47th ICAAC,
Chicago 2007

F1-1652

Friulimicin B, a Cyclic Lipopeptide, Exhibits Potent Efficacy in a Murine Pneumococcal Pneumonia Model

R. ENDERMANN[1], A. VENTE[2], H. LABISCHINSKI[2]

[1]Bayer HealthCare AG, Contract Res. for Combinature Biopharm AG, Wuppertal, Germany,[2]Combinature Biopharm AG, Berlin, Germany

Contact information:
Dr. Reiner Endermann
Bayer HealthCare AG
Wuppertal
Germany
Phone +49 (0) 202-368480
Fax +49 (0) 202-365395
E-mail:rainer.endermann@bayerhealthcare.com

Revised Abstract

Background: With the emergence of resistance to currently available antibiotics, the development of novel antibiotics has become of major importance. Friulimicin B (FRI), a cyclic lipopeptide, is intended for the treatment of severe infections caused by Gram positive pathogens. We studied the efficacy of FRI compared with ceftriaxone (CRO), daptomycin (DAP) and linezolid (LIN) in an intranasal lung infection caused by *Streptococcus pneumoniae* L3TV.

Methods: A pulmonary infection model was used (JID, 2005:191, 2149 - 2152). Isoflurane anaesthetised mice were inoculated intranasally with 1 x 10^6 *S. pneumoniae* L3TV/mouse. Mice (n = 5) were assigned to treatment with either antibiotics or vehicle. Treatment started at 1 h post infection (p.i.) with a further dosing at 4 h p.i.. Bacterial counts in lungs from infected mice were determined at 24 h p.i.. Lungs were removed aseptically, weighed, homogenised and plated. CFUs were counted after overnight incubation at 37°C under 5% CO_2.

Results: The therapeutic efficacy in the murine pneumococcal pneumonia was measured by reduction of the CFUs in the lung. With low doses of FRI (1.25 or 5 mg/kg), CFUs in the lung were lower than with DAP or LIN therapy. In the presence of a formulation agent, the efficacy of FRI was slightly higher. DAP and LIN were less active and CRO slightly more active.

Conclusions: FRI was highly efficacious in reducing the viable counts of *S. pneumoniae* L3TV in a murine pulmonary infection model and was more effective than DAP or LIN.

Introduction

Friulimicin B (FRI) is a novel cyclic lipopeptide antibacterial agent with potent activity against *S. pneumoniae* including multidrug-resistant strains. The compound also has good activity against a variety of other Gram-positive bacteria[1-4]. It is structurally similar to the lipodepsipeptide antibiotic daptomycin but has a distinct mode of action [5-6]. No cross resistance is shown with daptomycin. Its chemical structure is shown in Figure 1.

Streptococcus pneumoniae is the most frequently isolated pathogen in community acquired pneumonia, and is a significant cause of morbidity and mortality in humans.

In the present work, we report the *in vivo* activity of FRI in comparison with ceftriaxone, daptomycin and linezolid in an *in vivo* mouse model of acute pneumonia caused by *Streptococcus pneumoniae*.

Methods

Test compounds:
The test compounds were obtained from the following sources: ceftriaxone (Hexal AG, Germany), daptomycin (Novartis Pharma GmbH, Germany), linezolid (Pharmacia GmbH, Germany), FRI (Combinature Biopharm AG, Germany).

Bacterial strain:
The pneumococcal strain L3 TV (serotype 3), a clinical isolate, was taken from the culture collection of Bayer HealthCare AG, Germany.

Susceptibility testing:
The MICs of FRI and DAP were determined by agar dilution method. Mueller-Hinton agar substituted with 184 mg/l $CaCl_2*H_2O$ was used. Agar plates were inoculated with bacterial spots (Denley multipoint inoculator) containing ~1-5 x 10^4 colony forming units (CFUs) each. Incubation was for 16-20 hrs. at 37°C under 5% CO_2. The lowest antibiotic concentration yielding no growth was read as the MIC. The MICs of the other test compounds were determined by micro dilution method using CLSI-guidelines[7].

