47th ICAAC,
Chicago 2007

F1-1651

Friulimicin B Shows Good Efficacy in a Staphylococcal Murine Abscess Infection

H. LABISCHINSKI[1], M. GLENSCHEK-SIEBERTH[2], R. ENDERMANN[2]

[1]Combinature Biopharm AG, Berlin, Germany, [2]Bayer HealthCare AG, Contract Res. for Combinature Biopharm AG, Wuppertal, 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: Friulimicin B (FRI) is a novel lipopeptide antibiotic and is intended for the treatment of severe infections caused by Gram-positive pathogens. The compound shows structural similarities with daptomycin (DAP). The present study compared FRI with DAP and vancomycin (VAN) in the mouse granuloma pouch model using a MSSA strain. This model is regarded as an example of a SSTI.

Methods: The infecting strain used was *S. aureus* DSM 11823. Pouches were induced by injection of air and croton oil into loose subcutaneous tissue of the backs of mice. Three days later, air was evacuated and substituted by soft agar (0.25 %). On day 5, pouches were inoculated with a staphylococcal suspension (3 x 10^5 CFU/mouse). Infected pouches were treated IV bid for two days. Therapeutic efficacy was assessed by reduction of CFUs in the pouch exudates determined 18h after the last treatment.

Results: In the untreated control group, bacteria grew to a density of ~ 6 x 10^8 CFU/ml of pouch exudates on day 3 after infection. Both FRI and DAP were more effective than VAN.

Conclusions: In this study, FRI shows good efficacy in a mouse model of SSTI, the granuloma pouch, caused by MSSA.

Introduction

Friulimicin B (FRI) is a novel lipopeptide antibacterial agent that exhibits potent activity against a variety of Gram-positive bacteria[1-4]. It is structurally similar to the lipodepsipeptide antibiotic daptomycin (DAP) but has a distinct mode of action[5-6]. Its chemical structure is shown in Figure 1.

The aim of this study was to investigate the potential of FRI in experimental skin and soft tissue infections (SSTI). The efficacy of FRI in the murine granuloma pouch model using a susceptible *Staphylococcus* strain was determined in comparison with that of vancomycin (VAN) and DAP. This model is regarded as an example of an SSTI[7].

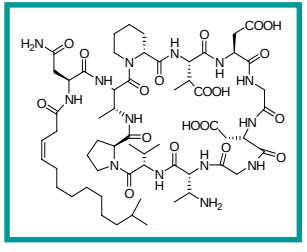

Fig. 1 Friulimicin B

Methods

Test compounds:
The test compounds were obtained from the following sources: FRI (Combinature Biopharm AG, Germany), VAN (Lilly Deutschland GmbH, Germany), DAP (Novartis Pharma GmbH, Germany).

Bacterial strain:
The staphylococcal strain DSM 11823, a clinical isolate, was taken from the culture collection of Bayer HealthCare AG, Germany.

MIC values for FRI, VAN 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 using a Denley multipoint inoculator, containing ~1 - 5 x 10^4 colony forming units (CFUs) each. Plates were incubated for 16-20 hrs. at 37°C under 5% CO_2. The lowest antibiotic concentration yielding no growth was read as the MIC.

The organisms for the inoculum were grown overnight in Thioglycollate broth under anaerobic conditions at 37°C. The inoculum was prepared from a subculture in Columbia broth after 4-5 h incubation at 37°C under anaerobic conditions. The final bacterial suspension for the inoculum was adjusted in Columbia broth with 0.25% agar.

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.

Abscess Model: Granuloma Pouch (Figure 2)
Pouches were formed in mice by injecting 5 ml of air and 0.5 ml of 0.1% croton oil in olive oil under the skin of the back. After 72 h, the air was replaced by 1 ml of 0.25% agar in saline. A bacterial suspension (500 µL; ~10^5 CFU/mouse) was injected into the pouch an additional 48 h later (5 days after inducing the granuloma pouch).

Six mice were used in each treatment group. In a few mice a pouch did not develop.

Antibiotics were given intravenously at doses of 1.25, 2.5, 5 and 10 mg/kg at 0.5, 4, 24 and 32 h post-infection (i.e. BID for 2 days). The viable bacterial load in the pouch exudates was determined at 48 h post-infection by plating serial tenfold dilutions on sheep blood agar plates. Bacterial CFUs were counted after overnight incubation of the plates at 37°C.

