automatic MIC-2000 multipin inoculator (Dynatech Laboratories, Inc., Alexandria, VA.) to yield 10^4 CFU per spot. MICs were determined after 18 h of incubation at 35 ℃. MIC was considered to be the lowest concentration that completely inhibited growth on agar plates, disregarding a single colony or a faint haze caused by the inoculum.

***In vivo* activity against systemic infection in neutropenic mice**

The therapeutic efficacy of LB20304a was examined against experimental systemic infection induced by methicillin-resistant *S. aureus* K283 (MRSA) in neutropenic mice. Test organism for infection was cultured in Tryptic Soy Agar Medium (Difco) at 35 ℃ for 18 h and was suspended in 7.5% gastric mucin (Difco). For trials using immunosuppressed mice, 150 mg/kg of cyclophosphamide per kg of body weight was injected intraperitoneally twice, at 4 days and 1 day before infection (Ling *et al.*, 1993). This procedure produced a greater than 90% reduction in circulating neutrophils. Male neutropenic ICR mice weighing 19 to 21 g (Biotech Research Institute, LG Chem Ltd., Taejon, Korea) were injected intraperitoneally with 0.5 ml (ca. 10^7 cfu) of the bacterial suspension corresponding to an inoculum range of 5 to 10 times the MLD (Minimal Lethal Dose) of bacteria. Four dose levels were used for each antibiotic, depending on *in vitro* antimicrobial activity of the compounds. Mice were orally administered twice, at 1 and 4 hr post-infection, with various dose regimens of antibiotics. Mortality was recorded for 7 days, and the median effective dose needed to protect 50% of mice (ED_{50}) was calculated by the Probit method (Bliss, 1985). All untreated mice died within 2 days after infection.

***In vivo* activity against urinary tract infection in mice**

For the study of *in vivo* activity of LB20304a in urinary tract infection (UTI) animal model, infections in urinary tract were produced by the modification of the method of Nish and Tsuchiya (Nish *et al.*, 1978). Test organism were cultured in Trypticase soy broth (TSB) at 37 ℃ for 18 hr and suspended in TSB. After restriction of water intake for 20 h, female ICR mice weighing 20 to 22 g were anesthetized by inhalation of ethyl ether and forced to void urine by compression of the bladder through the external abdominal wall. Experimental urinary tract infection was produced by inoculating 0.05 ml of *E. coli* 851E suspension (2.0×10^9 CFU/ml) or *P. aeruginosa* 1912E suspension (1.9×10^7 CFU/ml) transurethrally into the bladder. Immediately after the inoculation, the external urethral meatus was clamped for 2 hr. Drug was administered orally at 4 hr after infection. The kidneys were removed aseptically at 24 hr after administration of drug and homogenized, and then the bacterial counts in the homogenates were determined after 24 hr incubation at 37 ℃. The detection limit in this assay was 10 CFU/kidney.

RESULTS

***In vitro* antibacterial activity**

LB20304a showed a broad spectrum antibacterial activity against a wide range of bacteria covering gram-positive and gram-negative bacteria. Table I shows *in vitro* MICs of LB20304a, grepafloxacin, Q-35, ciprofloxacin and sparfloxacin against 380 recent clinical isolates. LB 20304a demonstrated the most potent antibacterial activity against gram-positive bacteria among the compounds tested. Against the methicillin-susceptible strains of *Staphylococcus aureus* (MSSA), MIC_{90} of LB20304a was 0.063 μg/ml. It was 8-fold more potent than ciprofloxacin. Against the methicillin-resistant strains of *Sta-*

Table I. Comparative *in vitro* activities of LB20304a against 380 clinical isolates.

