J Infect Chemother 25 (2019) 311–313

Contents lists available at ScienceDirect

Journal of Infection and Chemotherapy

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

Note

Antimicrobial activity of solithromycin and levofloxacin against a murine pneumonia mixed-infection model caused by *Streptococcus pneumoniae* and anaerobic bacteria*

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ARTICLE INFO

Article history:
Received 19 March 2018
Received in revised form
2 August 2018
Accepted 4 September 2018
Available online 2 October 2018

Keywords:
Solithromycin
In vivo murine pneumonia mixed-infection model
Streptococcus pneumoniae
Anaerobic bacteria

ABSTRACT

Introduction: Solithromycin is a novel fluoroketolide developed to treat pneumonia. But, few studies evaluating its antimicrobial activity against *S. pneumoniae* in a mixed-infection model with anaerobes are available, while community-acquired pneumonia can involve mixed-infection of *Streptococcus pneumoniae* and anaerobic bacteria. This study evaluated the antimicrobial activity of solithromycin against macrolide-resistant *S. pneumoniae* and anaerobic bacteria with a murine pneumonia mixed-infection model.

Material and methods: We evaluated antimicrobial activity of solithromycin (10 and 20 mg/kg) and levofloxacin (126 mg/kg) against *S. pneumoniae* with a three-point mutation in penicillin-binding protein and an *ermB* gene, and *Parvimonas micra*. Antimicrobial efficacy was calculated for each isolate as the change in bacterial count ($\Delta\log_{10}$ CFU/mL) obtained in the treated mice after 24 h compared with the count in the starting control animals.

Results: The solithromycin and levofloxacin minimum inhibitory concentrations (MICs) for *S. pneumoniae* were 0.03 and 0.5 μg/mL, respectively. The solithromycin and levofloxacin MICs for *P. micra* were 0.015 and 0.12 μg/mL, respectively. In a mixed-infection model, solithromycin showed significantly higher antimicrobial activity against *S. pneumoniae* than levofloxacin (solithromycin 20 mg/kg; -2.87 ± 1.33 $\log_{10}$ CFU/mL vs. levofloxacin; -1.35 ± 0.37 $\log_{10}$ CFU/mL, $p = 0.0397$). Similarly, solithromycin showed significantly higher antimicrobial activity against *P. micra* than levofloxacin (solithromycin 20 mg/kg; -2.78 ± 0.98 $\log_{10}$ CFU/mL vs. levofloxacin; -1.57 ± 0.47 $\log_{10}$ CFU/mL, $p = 0.0400$).

Discussion: Solithromycin showed higher antimicrobial activities against macrolide-resistant *S. pneumoniae* and *P. micra* than levofloxacin, even though they were coexisted in murine lung tissue. Our results suggest that solithromycin could be effective for pneumonia patients due to *S. pneumoniae* to reduce bacterial density in lung tissue.

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Solithromycin is a novel macrolide and the first fluoroketolide. It has been developed as an oral and intravenous antimicrobial related to 14-member-ring macrolides. The highlights of solithromycin include a wider spectrum of *in vitro* activity against respiratory tract pathogens associated with community-acquired pneumonia (CAP), including those resistant to other macrolides [1,2]. The overall antimicrobial potency of solithromycin against Gram-positive aerobes is greater than that of other macrolides [3]. Anaerobic bacteria were found mostly as part of a mixed infection in pneumonia patients [4]. Tokuyasu et al. reported that the overall detection rate of bacteria was 87.1% (monomicrobial, 32.3%; polymicrobial, 54.8%) and anaerobic bacteria accounted for 19.8% [5] in elderly aspiration pneumonia patients. Hence, especially for the antimicrobials used for the treatment of pneumonia in clinical situation, the evaluation of antimicrobial activity in polymicrobial

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https://doi.org/10.1016/j.jiac.2018.09.003
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infections would be important to consider. However, few studies evaluating antimicrobial activities of solithromycin against *S. pneumoniae* in a mixed-infection model with anaerobes are available. Therefore, in this *in vivo* study, the primary object was to reveal antimicrobial activity of solithromycin against macrolide-resistant *S. pneumoniae* and an anaerobe (*Parvimonas micra*) in lung tissues with a murine pneumonia mixed-infection model.

