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Bactericidal Activities of LB20304, a New Fluoroquinolone

Kyoung-Sook Paek, Mu-Yong Kim, In-Chull Kim and Jin-Hwan Kwak

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

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The time-kill curves of LB20304, a novel fluoroquinolone that has potent antibacterial activity against gram-positve and gram-negative bacteria, were calculated at the concentrations of 1/4-, 1/2-, 1-, 2- and 4-times the MIC against *Staphylococcus aureus* 77, *Escherichia coli* 3739E, *Pseudomonas aeruginosa* 1912E. The bactericidal activity of LB20304 for these strains was very rapid and comparable to that of ciprofloxacin. LB20304 produced a decrease in the $\log_{10}$ CFU per milliliter of ≥ 3 within 2 h at 4-times the MIC for all strains and consitently prevented regrowth of bacteria after 24 h of incubation. The MBCs (Minimal Bactericidal Concentration) of LB20304 against test organisms were equal to or at most four-times higher than the MICs.

Key words : LB20304, Fluoroquinolone, Bactericidal activity, Time-kill, MBC

INTRODUCTION

Although new fluoroquinolones, such as ciprofloxacin, lomefloxacin, ofloxacin and fleroxacin, have excellent antibacterial activities against most of the gram-negative bacteria, these quinolones have only moderate activities against many gram-positive cocci, including *Streptococcus pneumoniae* and methicillin resistant *Staphylococcus aures* (MRSA) which are major causative strains of respiratory tract infections (Raviglione *et al.*, 1990). Because of the increasing incidence of methicillin- and ciprofloxacin-resistant gram-positive bacteria including staphylococci and streptococci (Blumberg *et al.*, 1991), there is a pressing need to investigate novel quinolone compounds for their potential activities against these organisms (Piddock, 1994; Fuchs *et al.*, 1991).

LB20304 is a new fluoronaphthyridone antibacterial agent containing novel oxime functionalized aminomethyl pyrrolidine (Fig. 1). This compound has shown improved antibacterial activity against gram-positive strains while retaining the broad spectrum activity of ciprofloxacin. The *in vitro* activities of LB20304 were superior to those of the currently available quinolones against most bacterial strains including gram-positive bacteria and anaerobes (Kim *et al.*, 1996; Oh *et al.*, 1996; Paek *et al.*, 1996).

It is well known that fluoquinolones have a bactericidal activity against bacteria. The bactericidal effect of quinolones is attributed to inhibition of bacterial DNA gyrase (bacterial topoisomerase II), an enzyme that regulates supercoiling and uncoiling of DNA (Hooper, 1995). In this paper, we examined the bactericidal activities of LB20304 against representative strains and compared with those of ciprofloxacin.

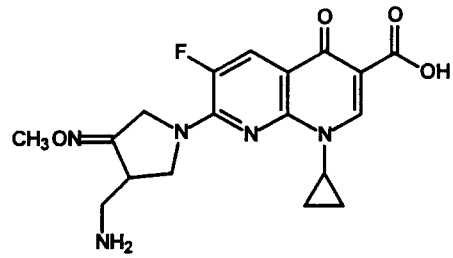

Fig. 1. Chemical structure of LB20304

MATERIALS AND METHODS

Antimicrobial agents

LB20304 was synthesized at the Biotech Research Institute, LG Chem Research Park, LG Chemical Ltd., Taejon, Korea. All comparative quinolone compounds were obtained directly from their manufacturers.

Bacterial strains

Staphylococcus aureus 77 used in this study was a clinical isolate collected from the hospital in Korea.

Correspondence to: Jin-Hwan Kwak, Biotech Research Institute, LG Chem Research Park, LG Chemical Ltd.,104-1 Moonji-Dong, Yuseong-Ku, Taejon 305-380, Korea

Escherichia coli 3739E and *Pseudomonas aeruginosa* 1912E were laboratory standard strains obtained from Glaxo Group Research Ltd. Test organisms were stored frozen at -70°C.