Microorganism (No. of strains)	Antimicrobial Agents	[a]MIC (μg/ml)			[b]% Susceptibility at MIC (μg/ml)≤1
		Range	50%	90%	
MSSA (20)	LB20304a	≤0.008-0.063	0.031	0.063	100
	Ciprofloxacin	0.063-1	0.5	0.5	100
	Grepafloxacin	0.031-0.25	0.063	0.13	100
	Sparfloxacin	0.031-0.13	0.063	0.13	100
	Q-35	0.063-0.25	0.13	0.25	100
MRSA (20)	LB20304a	0.031->8	1	4	80
	Ciprofloxacin	0.5->8	>8	>8	35
	Grepafloxacin	0.063->8	>8	>8	35
	Sparfloxacin	0.063->8	8	8	35
	Q-35	0.13->8	2	4	35
Coagulase Negative *Staphylococci* oxacillin-susceptible (13)	LB20304a	0.016-0.031	0.031	0.031	100
	Ciprofloxacin	0.13-0.5	0.25	0.5	100
	Grepafloxacin	0.063-0.25	0.13	0.13	100
	Sparfloxacin	0.063-0.25	0.13	0.13	100
	Q-35	0.063-0.25	0.13	0.25	100
oxacillin-resistant (13)	LB20304a	0.031-4	0.5	2	69
	Ciprofloxacin	0.25->8	8	>8	38
	Grepafloxacin	0.063->8	8	>8	38
	Sparfloxacin	0.13->8	4	8	38
	Q-35	0.063-4	2	2	46
Streptococcus pneumoniae (8)	LB20304a	0.008-0.063	0.016		100
	Ciprofloxacin	0.25-2	0.5		88
	Grepafloxacin	0.063-0.25	0.13		100
	Sparfloxacin	0.13-0.25	0.25		100
	Q-35	0.13-0.5	0.25		100
Streptococcus pyogenes (20)	LB20304a	0.016-0.13	0.031	0.13	100
	Ciprofloxacin	0.25-8	0.5	1	90
	Grepafloxacin	0.25-2	0.5	0.5	95
	Sparfloxacin	0.25-2	0.5	0.5	95
	Q-35	0.25-2	0.5	0.5	95
Enterococcus faecalis (15)	LB20304a	0.063-4	0.25	4	73
	Ciprofloxacin	0.5->8	2	>8	20
	Grepafloxacin	0.25->8	0.5	>8	73
	Sparfloxacin	0.5->8	1	>8	73
	Q-35	0.5->8	1	>8	67
Enterococcus faecium (16)	LB20304a	0.031-4	1	4	56
	Ciprofloxacin	0.5-8	8	8	6
	Grepafloxacin	0.25-8	4	8	19
	Sparfloxacin	0.25-8	4	4	19
	Q-35	0.5->8	8	>8	19
Escherichia coli (20)	LB20304a	≤0.008->8	0.13	>8	70
	Ciprofloxacin	≤0.008->8	0.13	>8	65
	Grepafloxacin	≤0.008->8	0.25	>8	65
	Sparfloxacin	≤0.008->8	0.25	>8	65
	Q-35	0.031->8	1	>8	55
Enterobacter cloacae (20)	LB20304a	0.031-4	0.5	4	75
	Ciprofloxacin	≤0.008->8	1	8	60
	Grepafloxacin	0.016->8	1	>8	50
	Sparfloxacin	0.016->8	1	>8	50
	Q-35	0.13->8	8	8	30

Table I. Continued.