Solithromycin (Toyama Chemical Co., Ltd., Tokyo, Japan) and levofloxacin (Sigma-Aldrich, Tokyo, Japan) were reconstituted and diluted with 0.05% acetic acid or normal saline, respectively, to achieve the desired concentration immediately prior to each experiment. *S. pneumoniae* isolate was provided by Toyama Chemical Co., Ltd. (Tokyo, Japan). The isolate represented three-point mutation of penicillin-binding protein (genotypic penicillin-resistant *S. pneumoniae*; gPRSP) as the genetic phenotype and had an *ermB* gene [6]. The *P. micra* isolate used in this *in vivo* study was a clinical isolate from Aichi Medical University Hospital (Aichi, Japan). These isolates were stored at −70 °C in skim milk. *S. pneumoniae* was subcultured twice onto Trypticase Soy Agar with 5% sheep blood (Becton, Dickinson & Co.; Sparks, MD), and grown for 24 h at 37 °C under 5% CO_2. *P. micra* was subcultured twice onto Burusera HK nutrient agar (Kyokuto, Tokyo, Japan), and placed into an incubator at 37 °C under <1% O_2 for 72 h. The minimum inhibitory concentration (MIC) values of solithromycin and levofloxacin were determined by E-test according to the manufacturer's specifications (BioMerieux North America, Durham, NC) or microdilution methodology, as outlined by the Clinical and Laboratory Standards Institute (CLSI) [7]. The MIC studies were conducted a minimum of three times, and the geometric MIC was reported. The MICs of *S. pneumoniae* and *P. micra* were read at 20–24 h and 70–74 h after incubation at 37 °C. Pathogen-free female ICR Swiss mice weighted approximately 22 g were purchased from Charles River Laboratories Japan, Inc. (Yokohama, Japan) and were utilized throughout this study. The study was reviewed and approved by the Aichi Medical University Hospital Institutional Animal Care and Use Committee. The murine pneumonia model as previously reported was adopted in this study [8]. Mice were rendered neutropenic with 150 and 100 mg/kg of body weight intraperitoneal injections of cyclophosphamide (Sigma-Aldrich), administered 4 and 1 days prior to inoculation, respectively [9]. Mice were anesthetized with butorphanol (Meiji Seika Pharma Co., Ltd., Tokyo, Japan), medetomidine (ZENOAQ, Fukushima, Japan) and midazolam (Astellas Pharma Inc., Tokyo, Japan). Bacterial suspensions were prepared with the second subculture of each pathogens. They were incubated for 24 h and 72 h and diluted in thioglycollate medium (Sigma-Aldrich, Tokyo, Japan) to achieve an inoculum of 10^8 CFU/mL. Two hours prior to the initiation of antimicrobial therapy, mice were inoculated orally with 60 μL of a solution containing approximately 10^8 CFU/mL of the each test isolates, respectively. While the mice were anesthetized, the bacterial suspension was orally instilled and nares were blocked. The mouse aspirated suspension into the lungs while being held vertically for 60 s. Mice were randomized into vehicle or antimicrobial treatment groups consisting of 8 infected mice each. At specified time points, the animals were sacrificed by CO_2 asphyxiation. After the mice were sacrificed, the lungs were immediately removed and homogenized in thioglycollate medium. After we conducted serial dilutions, fixed amount of these solutions were put on the plates. Then, we counted the number of viable bacteriafor CFU/mL determination. Antimicrobialactivity was calculated for each isolate as the change in bacterial numbers ($\Delta\log_{10}$ CFU/mL) obtained in the treated mice after 24 h compared with the numbers in the starting control animals (0 h). To control the impact of the host and the resulting variability in 24 h control bacterial densities between isolates, animals with bacterial densities beyond 1.5 times the interquartile range were identified as outliers and were excluded from the group averages [10]. Two hours after inoculation, mice were administered each antimicrobials with subcutaneous dose (0.2 mL). The following doses of solithromycin were evaluated: 10 and 20 mg/kg as subcutaneous injections. And the following doses of levofloxacin were evaluated: 126 mg/kg as subcutaneous injections [11]. Data were analyzed with JMP version 10.0 (SAS, Tokyo, Japan). To compare antimicrobial efficacy between each drugs, the statistical analyses were performed using one-way ANOVA. A difference was considered statistically significant at a p value of <0.05.

The solithromycin and levofloxacin MICs for *S. pneumoniae* were 0.03 and 0.5 μg/mL, respectively. The solithromycin and levofloxacin MICs for *P. micra* were 0.015 and 0.12 μg/mL, respectively. The results of the efficacy studies of *S. pneumoniae* and *P. micra* in the pneumonia mixed-infection model are shown in Fig. 1 (*S. pneumoniae*: Fig. 1A, *P. micra*: Fig. 1B). The bacterial densities of *S. pneumoniae* in lungs ranged from 7.00 to 7.95 $\log_{10}$ CFU/mLat 0 h in control. And, the bacterial densities of control mice at 24 h ranged from 7.48 to 8.75 $\log_{10}$ CFU/mL. The bacterial densities of *P. micra* recovered from the lungs of infected animals serving as 0 h controls ranged from 6.70 to 7.70 $\log_{10}$ CFU/mL and, the bacterial densities of control mice at 24 h ranged from 6.00 to 8.41 $\log_{10}$ CFU/mL. In the mixed-infection model, solithromycin showed significantly higher antimicrobial activity against *S. pneumoniae* than that of levofloxacin (solithromycin 20 mg/kg; -2.87 ± 1.33 $\log_{10}$ CFU/mL vs. levofloxacin; -1.35 ± 0.37 $\log_{10}$ CFU/mL, $p = 0.0397$: Fig. 1A). Similarly, solithromycin showed significantly higher antimicrobial