Statistical significance of differences in the numbers of organisms in the pouches of the different treatment groups was determined by the Mann and Whitney test.

This model is regarded as a good example of a skin and soft tissue infection.

Friulimicin B	Vancomycin	Daptomycin
0.5	0.5	0.25

Table 1 MIC values (mg/L) of test compounds against *Staphylococcus aureus* DSM 11823

Results and Discussion

Fig. 2 Murine granuloma pouch model

The MIC values for the three test compounds are shown in Table 1.

The therapeutic efficacy of compounds in the granuloma pouch model was measured by the reduction of the numbers of staphylococcae in the exudates.

Figure 3 shows the counts in the individual granuloma pouches of each group at 2 days post infection and Table 2 shows the change in the numbers of viable organisms compared with control animals receiving no therapy.

The growth of the infecting organism in the control group was consistent and reached approximately 6 x 10^8 CFU/ml of homogenate by 2 days post-infection.

All test compounds showed a dose-dependent reduction of the bacterial load. DAP was more active than the comparators. Compared with VAN, the bactericidal efficacy of FRI was higher over the whole dose range (1.25 - 10 mg/kg). Both FRI and DAP produced a 6 log fall in bacterial numbers at the highest dose. FRI reduced the count by 1.12 logs at the lowest does, in contrast to VAN which only reduced the count by 0.4 logs.

A CFU-reduction of about 4 log units was achieved with 1.25 mg/kg of DAP while 5 mg/kg of VAN or 2.5 – 5 mg/kg of FRI were needed for the same efficacy.

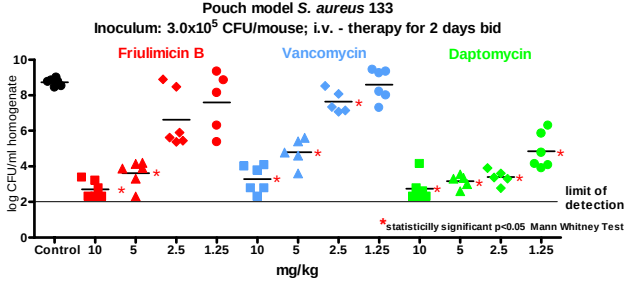

Fig. 3 Numbers of organisms in granuloma pouches following treatment with FRI, VAN or DAP

Dose	Friulimicin B		Vancomycin		Daptomycin	
mg/kg	Δ log CFU/ml homogenate	P value	Δ log CFU/ml homogenate	P value	Δ log CFU/ml homogenate	P value
1.25	-1.12	0.5368	-0.14	1.000	-3.88	0.0022
2.5	-2.11	0.0649	-1.09	0.0087	-5.34	0.0043
5	-5.11	0.0022	-3.93	0.0043	-5.57	0.0043
10	-6.02	0.0022	-5.44	0.0022	-5.99	0.0022

Table 2 Reduction in viable counts in granuloma pouches sampled two days post infection

Conclusions

- Using an experimental model of an SSTI – the mouse granuloma mouse model infected with a methicillin susceptible strain of *Staphylococcus aureus,* friulimicin B demonstrated excellent efficacy
- A range of doses (1.25, 2.5, 5.0 and 10 mg/kg) was administered intravenously BID for 2 days post infection and the highest two doses (5 and 10 mg/kg) of friulimicin B produced a dramatic bactericidal effect
- Overall friulimicin B showed a higher reduction in the bacterial counts in the exudates than vancomycin, but was less potent than daptomycin at the lower doses

Literature

[1] P. Bremer et al., *AAC* 47 (2003) 3025-9
[2] R. Schaumann et al., 47th ICAAC, Chicago 2007, Poster No. F1-1644
[3] P. McGhee et al., 47th ICAAC, Chicago 2007, Poster No. F1-1648
[4] S. Schubert et al., 47th ICAAC, Chicago 2007, Poster No. F1-1649
[5] T. Schneider et al., 47th ICAAC, Chicago 2007, Poster No. F1-1640
[6] D. Zuehlke et al., 47th ICAAC, Chicago 2007, Poster No. F1-1641
[7] Experimental models in antimicrobial chemotherapy; Vol. 1; Academic Press 1986