J Infect Chemother 23 (2017) 131–135

Contents lists available at ScienceDirect

Journal of Infection and Chemotherapy

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

Original Article

In vivo antianaerobe activity of DS-8587, a new fluoroquinolone, against *Fusobacterium necrophorum* in a mouse model

Kentaro Nagaoka [a, b, d], Katsunori Yanagihara [a, b, *], Yoshitomo Morinaga [a, b], Yuichi Kurosaka [e], Kazuki Hoshino [e], Shigeru Kohno [b, c]

[a] Department of Laboratory Medicine, Nagasaki University Graduate School of Biomedical Sciences, Nagasaki, Japan
[b] Second Department of Internal Medicine, Nagasaki University Graduate School of Biomedical Sciences, Nagasaki, Japan
[c] Global COE Program, Nagasaki University, Nagasaki, Japan
[d] First Department of Internal Medicine, Hokkaido University Hospital, Hokkaido, Japan
[e] Biological Research Laboratories, Daiichi Sankyo Co., Ltd., Tokyo, Japan

ARTICLE INFO

Article history:
Received 24 July 2016
Received in revised form
11 September 2016
Accepted 20 September 2016
Available online 18 January 2017

Keywords:
Anaerobe
Fusobacterium necrophorum
Fluoroquinolone
Sitafloxacin

ABSTRACT

DS-8587 is a novel parenteral fluoroquinolone, which has an activity equivalent to sitafloxacin against various pathogens including anaerobes. We examined the *in vivo* anti-anaerobic activity of DS-8587, and compared it with that of levofloxacin (LVFX), using a murine model of *Fusobacterium necrophorum*-induced liver abscess developed *via* blood borne infection. Mice with liver abscess infection caused by *F. necrophorum* were treated with saline (control), DS-8587 (0.8, 4, and 20 mg/kg twice daily), or LVFX (20 and 100 mg/kg) for a day. After treatment, the number of viable bacteria in liver was analyzed. We also analyzed the pharmacokinetics of these agents in plasma and the liver after initial treatment. The MICs of DS-8587 and LVFX were 0.015 and 1 mg/mL, respectively. DS-8587 eradicated the viable bacteria in the liver even at doses as low as 4 mg/kg. In contrast, the liver bacteria were not eradicated in any of the LVFX-treated mice even at a dose of 100 mg/kg ($P < 0.05$ compared with DS-8587, 4 or 20 mg/kg). The pharmacokinetic parameter AUC/MIC ratios for DS-8587 (4 mg/kg) and LVFX (100 mg/kg) were 96.7 and 60.8 in plasma and 600 and 145.6 in the liver, respectively. The AUC/MIC ratio showed the best correlation with efficacy of DS-8587. DS-8587 significantly reduced the number of viable bacteria in a murine model of *F. necrophorum*-induced liver abscess compared to LVFX. Our study demonstrated that the anti-anaerobic activity of quinolones *in vivo* was different from the MICs *in vitro*.

1. Introduction

Anaerobic bacterial infections are known to be associated with significant mortality in severe bacteremia or untreated cases [1,2]. Anaerobes are often present in polymicrobial infections and form abscesses in the human body. The low pH or impairment of the action of bactericidal compounds inside the abscess environment, can lead to anaerobic infections that are resistant to antibiotics with low *in vitro* anti-anaerobic activity, such as aminoglycosides, 1st and 2nd generation cephalosporins, except cephamycins and oxacephems, 1st generation fluoroquinolones, macrolides, and tetracyclines [3]. In addition, anaerobic infections associated with abscesses are occasionally exacerbated even with the administration of antibiotics with anti-anaerobic activity. These infections often require surgical debridement, which is a cornerstone of the treatment [4]. Therefore, the evaluation of anaerobic spectrum of antibiotics in clinical setting is controversial.

DS-8587 (Daiichi Sankyo Co., Ltd) is a novel parenteral fluoroquinolone, which has an activity similar to that of sitafloxacin (STFX) against various pathogens including anaerobes [5]. STFX is reported to have potent bactericidal activity against anaerobes *in vitro* [6–8]. Although the presence of anti-anaerobic activity was considered an advantage of newly developed antibiotics [9–11], few *in vivo* studies have been reported to date, and the *in vivo* anti-anaerobic activities of STFX and DS-8587 are still not elucidated.

In this study, we examined the efficacy of DS-8587 in a murine model of *Fusobacterium necrophorum*-induced liver abscess, which

* Corresponding author. Department of Laboratory Medicine, Nagasaki University Graduate School of Biomedical Sciences, 1-7-1 Sakamoto, Nagasaki 852-8501, Japan. Fax: +81 95 819 7257.
E-mail address: k-yanagi@net.nagasaki-u.ac.jp (K. Yanagihara).

http://dx.doi.org/10.1016/j.jiac.2016.09.007
1341-321X/

is a pathogenic obligate anaerobe. In order to evaluate the anti-anaerobic activity of antibiotics, we recently developed a mouse model of liver abscess induced by *F. necrophorum* using a blood borne infection [12]. In this model, the abscess formation by *F. necrophorum* occurs uniformly, and the technical process of the infection procedure is convenient, which makes the model more suitable for examining the anti-anaerobic effect of antibiotics. Using this animal model, we compared the *in vivo* anti-anaerobic activities of DS-8587 and levofloxacin (LVFX). We also analyzed the pharmacokinetics of these agents in plasma and the liver.