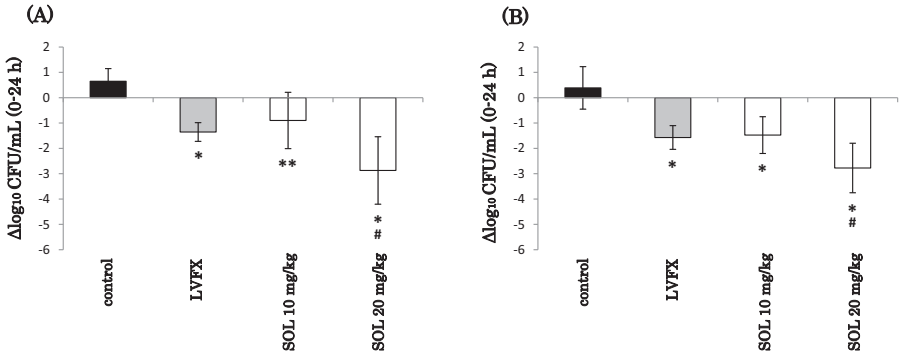

Fig. 1. Impact of pharmacodynamics regression of the *in vivo* solithromycin and levofloxacin studies against *S. pneumoniae* (A) and *P. micra* (B). Change in $\log_{10}$ CFU/mL for human simulated regimens of solithromycin and levofloxacin against *S. pneumoniae* and *P. micra*in the murine pneumonia mixed-infection model. Each value represents the mean ± SD of 8 mice. SOL; solithromycin, LVFX; levofloxacin. *: $p < 0.01$ (vs control), **: $p < 0.05$ (vs control), #: $p < 0.05$ (vs LVFX).

activity against *P. micra* than levofloxacin (solithromycin 20 mg/kg; −2.78 ± 0.98 $\log_{10}$ CFU/mL vs. levofloxacin; −1.57 ± 0.47 $\log_{10}$ CFU/mL, $p = 0.0400$: Fig. 1B).

This is the first study to evaluate antimicrobial activities of solithromycin in lung tissues with the murine pneumonia mixed-infection model. In this study, macrolide resistant PRSP was used. Because, previous clinical surveillance study revealed that the number of PRSP and macroride-resistant *S. pneumoniae* isolates are increasing (more than 50%) [12]. And, solithromycin MIC_{90} for *S. pneumoniae* isolates was 0.125 μg/mL [range: 0.03–0.125 μg/mL] [13]. And, our study revealed that the median MIC of solithromycin against 23 clinical isolated *P. micra* was 0.015 μg/mL (95.6%) [0.015–0.03 μg/mL].Consequently, solithromycin showed the significantly greater antimicrobial activity, compared with levofloxacin (Fig. 1). Generally, macrolides are bacteriostatic or slowly bactericidal antimicrobials. But, solithromycin has great pulmonary penetration [14]. Hence, the concentrations in lung tissue could be well above the MICs for likely respiratory pathogens, including those resistant to currently available macrolides. And, the characteristic might lead higher antimicrobial activities of solithromycin, compared to levofloxacin in this *in vivo* study. Limitations of this study include the utilization of only one *S. pneumoniae* and *P. micra* isolate for our testing, respectively. Another limitation was that we did not perform a pharmacokinetics study. Therefore, we referred to previous reports [11]. It will be important in future studies to perform pharmacokinetcs-pharmacodynamics analyses with free-drug exposures of solithromycin in epithelial lining fluid and its antimicrobial activities. And, some evaluations of solithromycin antimicrobial activities with various bacterial phenotypes, such as antimicrobial susceptibility and pathogenicity, will also need in future studies to show its effectiveness in clinical situations.

In conclusion, solithromycin showed significantly higher antimicrobial activities against macrolide-resistant PRSP and *P. micra,* even though they were coexisted in murine lung tissue. Our results suggest that solithromycin could be effective for pneumonia patients due to *S. pneumoniae* to reduce bacterial density in lung tissue.

Funding

None.

Conflict of interest

No conflict of interest is declared.

Acknowledgments

All authors thank all clinicians and pharmacists that have assisted with the provision of data for this project.

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