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Comparative *in vitro* Activity of the Novel Lipopeptide Friulimicin B with Daptomycin; the Effect of Inoculum, Pulmonary Surfactant and Calcium

H. PRIEFERT[1], L. BYLUND[2], S. PELZER[1], A. VENTE[1], H. LABISCHINSKI[1]

[1]Combinature Biopharm AG, Berlin, Germany, [2]AB Biodisk, Solna, Sweden

Contact Information:
Dr. Stefan Pelzer
Combinature Biopharm AG
Robert-Roessle Str. 10
D-13125 Berlin
Phone +49-30-9489-4050
Fax +49-30-9489-4051
E-Mail: pelzer@combinature.com

Revised Abstract

Background: Friulimicin B (FRI), an acidic, cyclic lipopeptide, is intended for the treatment of severe infections caused by Gram-positive pathogens. FRI shows structural similarities with the lipodepsipeptide daptomycin (DAP). We compared their *in vitro* activity against selected aerobic Gram-positive bacteria under different test conditions.

Methods: CLSI broth microdilution (BMD) method for DAP was used to compare the compounds using Mueller Hinton (MH) broth including 50 mg/L Ca^{2+} and 0.002% Tween 80. Ca^{2+} dependency, inoculum effect and influence of pulmonary surfactant (Survanta®) was measured using MH broth containing 50 mg/L Ca^{2+}.

Results: MIC determination of FRI is strongly influenced by the procedure and parameters used. The optimal procedure for MIC determination of FRI is BMD with MH supplemented with 50µg/ml Ca^{2+} and addition of 0.002% Tween 80. MIC values against selected aerobic Gram+ bacteria were comparable to DAP (see table). MIC of DAP against *S. aureus* increased 8-fold with increasing inoculum density from 4.7 x 10^3 to 4 x 10^7 CFU/mL. In contrast, the *in vitro* activity of FRI stayed constant across a similar range of inocula. The pulmonary surfactant had only a marginal effect on the activity of FRI against *S. aureus,* in contrast the activity of DAP was decreased 32-fold.

Conclusions: The *in vitro* activity of FRI against Gram+ pathogens is Ca^{2+} dependent and at least comparable to DAP using an optimized CLSI BMD method. In contrast to DAP, activity of FRI is not significantly inhibited by high inoculum or pulmonary surfactant.

Introduction

Friulimicin B (FRI, Fig. 1) is a novel lipopeptide antibiotic that is produced by *Actinoplanes friuliensis.* FRI is structurally similar with the lipodepsipeptide daptomycin (DAP), but has a distinct molecular mode of action[1]. In contrast to the membrane interfering DAP[2] FRI inhibits late-stage cell wall synthesis[3].

FRI displays a similar antibacterial activity spectrum like DAP addressing all important Gram-positive pathogens such as staphylococci, enterococci, pneumococci (including multi-resistant and DAP-resistant strains)[4-6] and obligatory anaerobic bacteria[7].

The purpose of the studies presented here, was to compare the influence of various test parameters, like the inoculum titre, the presence of Ca^{2+}, polysorbate 80 (Tween80) and lung surfactant on the *in vitro* activity of FRI and DAP, respectively.

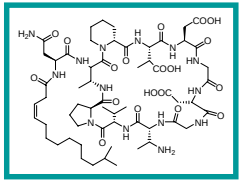

Fig. 1 Friulimicin B

Methods

Bacterial strains:
A total of 33 Gram-positive aerobes, comprising clinical isolates and quality control strains were used for the development and validation of MIC methods: 6 *E. faecalis* (including 2 VanB); 3 *E. faecium* (1 VanA, 1 VanB); 13 *S. aureus* (4 MSSA, 9 MRSA, 6 GISA); 8 coagulase-negative *Staphylococci* (1 hGISE); 2 *S. epidermidis* (1 FQR); 1 *S. haemolyticus* (hGIS).

Susceptibility tests:
MICs were determined by standard broth microdilution (BMD) in cation-adjusted Mueller-Hinton broth (CAMHB) according to CLSI guidelines[8]. For standard testing of FRI and DAP the calcium content of MHB was adjusted to a final concentration of 50 mg/l Ca^{2+} yielding $CAMHB^{50}$. For the FRI reference method $CAMHB^{50}$ was supplemented with 0.002% (v/v) polysorbate 80 (Tween80). The influence of pulmonary surfactant on the antibacterial activity of FRI and comparator drugs was determined by BMD, supplementing $CAMHB^{50}$ with Survanta® (Abbott GmbH & Co. KG, Wiesbaden, Germany) to final concentrations of 0.25%, 0.5%, 1%, 5%, 10%, and 15% (v/v). Due to the turbidity of the medium at concentrations > 1% (v/v) Survanta®, MIC values were read as colour change from yellow to purple-blue, after the addition of thiazolyl blue tetrazolium bromide (MTT, final concentration 0.5 mg/ml) to the test wells and a further incubation at 35°C for 45 min.
FRI was obtained from Combinature Biopharm AG, Berlin, Germany, and other drugs from their respective manufacturers.

Results and Discussion

Influence of Ca^{2+} concentrations on MIC
The antibacterial activity of FRI and DAP against *S. aureus* ATCC29213 and *E. faecium* ATCC 19434 was strongly dependent on the presence of free Ca^{2+} ions. At Ca^{2+} concentration of 50 mg/L (corresponding to physiological concentration in blood), FRI displayed strong activity which reached its maximum at 100 mg Ca^{2+}/l (Table 1). For DAP, the highest activity against *E. faecium* was reached at 200 mg Ca^{2+}/l.