In vitro MIC tests

The MICs of LB20304 and ciprofloxacin against test organisms were determined by the agar dilution method as described by the National Committee for Clinical Laboratory Standards M7-A3 (NCCLS, 1993). Test strains were grown for 18 h in Mueller-Hinton broth, and then these overnight cultures were diluted with the same fresh medium to the density of approximately 10^7 CFU/ml. These strains were applied to Mueller-Hinton agar plates containing serial dilutions of antimicrobial agent, by using an automatic MIC-2000 multipin inoculator (Dynatech Laboratories, Inc., Alexandria, VA.) to yield 10^4 CFU per spot. The MICs were determined after 18 h of incubation at 35°C. The MIC was considered to be the lowest concentration that completely inhibited bacterial growth on agar plates, disregarding a single colony or a faint haze caused by the inoculum.

Time-kill studies

Time-kill measurements were performed by the method of the National Committee for Clinical Laboratory Standards M26-T (NCCLS, 1992). Test organisms incubated in Muller-Hinton broth for 18 h at 35°C were diluted with fresh broth to approximately 10^5 CFU/ml, and the diluted cultures were pre-incubated for 2 h. Each drug was added to the cultures at concentration of 1/4-, 1/2-, 1-, 2- and 4-times the MIC. Aliquots (0.1 ml) of the cultures were removed at 0, 2, 4, 6, and 24 h of incubation, and serial 10-fold dilutions were prepared in saline as needed. Drug carryover effects were reduced by 100-fold dilution of the sample with agar. The number of viable cells were determined on drug-free Muller-Hinton agar plate after 24 h of incubation. The lower limit of sensitivity of the method was 10 CFU/ml. The MBC was determined also by subculturing 100 µl of culture broth onto antibiotic free Muller-Hinton agar plate and antibiotic supplemented Muller-Hinton agar plate. And the MBC was defined as the lowest concentration which induced more than 99.9% reduction in CFU after 24 h of incubation at 35°C.

RESULTS

Time-kill curve studies

The MICs of LB20304 and ciprofloxacin for each strain are shown in the corresponding panels of Figures 2 to 4. The bactericidal activity of LB20304 for *S. aureus* 77 was compared with that of ciprofloxacin in Fig. 2. LB20304, at the concentration of 1× or 2× MIC, showed a rapid bactericidal activity against *S. aureus* 77. There was at least a 3-$\log_{10}$-unit decrease in CFU per milliliter within 2 h at two and four times the MICs of LB20304. The regrowth of *S. aureus* 77 was prevented completely by LB20304 at 1×, 2× and 4×MIC concentration, respectively, by 24 h, al-

