International Journal of Antimicrobial Agents 40 (2012) 539–543

Contents lists available at SciVerse ScienceDirect

International Journal of Antimicrobial Agents

journal homepage: http://www.elsevier.com/locate/ijantimicag

Short communication

Antibacterial activity of LCB01-0062, a novel oxazolidinone

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ARTICLE INFO

Article history:
Received 1 May 2012
Accepted 23 July 2012

Keywords:
LCB01-0062
Oxazolidinone
MIC
Time–kill
Systemic infection

ABSTRACT

LCB01-0062, a novel oxazolidinone, has potent antibacterial activity against clinical isolates of Gram-positive bacteria. The in vitro activity of LCB01-0062 was compared with that of linezolid, oxacillin, erythromycin, ciprofloxacin, vancomycin and quinupristin/dalfopristin. Among the tested agents, LCB01-0062 showed the most potent antibacterial activity against meticillin-resistant *Staphylococcus aureus*, meticillin-resistant coagulase-negative staphylococci and vancomycin-resistant enterococci. LCB01-0062 was 4–8-fold more active than linezolid, the first oxazolidinone drug, against Gram-positive bacteria. The time–kill curves of LCB01-0062 were analysed at concentrations of 0.5×, 1×, 2×, 4× and 8× the minimum inhibitory concentration against *S. aureus* strains. LCB01-0062 showed bacteriostatic activity during 24 h. LCB01-0062 was also more effective than linezolid against *S. aureus* in a systemic mouse model of infection.

1. Introduction

The emergence of multidrug-resistant (MDR) Gram-positive pathogens, such as meticillin-resistant *Staphylococcus aureus* (MRSA), meticillin-resistant coagulase-negative staphylococci (MR-CoNS), penicillin-resistant *Streptococcus pneumoniae* (PRSP) and vancomycin-resistant enterococci (VRE), has caused serious problems in the medical community. New effective antibacterial agents against pathogenic bacteria resistant to the current antibiotics are needed [1,2].

The oxazolidinones are totally synthetic antibiotics that are effective against Gram-positive bacteria, including MDR pathogens [3]. They bind to the 50S ribosomal subunit and inhibit formation of the initiation complex for protein synthesis [4,5]. Linezolid (Zyvox®) is the first member of the oxazolidinone class and was approved by the US Food and Drug Administration (FDA) in 2000 [6]. The success of linezolid and the occurrence of strains resistant to linezolid have inspired further efforts to develop new oxazolidinones with improved antibacterial activity and safety.

LCB01-0062 is a novel oxazolidinone synthesised by LegoChem Biosciences, Inc. (Daejeon, South Korea) (Fig. 1). In this study, the in vitro activity of LCB01-0062 was compared with that of six different antibacterial agents against 434 clinical isolates collected from several general hospitals in South Korea. Time–kill studies of LCB01-0062 against *S. aureus* Giorgio [meticillin-susceptible *S. aureus* (MSSA)] and *S. aureus* P197 (MRSA) were investigated. The in vivo efficacy of LCB01-0062 was also examined against systemic infection caused by *S. aureus* Giorgio in mice.

2. Materials and methods

2.1. Antimicrobial agents and bacterial strains

LCB01-0062 and linezolid were synthesised at LegoChem Biosciences, Inc. Quinupristin/dalfopristin (Synercid®) and vancomycin were obtained from CrystalGenomics (Seoul, South Korea). Ciprofloxacin was obtained from the R&D Centre, Dong Wha Pharmaceutical Co., Ltd. (Anyang, South Korea). Oxacillin and erythromycin were purchased from Sigma-Aldrich (St Louis, MO). Test organisms used in this study were originally isolated from human clinical specimens. They were obtained from several hospitals in Seoul during 2007–2010. MRSA P197 were selected by screening clinical isolates.

2.2. Antimicrobial susceptibility testing

Minimum inhibitory concentrations (MICs) were determined by the agar dilution method as described by the Clinical and Laboratory Standards Institute (CLSI) [7]. Mueller–Hinton agar (MHA) was used to test antibiotic susceptibility of aerobic and facultative organisms. *Streptococcus pneumoniae* and *Streptococcus pyogenes* were grown on MHA supplemented with 5% defibrinated sheep blood (Komed,

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http://dx.doi.org/10.1016/j.ijantimicag.2012.07.017

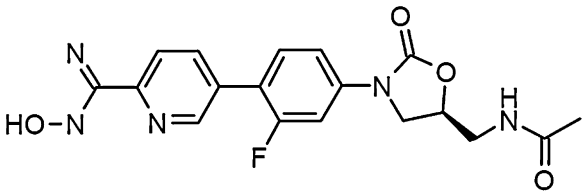

Fig. 1. Structure of LCB01-0062.

Sungnam, South Korea). MHA supplemented with 5% Fildes extracts (Oxoid, Basingstoke, UK) was used for *Haemophilus influenzae*. Test organisms were grown for 18 h and were diluted with the same fresh medium to a density of ca. 10^7 CFU/mL. Cultures were applied to agar plates containing serial dilutions of the antimicrobial agents using a multipin inoculator to yield 10^5 CFU/spot. Plates were incubated at 35 °C for 18 h and were examined for growth. The 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.