Microorganism (No. of strains)	Antimicrobial Agents	[a]MIC (μg/ml)			[b]% Susceptibility at MIC (μg/ml)≤1
		Range	50%	90%	
Enterobacter aerogenes (7)	LB20304a	0.063-1	0.13		100
	Ciprofloxacin	0.031-0.5	0.031		100
	Grepafloxacin	0.063-1	0.13		100
	Sparfloxacin	0.063-1	0.13		100
	Q-35	0.25-4	0.5		57
Citrobacter freundii (16)	LB20304a	0.031->8	0.5	>8	69
	Ciprofloxacin	0.031->8	0.13	>8	69
	Grepafloxacin	0.063->8	1	>8	63
	Sparfloxacin	0.063->8	1	>8	63
	Q-35	0.25->8	2	>8	25
Klebsiella pneumoniae (20)	LB20304a	0.031-8	0.063	1	90
	Ciprofloxacin	0.016-4	0.031	0.5	90
	Grepafloxacin	0.031->8	0.063	4	70
	Sparfloxacin	0.031-8	0.063	2	70
	Q-35	0.13->8	0.25	4	65
Proteus vulgaris (12)	LB20304a	0.13->8	0.25	>8	83
	Ciprofloxacin	0.031->8	0.031	>8	83
	Grepafloxacin	0.13->8	0.25	>8	83
	Sparfloxacin	0.13->8	0.25	>8	83
	Q-35	0.5->8	1	>8	67
Proteus mirabilis (16)	LB20304a	0.063-8	0.13	0.5	94
	Ciprofloxacin	0.031-2	0.031	0.25	94
	Grepafloxacin	0.25->8	0.5	0.5	94
	Sparfloxacin	0.063-8	0.5	0.5	94
	Q-35	0.5->8	2	2	31
Morganella morganii (14)	LB20304a	0.063->8	0.13	4	86
	Ciprofloxacin	≤0.008->8	0.016	1	93
	Grepafloxacin	0.063->8	0.25	4	86
	Sparfloxacin	0.063->8	0.25	8	86
	Q-35	0.25->8	0.5	>8	86
Serratia marcescens (20)	LB20304a	0.063-8	2	2	45
	Ciprofloxacin	0.063-8	1	2	50
	Grepafloxacin	0.13->8	4	4	45
	Sparfloxacin	0.13->8	2	4	45
	Q-35	0.5->8	8	>8	10
Salmonella spp. (20)	LB20304a	≤0.008-0.13	0.031	0.063	100
	Ciprofloxacin	≤0.008-0.063	0.031	0.031	100
	Grepafloxacin	0.016-0.25	0.063	0.063	100
	Sparfloxacin	≤0.008-0.13	0.031	0.063	100
	Q-35	0.13-1	0.25	0.5	100
Shigella spp. (11)	LB20304a	0.016-0.13	0.016	0.13	100
	Ciprofloxacin	≤0.008-0.13	0.016	0.13	100
	Grepafloxacin	0.016-0.13	0.016	0.13	100
	Sparfloxacin	≤0.008-0.13	0.016	0.13	100
	Q-35	0.13-1	0.13	0.5	100
Acinetobacter baumannii (23)	LB20304a	0.016->8	0.063	0.13	91
	Ciprofloxacin	0.063->8	0.25	1	91
	Grepafloxacin	0.031->8	0.063	0.25	91
	Sparfloxacin	0.016->	0.031	0.25	91
	Q-35	0.13->8	0.5	2	74

Table I. Continued.

Microorganism (No. of strains)	Antimicrobial Agents	[a]MIC (μg/ml)			[b]% Susceptibility at MIC (μg/ml)$\leq$1
		Range	50%	90%	
Acinetobacter calcoaceticus (14)	LB20304a	0.031->8	8	>8	36
	Ciprofloxacin	0.25->8	>8	>8	29
	Grepafloxacin	0.063->8	>8	>8	36
	Sparfloxacin	0.031->8	8	>8	29
	Q-35	0.25->8	>8	>8	29
Pseudomonas aeruginosa (20)	LB20304a	0.5->8	4	>8	10
CAZ or IMP resistant	Ciprofloxacin	0.25->8	4	>8	20
	Grepafloxacin	0.5->8	8	>8	10
	Sparfloxacin	1->8	8	>8	10
	Q-35	2->8	>8	>8	0
Stenotrophomonas maltophilia (15)	LB20304a	0.13-2	2	2	40
	Ciprofloxacin	1-4	4	4	20
	Grepafloxacin	0.13-2	1	1	93
	Sparfloxacin	0.063-1	0.5	1	100
	Q-35	0.5-8	4	8	20
Haemophilus influenzae (7)	LB20304a	0.008-0.031	0.016		100
	Ciprofloxacin	0.016	0.016		100
	Grepafloxacin	0.016-0.031	0.016		100
	Sparfloxacin	0.008-0.031	0.016		100
	Q-35	0.031-0.063	0.063		100

[a]50% and 90%-MICs at which 50 and 90% of isolates were inhibited, respectively.
[b]% susceptible result in parentheses relates to the NCCLS [1995] breakpoint for ciprofloxacin ($\leq$1 μg/ml).