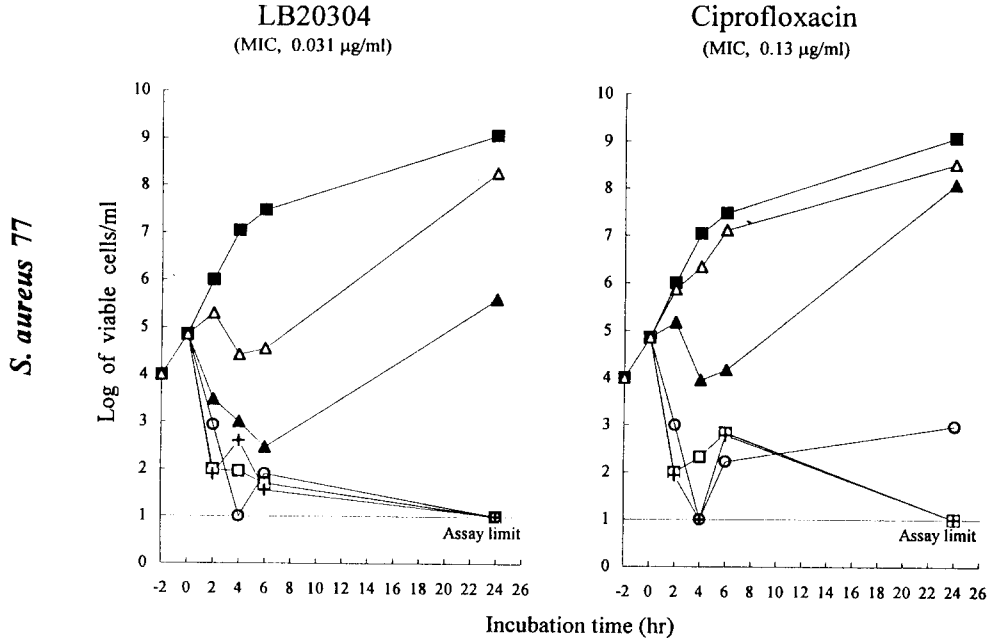

Fig. 2. Bactericidal activity of LB20304 against *S. aureus* 77. ■, untreated control; △, ¼× MIC; ▲, ½× MIC; ○, 1× MIC; +, 2× MIC; □, 4× MIC.

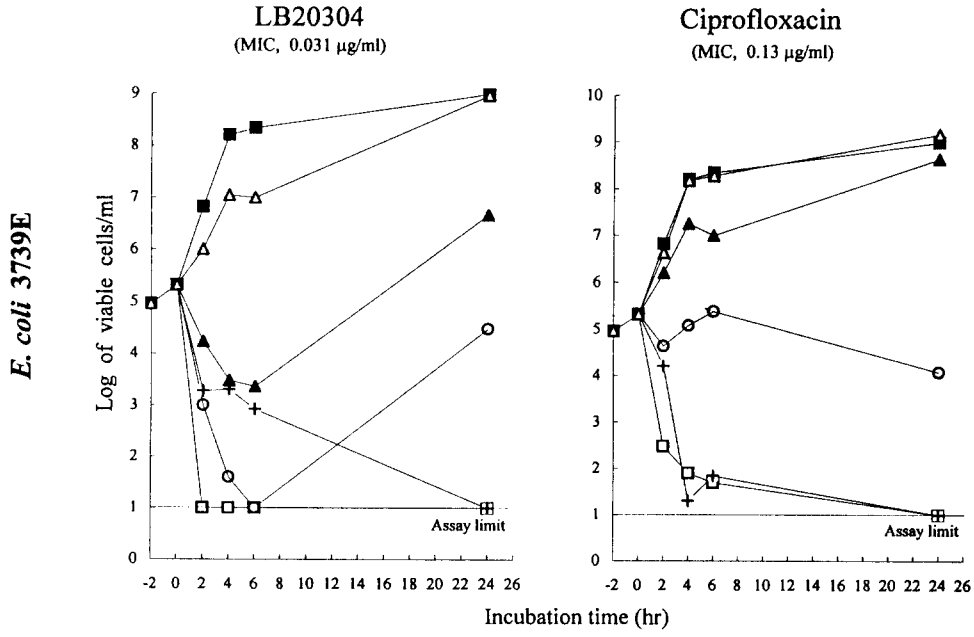

Fig. 3. Bactericidal activity of LB20304 against *E. coli* 3739E. ■, untreated control; △, ¼× MIC; ▲, ½× MIC; ○, 1× MIC; +, 2× MIC; □, 4× MIC.