Bacterial Strain	Antibiotic	Ca^{2+} concentration [mg/L] <20	20	50	100	200
S.aureus ATCC 29213	Friulimicin B	4	4	2	2	2
	Daptomycin	2	2	0.5	0.25	0.25
	Vancomycin	0.5	0.5	0.5	0.5	0.5
E. faecium ATCC 19343	Friulimicin B	8	8	4	2	2
	Daptomycin	16	16	4	2	1
	Vancomycin	0.5	0.5	0.5	0.5	0.5

Table 1: MICs (mg/L) at different Ca^{2+} concentrations

Optimal procedure for MIC determination of FRI
MIC determination of FRI is complex, since results are strongly influenced by the method (agar dilution showed a tendency to lower values against aerobic Gram-positive compared to BMD) and by the final concentration of Ca^{2+} and Tween80. The optimal procedure (reference method) for determining the MIC of FRI is BMD with MH broth supplemented with Ca^{2+} of 50 mg/L and 0.002% (v/v) Tween80[9]. Under these conditions FRI MIC values against 33 selected aerobic Gram-positive bacteria are comparable with DAP (Table 2).

Results and Discussion

Species	Organism	Comment	FRI	DAP
E. faecalis	EFE 763		1	4
E. faecalis	EFE 764		1	4
E. faecalis	EFE 94		2	1
E. faecalis	EFE 100	VanB	2	2
E. faecalis	EFE 101	VanB	1	4
E. faecalis	ATCC 29213		2	2
E. faecium	EFM 504		1	4
E. faecium	EFM 178	VanB	1	4
E. faecium	EFM 65	VanA	0,25	1
S. aureus	CDC 2161		0,25	0,125
S. aureus	CDC 2195		0,25	0,5
S. aureus	SCQ 21		0,25	0,5
S. aureus	ATCC 29213		0,25	0,5
S. aureus	SCP 504	GISA, MRSA	2	8
S. aureus	SCP 508		4	8
S. aureus	SCP 505		0,5	1
S. aureus	SCP 546	GISA	2	4
S. aureus	ATCC 43300		0,5	0,5
S. aureus	ATCC 700698		1	0,5
S. aureus	ATCC 700699	GISA, MRSA	2	2
S. aureus	New Jersey 992	hGISA, MRSA	2	1
S. aureus	Michigan 963	hGISA, MRSA	2	2
S. coag-	COR 29	MR	0,25	0,5
S. coag-	COR 30	MR	0,25	0,5
S. coag-	COR 31	MR	0,25	0,5
S. coag-	COR32	MR	0,5	0,5
S. coag-	COR 43	MR	0,5	0,5
S. coag-	COR 53	MR	0,5	2
S. coag-	COS 1		0,5	0,5
S. coag-	Wisconsin 759	hGISE	0,5	1
S. epidermidis	SEP 188		0,5	0,5
S. epidermidis	SEP 104	FQR	0,25	0,5
S. haemolyticus	Xiong 12	hGIS	0,25	0,25

Table 2: *In vitro* activity (MIC, mg/L) of FRI (BMD, reference method) compared to DAP (CLSI method)

Inoculum effect
MIC values of FRI and Vancomycin against *S. aureus* ATCC 29213 stayed constant at inoculum densities ranging from 4 x10^1 to 1 x 10^7 CFU/ml. However, an 8-fold increase in DAP MIC with increasing inoculum densities from 4.7 x 10^3 to 4 x 10^7 (Figure 2) was observed.

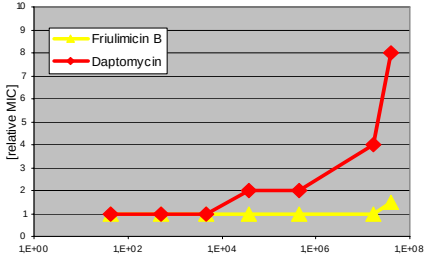

Fig. 2: Inoculum effect using *S. aureus* ATCC 29213

Influence of lung surfactant
The antibacterial activity of DAP is diminished by interaction with pulmonary surfactants which is the reason why DAP can not be used in community acquired pneumonia. The *in vitro* inhibition of antibacterial activity of FRI by pulmonary surfactant was much less pronounced than DAP which decreased 256-fold (Figure 3).

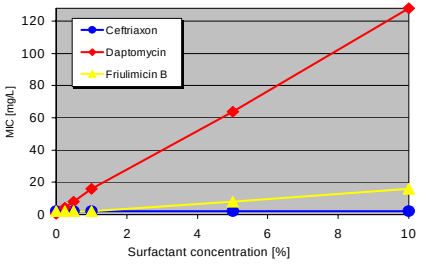

Fig. 3: MIC values against *S. aureus* ATCC 29213 in the presence of surfactant

Conclusions

- BMD using MH broth containing 50 mg/L Ca^{2+} and 0.002% (v/v) Tween80 is the optimal procedure to determine MICs for FRI
- Use of FRI reference method gives MICs against 33 aerobic Gram-positive bacteria comparable to DAP
- In contrast to DAP, FRI is not significantly inhibited by high inoculum densities or the presence of high pulmonary surfactant concentrations

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