phylococcus aureus (MRSA), LB20304a inhibited 80% of isolates at the concentration of $\leq$1 μg/ml. On the other hand, the other quinolones inhibited only 35% of isolates at the same concentration. Against coagulase negative staphylococci including both oxacillin-susceptible strains and oxacillin-resistant strains, LB20304a was at least four-fold more active than the other quinolones. Its MIC_{90} was 0.031 and 2 μg/ml, respectively. LB20304a was also most active among the test compounds against *S. pneumoniae, S. pyogenes, E. faecalis,* and *E. faecium.* LB20304a inhibited 100% of isolates against streptococci at the concentration of $\leq$1 μg/ml. However, enterococci were moderately resistant to LB20304a. LB 20304a was also highly active against gram-negative bacteria. Against *Enterobacter aerogenes, Klebsiella pneumoniae, Proteus mirabilis, Salmonella* spp., *Shigella* spp., *Acinetobacter baumannii,* and *Haemophillus influenzae,* LB20304a inhibited more than 90% of clinical isolates at the concentration of $\leq$1 μg/ml. LB20304a was as active as ciprofloxacin but more active than the other quinolones. LB20304a showed comparable activity to ciprofloxacin against *Enterobacter cloacae, Citrobacter freundii, Proteus vulgaris,* and *Morganella morganii.* But it was more active than the other quinolones. Against *Serratia marcescens, Acinetobacter calcoaceticus,* and *Stenotrophomonas maltophilia,* LB20304a showed a moderate activity like the other quinolones. However, clinical isolates of ceftazidime-resistant or imipenem-resistant *P. aeruginosa* were highly resistant to all quinolones tested. For resistant isolates of *P. aeruginosa,* there was a cross-resistance between quinolones and β-lactam antibiotics as found in the previous report (Kim *et al.*, 1996b).

***In vivo* activity against systemic infection in neutropenic mice**

The protective effect of LB20304a against systemic infections by MRSA in neutropenic mice was compared with those of ciprofloxacin and sparfloxacin in Table II. Against infections induced by methicillin-resistant *S. aureus* K283, oral therapy with LB20304a yielded the ED_{50} (the median effective dose needed to protect 50% of mice) of 31.06 mg/kg, while oral therapy with ciprofloxacin or sparfloxacin at the doses of up to 108 mg/kg had no effect on reducing the mortality. The protective effect of LB20304a in the mouse infection model was well-correlated with its *in vitro* activity.

***In vivo* activity against urinary tract infection in mice**

The therapeutic activity of LB20304a against experimental urinary tract infection (UTI) induced by *E.*

Table II. Protective effect of LB20304a against systemic infection caused by methicillin-resistant *S. aureus* K283 in neutropenic mice.

	Dose (mg/kg)[a]					MIC (μg/ml)	PD_{50} (mg/kg)
	control	4	12	36	108		
LB20304a	0/6[b]	0/6	0/7	5/7	7/7	0.5	31.06
Sparfloxacin	0/6	0/7	0/6	0/6	0/6	8	>108
Ciprofloxacin	0/6	0/6	0/6	0/6	0/7	8	>108

[a]Drug was administrated orally at 4 hr after infection.
[b]Survival ratio.

Table III. Therapeutic effect of LB20304a, ciprofloxacin, and sparfloxacin on ascending urinary tract infection caused by *E. coli* 851E in mice.

Drug[a]	MIC (μg/ml)	Viable cells/kidney ($\log_{10}$ CFU/kidney)[b]		
		None (control)	2.5 mg/kg	25 mg/kg
LB20304a	$\leq$0.008	4.16$\pm$0.28	3.00$\pm$0.76	2.01$\pm$0.35
Ciproflatoxin	0.016	4.16$\pm$0.28	3.32$\pm$0.78	2.55$\pm$0.40
Sparfloxacin	0.031	4.16$\pm$0.28	3.14$\pm$0.75	2.07$\pm$0.06

*Experimental urinary tract infection in mice was produced by transurethral inoculation of 0.05 ml suspension of *E. coli* 851E into the bladder.
[a]Drug was administrated orally at 4 hr after infection.
[b]Viable cells in kidney were counted at 24 hr after admnistration of drug. Data are given as means$\pm$standard deviations.