2. Material and methods

2.1. Antimicrobial agents

DS-8587, LVFX, and STFX were synthesized at the Daiichi Sankyo Co., Ltd (Tokyo, Japan). Moxifloxacin (MFLX) and azithromycin were purchased from the LKT Laboratories, Inc. (MN, USA). Clarithromycin, metronidazole, and clindamycin were purchased from Sigma–Aldrich Japan (Tokyo, Japan). Each drug was used as the anhydrous free base equivalent.

2.2. Mice

Eight-week-old male BALB/c specific-pathogen-free mice were obtained from the Charles River Laboratories Japan, Inc., (Kanagawa, Japan). All mouse experiments were performed according to the guidelines of the Laboratory Animal Center for Biomedical Research, Nagasaki University School of Medicine and the Institutional Animal Care and Use Committee of Daiichi Sankyo Co., Ltd. The Animal Care Ethics Review Committees of our institutions approved the experimental protocol.

2.3. Organism

A clinical isolate of *F. necrophorum* subp. *funduliform* (strain FNU-89), maintained as a stock culture in the Department of Laboratory Medicine, Nagasaki University Hospital, Nagasaki, Japan, was used in this study. The minimum inhibitory concentrations (MICs) of the antibiotics against the organism were determined by the broth microdilution method, according to the reference procedure recommended by the Clinical and Laboratory Standards Institute (CLSI) guidelines.

2.4. Intravenous infection procedure

We intravenously infected the mice with *F. necrophorum* as described previously [12]. The *F. necrophorum* strain was cultured in PV (paromomycin, vancomycin) *Brucella* HK Agar (Kyokuto Pharmaceutical Industrial Co., Tokyo, Japan) for 48 h under anaerobic conditions, and then scraped and suspended in modified Gifu anaerobic medium (GAM) broth (Nissui Pharmaceutical Industrial Co., Tokyo, Japan) and cultured in an anaerobic chamber for 18 h. The bacteria were harvested by centrifugation (3000 rpm, 10 min), and resuspended in normal saline. The final density of the bacterial suspension prepared was approximately 10^7–10^8 colony-forming units (CFU)/mL, as determined by the optical density method. The infection was induced by a caudal vein injection of 0.2 mL of a bacterial suspension containing about 8×10^8 CFU/mL (1.6×10^8 CFU/mouse). The nutritional status study involved observing the mice and assessing their body weight for 14 days.

2.5. Bacteriological examination

Each group of animals was euthanized at a specific time point by cervical dislocation. After exsanguination, the livers were dissected and removed under aseptic conditions. The organs used for bacteriological analyses were homogenized with a Polytron homogenizer, and quantitatively inoculated into PV *Brucella* HK Agar by serial dilution. Since the relatively high amount of viable bacteria was gained by this method, all these procedures were performed under aerobic condition.

2.6. Drug treatment

The effects of treatment with DS-8587 and LVFX were evaluated in the liver abscess model. One day after the infection, DS-8587 (0.8, 4, and 20 mg/kg) and LVFX (20 and 100 mg/kg) were injected intraperitoneally twice daily. Treatment was administered on three consecutive days for the body weight observation and 1 day for the CFU assay. The placebo-treated mice received sterile physiological saline at the same volume as the saline used to dissolve DS-8587 and LVFX. The change in body weight and the liver bacterial counts were analyzed for each group. Groups of eight mice each were used for the determination of body weight change, and the CFU assay groups consisted of four to five mice.

2.7. Pharmacokinetics studies

One day after the infection, DS-8587 and LVFX were injected intraperitoneally once a day. The blood and liver samples were collected from three mice each, at various time intervals (0.25, 0.5, 1, 3, 6, 12, and 24 h) after drug administration. The drug concentrations in the plasma and supernatant of the liver homogenates were measured using a Liquid chromatography-tandem mass spectrometry (LC/MS/MS) system. A Waters Acquity UPLC system (Waters Co., MA, USA) and an AB SCIEX API 4000 triple quadrupole mass spectrometer (AB SCIEX Co., MA, USA) were used for all analyses. For preparation of the quantification, the sample was added to acetonitrile/methanol (75/25, v/v) containing phenacetin (1 ng/mL) as internal standard, and then filtrated (0.45 μm, EMD Millipore, MA, USA). Gradient elution conditions were used on a Unison UK-C18 column (2.0 mm × 50 mm, 3 μm, Imtakt Co. Kyoto, Japan) at 50 °C. The mobile phase A was acetonitrile-water (5:95, v/v) containing 5 mM ammonium acetate and 0.2% formic acid, and the mobile phase B was acetonitrile-water (95:5, v/v) containing 5 mM ammonium acetate and 0.2% formic acid. A 5 μL injection volume was loaded onto the column at 0.8 mL/min. The detection was performed on an API 4000 tandem mass spectrometer coupled with heated electrospray ionization source in the positive mode. Quantitation of drug level in sample was conducted using the transitions *m/z* 422 → 245 for DS-8587, 362 → 261 for levofloxacin, and 180 → 110 for phenacetin. The analytical data were processed using Analyst 1.5.2 software in the API 4000. The highest mean value of the concentration of each group and the time required to achieve it were defined as the C_{max} and T_{max}, respectively. The area under the concentration–time curve from 0 to 24 h (AUC_{0-24h}) was calculated based on the mean value of the drug concentrations using the trapezoidal method.

2.8. Pharmacokinetics (PK)/pharmacodynamics (PD)

The intraperitoneal injection of DS-8587 was administered one day after the infection. Groups of three mice were treated for 24 h with nine different dosing regimens, and four-fold increasing total doses divided into 1, 2, or 4 doses. The total doses of DS-8587 administered were 0.5, 2, and 8 mg kg^{-1} 24 h^{-1}. We also