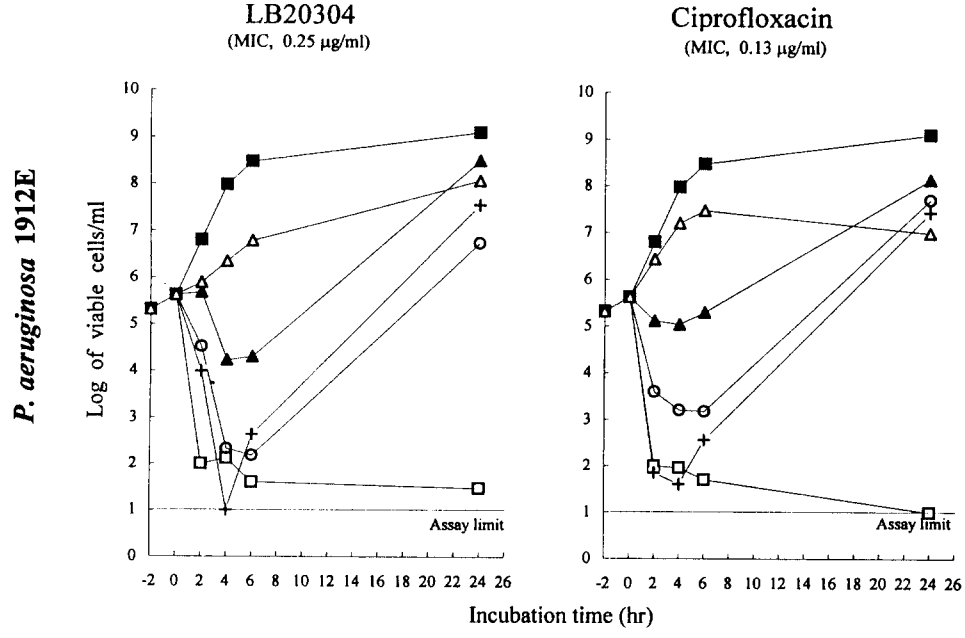

Fig. 4. Bactericidal activity of LB20304 against *P. aeruginosa* 1912E. ■, untreated control; △, ¼× MIC; ▲, ½× MIC; ○, 1× MIC; +, 2× MIC; □, 4× MIC.

though regrowth occured in the presence of ciprofloxacin of 1× MIC concentration. For *E. coli* 3739E, the bactericidal activity of LB20304 was also very potent (Fig. 3). There was no detectable viable cell within 2 h at four times the MIC of LB20304. On the other hand, there was only 3- or 4-$\log_{10}$-unit decrease in CFU per milliliter at four times the MIC of ciprofloxacin. *P. aeruginosa* 1912E was killed rapidly by the presence of LB20304 or ciprofloxacin as shown in Fig. 4. However, a persistent effect could only be demonstrated at four times the MICs of both quinolones. The regrowth of *P. aeruginosa* 1912E occured at two times MIC of LB20304 and ciprofloxacin, respectively. The MBCs of LB20304 in Muller-Hinton broth for all strains tested were identical to or at most two times as high as MICs.

DISCUSSION

LB20304 is a new fluoroquinolone antibiotic which has potent activity against gram-positive, gram-negative bacteria and anaerobes *in vitro* and *in vivo*. Also, it has improved pharmacokinetic profiles in animals compared with ciprofloxacin (Oh *et al*, 1995).

The *in vitro* MIC of LB20304 against *S. aureus* 77 and *E. coli* 3739E was 0.031 μg/ml and it was four-fold lower than that of ciprofloxacin. However, ciprofloxacin was two times more active than LB20304 against *P. aeruginosa* 1912E. The bactericidal effect of LB20304 against *S. aureus, E. coli* and *P. aeruginosa* was very active and no regrowth was detected at two and four times the MIC by 24 h of incubation except for *P. aeruginosa* 1912E. The regrowth of *P. aeruginosa* 1912E observed at two times MIC of LB 20304 or ciprofloxacin might be due to the selection of resistant subpopulations. Mutational frequency of *P. aeruginosa* 1912E to LB20304 and ciprofloxacin at a selecting concentration of four times MIC was 5.6×10^{-8} and 3.3×10^{-8}, respectively (Paek *et al*, 1996). It was much higher than the frequency of mutants resistant to LB20304 and ciprofloxacin observed in *S. aureus* 77 and *E. coli* 3739E.

It was reported that all quinolones have a paradoxical lethal effect on bacteria. There is an increase in bactericidal activity with an increase in drug concentration up to an optimum (the optimaum bactericidal concentration), after which higher concentrations are less bactericidal (Hooper *et al*, 1993). The optimum bactericidal concentration of new fluoroquinolones is usually 30- to 40-fold higher than the MIC of same agents. LB20304 showed also the dose dependent bactericidal activity against all test organisms up to the concentration of four times the MIC.

In conclusion, LB20304 inhibited the growth of bacteria tested at low concentrations (0.031 to 0.25 μg/ml) and induced rapid killing of such organisms at 1× or 2× MICs. Regrowth did not occur by 24 h at four times the MIC.

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