Table IV. Therapeutic effect of LB20304a, ciprofloxacin, and sparfloxacin on ascending urinary tract infection caused by *P. aeruginosa* 1912E in mice.

Drug[a]	MIC (μg/ml)	Viable cells/kidney ($\log_{10}$ CFU/kidney)[b]		
		None (control)	2.5 mg/kg	25 mg/kg
LB20304a	0.25	7.14$\pm$1.07	1.83$\pm$1.33	1.17$\pm$0.46
Ciproflatoxin	0.13	7.14$\pm$1.07	1.57$\pm$0.98	-[c]
Sparfloxacin	1	7.14$\pm$1.07	3.22$\pm$2.81	1.27$\pm$0.46

*Experimental urinary tract infection in mice was produced by transurethral inoculation of 0.05 ml suspension of *P. aeruginosa* 1912E into the bladder.
[a]Drug was administrated orally at 4 hr after infection.
[b]Viable cells in kidney were counted at 24 hr after admnistration of drug. Data are given as means$\pm$standard deviations.
[c]Viable cell counts in two of total seven mice were 1 $\log_{10}$ CFU/kidney, but those in the other mice were below the assay limit.

coli 851E and *P. aeruginosa* 1912E in mice was compared with those of ciprofloxacin and sparfloxacin. Against ascending urinary tract infection by *E. coli*, the therapeutic effect of LB20304a was more active than those of sparfloxacin and ciprofloxacin (Table III). The viable cell counts ($\log_{10}$ CFU/kidney) at a dose of 25 mg/kg were 2.01$\pm$0.35, 2.55$\pm$0.40, and 2.07$\pm$0.06, respectively, for LB20304a, ciprofloxacin, and sparfloxacin, whereas it was 4.16$\pm$0.28 for the untreated control. Against infection by *P. aeruginosa*, LB20304a was slightly less active than ciprofloxacin but more active than sparfloxacin (Table IV). The number of viable cells ($\log_{10}$ CFU/kidney) at a dose of 2.5 mg/kg were 1.83$\pm$1.33, 1.57$\pm$0.98, and 3.22$\pm$2.81, respectively, for LB 20304a, ciprofloxacin, and sparfloxacin, whereas it was 7.14$\pm$1.07 for the untreated control. And most of the bacteria in kidney were killed rapidly at a dose of 25 mg/kg of all compounds tested.

DISCUSSION

New fluoroquinolones, such as ciprofloxacin, ofloxacin and lomefloxacin, have been used widely as effective antibiotics for the therapy of both gram-negative and gram-positive bacterial infections. However, there is still a need to develop new compounds which have more potent activities against *S. pneumoniae* and methicillin-resistant *Staphylococci*, because the increasing use of fluoroquinolones has apparently led to a dramatic increase in quinolone resistance among gram-positive bacteria, particularly methicillin-resistant *S. aureus* and *S. epidermidis* (Blumberg *et al.*, 1991; Jones, 1992).

LB20304a is a new quinolone with a novel oxime functionalized aminomethyl-pyrrolidine side chain which is believed to be associated with enhanced antibacterial activity against gram-positive bacteria (Domagala *et al.*, 1993; Domagala, 1994). This compound, with potent gram-negative activity still being maintained, was more active than grepafloxacin, Q-35, ciprofloxacin, and sparfloxacin against gram-positive pathogens, such as MRSA, coagulase-negative staphylococci, *S. pneumoniae, S. pyogenes*. In addition, LB20304a demonstrated better protective activity against systemic infection caused by MRSA in neutropenic mice than sparfloxacin and ciprofloxacin. Against urinary tract infections by gram-negative bacteria, LB20304 also exhibited excellent therapeutic activities like other quinolones. This finding showed that *in vivo* activities of LB20304a was well-correlated with its *in vitro* activities. In view of its strong activity against not only gram-positive strains but also the family *Enterobacteriaceae*, LB20304a could be used to treat a broad spectrum of human infections, such as respiratory tract, urinary tract, skin and skin structures, bone and gastro-intestinal tract infections. Further studies would be necessary to establish the clinical usefulness of this compound.

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