Animals:
For all experiments described, female CFW-1 mice (18-20 g body weight) were used (Harlan-Winckelmann, Germany). The animals were kept under conventional housing conditions.

Pneumococcal pneumonia:
Isoflurane-anaesthetized mice were inoculated intranasally (i.n.) with 20 µl of physiological saline producing an infective dose of I x 10^6 CFU/mouse of *S. pneumoniae* strain L3TV i.n. The animals were treated with FRI, DAP, LIN or CRO intravenously, 1 and 4 h post infection (p.i.). All four drugs were adminstered at doses of 1.25, 5 and 20 mg/kg to groups of five mice.

The bacterial counts in the lung were determined at 24 h p.i. For determination of the viable bacterial load in the lungs, the lungs were removed aseptically and homogenised with an Ultra-Turrax (IKA-Werk, Germany) in sterile saline. Diluted samples were spread on blood agar plates and the resultant CFUs were counted after overnight incubation at 37°C in the presence of 5% CO_2.

Results and Discussion

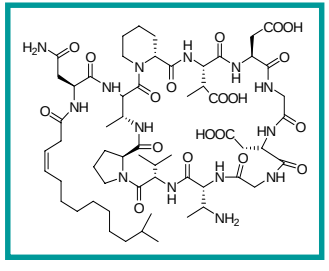

Fig. 1 Friulimicin B

Results and Discussion

The susceptibility of the infecting strain *S. pneumoniae* is detailed in Table 1. It was highly susceptible to ceftriaxone (MIC 0.03 mg/L). DAP was slightly less active than FRI and both were markedly more active than linezolid.

Compound	MIC [mg/L]
Friulimicin B	≤ 0.125
Daptomycin	0.25
Linezolid	2
Ceftriaxone	0.03
Ampicillin	0.25
Erythromycin	0.125

Table 1 Susceptibility of *S. pneumoniae* L3TV against test compounds

The therapeutic efficacy in the mouse model of pneumococcal pneumonia was measured by reduction of the CFUs in the lung at 24 h p.i.. The mean values (and standard deviation) for each group are illustrated in the histogram (Figure 2). Individual counts comparing the FRI and DAP treatment are illustrated in Figure 3, with a line indicating the mean value.

For all dosage groups tested FRI was more active than either DAP or LIN. CFUs in the lung of the FRI treated mice were at least 1 log-unit lower than with DAP or LIN therapy at all doses.

Overall cetriaxone was slightly more active than FRI, reflecting its good activity *in vitro*.

In spite of having activity *in vitro* that was close to that of FRI, the activity of DAP *in vivo* was markedly inferiour.

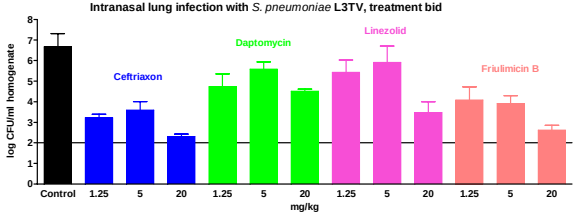

Fig. 2 Efficacy in the mouse model of *S. pneumoniae* pneumonia. The viable bacterial load in the lungs of antibiotic-treated mice compared with untreated control animals (mean values - n = 5 mice)

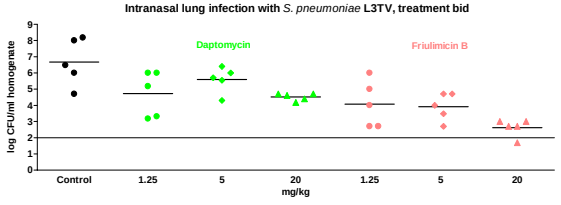

Fig. 3 Comparative efficacy of FRI and DAP in mouse model of pneumococcal pneumonia. Viable bacterial loads in the lungs of single mice compared with control animals

Conclusions

- Treatment with friulimicin B is highly effective in an animal model of pneumococcal pneumonia
- Friulimicin B demonstrated superior efficacy to daptomycin or linezolid as measured by a reduction in CFUs in the lungs
- Friulimicin B appears to be a promising new antimicrobial agent for the treatment of respiratory tract infections caused by Gram - positive organisms

Literature

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