2.3. Time–kill studies

Time–kill analyses were performed according to the method of the CLSI [8]. Test organisms incubated in Mueller–Hinton broth (MHB) for 18 h at 35 °C were diluted with fresh MHB to ca. 10^5 CFU/mL and the diluted cultures were pre-incubated for 2 h. Each drug was added to the cultures at concentrations of 0.5×, 1×, 2×, 4× and 8× MIC. Samples (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. The number of viable cells was determined on drug-free MHA plates after 24 h of incubation. The compound was considered bactericidal at a concentration that reduced the original inoculum by 3 $\log_{10}$ CFU/mL (99.9%) at each of the time periods, or was considered bacteriostatic if the inoculum was reduced by 0–3 $\log_{10}$ CFU/mL.

2.4. Systemic infection mouse model

Four-week-old male ICR mice (Dae Han Bio Link Co., Ltd., Eumseong, South Korea) weighing 18–22 g were used for a systemic infection model. Mice were housed in animal rooms maintained at 23 ± 2 °C with 55 ± 20% relative humidity. *Staphylococcus aureus* Giorgio cultured on a MHA plate at 35 °C for 18 h was used as the challenge organism. For inoculation, the test bacterial strain was suspended in 0.9% NaCl containing 5% mucin. Groups of five mice were challenged intraperitoneally with 0.5 mL of the bacterial suspension, corresponding to an inoculum range from 10 to 100 times the minimum lethal dose of bacteria. Four dose levels were used for each antibiotic, depending on the in vitro antimicrobial activity of the compound. LCB01-0062 and linezolid were administered orally to mice twice at 1 h and 4 h post infection. Mortality was recorded over 7 days and the median effective dose needed to protect 50% of the mice (ED_{50}) was calculated by the Probit method [9]. The challenge inoculum was sufficient to kill 100% of the untreated control mice, which died within 48 h post infection.

3. Results

The comparative in vitro antibacterial activity of LCB01-0062 is shown in Table 1. LCB01-0062 had potent activity against Gram-positive bacteria. The MIC_{90} values (MIC at which 90% of the strains are inhibited) of LCB01-0062 against MSSA and MRSA were 0.25 mg/L and 0.5 mg/L, respectively. LCB01-0062 showed the most potent activity among the antibiotics tested. It was 4–8-fold more

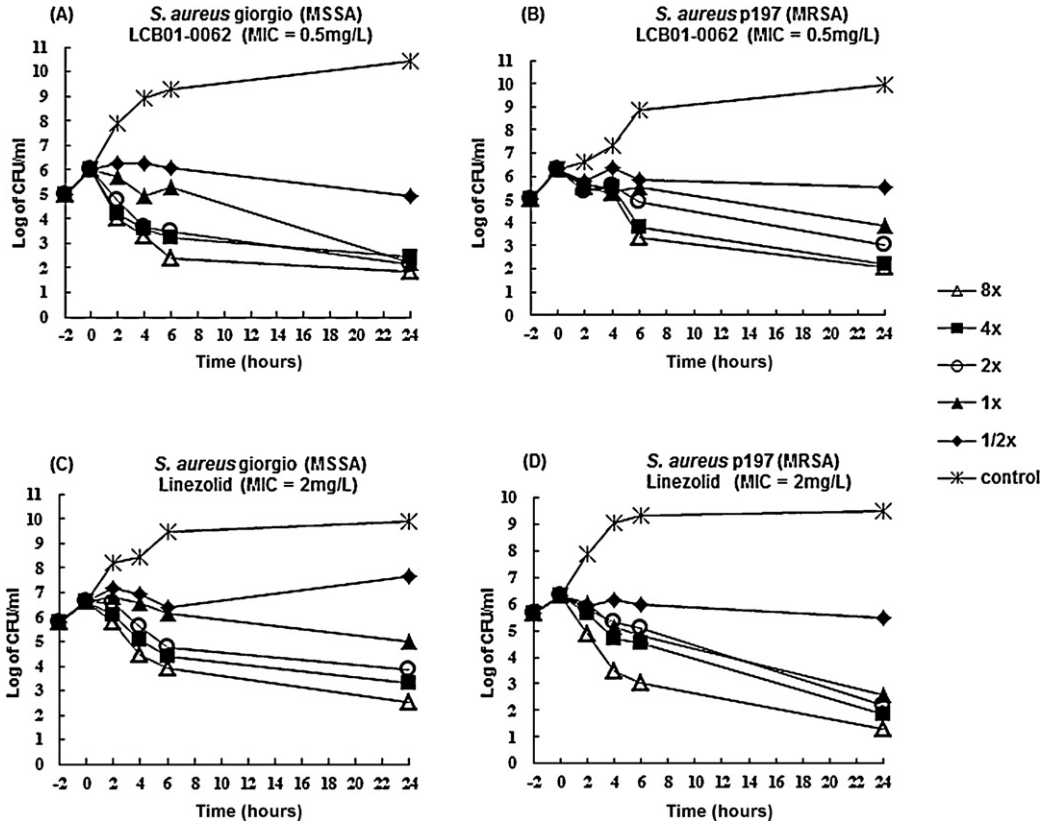

Fig. 2. Time–kill curves against (A and C) *Staphylococcus aureus* giorgio (MSSA) and (B and D) *S. aureus* P197 (MRSA) exposed to LCB01-0062 (A and B) and linezolid (C and D). MSSA, meticillin-susceptible *S. aureus*; MRSA, meticillin-resistant *S. aureus*; MIC, minimum inhibitory concentration.