47th ICAAC,
Chicago 2007

F1-1642

Comparative In Vitro Activities of the Novel Antibacterial Friulimicin B and Other Antibacterial Agents Against Selected Aerobic Gram-positive Bacteria

M. KRESKEN[1], J. BRAUERS[1], B. KÖRBER-IRRGANG[1], H. LABISCHINSKI[2], S. PELZER[2]

[1]Antiinfectives Intelligence GmbH, Rheinbach, Germany, [2]Combinature Biopharm AG, Berlin, Germany

Contact information:
Dr. Michael Kresken
Antiinfecives Intelligence GmbH
Campus University of Applied Sciences
von-Liebig-Strasse 20, D-53359 Rheinbach
Phone +49 (0) 2226.908.912/Fax +49 (0) 2226.908.918
Email: michael.kresken@antiinfectives-intelligence.de

Revised Abstract

Background: Friulimicin B (FRI) is a novel lipopeptide antibacterial agent. The objective of the present study was to determine the *in vitro* activities of FRI against 242 Gram-positive cocci.

Methods: Organisms tested were methicillin-susceptible *S. aureus* (MSSA), methicillin-resistant *S. aureus* (MRSA), *S. epidermidis*, *E. faecalis* incl. 6 VREf, *E. faecium* incl. 8 VREm, *S. pneumoniae* incl. 26 PNSP, and *S. pyogenes*. Antibacterial agents tested were FRI, daptomycin (DAP), linezolid (LZD), vancomycin (VAN) and other drugs. The CLSI broth microdilution method was used to determine MICs for all bacterial isolates. MICs of FRI and DAP for 20 isolates were also determined using the CLSI agar dilution method. Furthermore, the impact of polysorbate 80 (0.002%) and human serum (50% v/v) on the MICs of FRI was investigated against 25 isolates.

Results: Applying the broth microdilution method, MICs of FRI ranged from 0.5 to 8 mg/L. $MIC_{50/90}$s (mg/L) are given in the Table.

	FRI	DAP	VAN	LZD
MSSA (n=25)	2 / 2	1 / 2	1 / 1	2 / 2
MRSA (n=27)	2 / 4	1 / 2	1 / 2	2 / 4
S. epidermidis (n=22)	2 / 2	1 / 1	2 / 2	1 / 2
E. faecalis (n=30)	8 / 8	2 / 4	2 / >128	2 /2
E. faecium (n=25)	4 / 8	4 / 8	1 / >128	2 / 2
S. pneumoniae (n=56)	1 / 2	0.5 / 1	0.5 / 0.5	1 / 1
S. pyogenes (n=57)	4 / 8	0.25 / 0.5	0.5 / 0.5	1 / 2

Adding polysorbate 80 to Mueller-Hinton broth gave 1 dilution step lower MICs of FRI for 8/25 strains, whereas the addition of human serum elevated the MICs of all strains by 1-3 dilution steps. MICs of both FRI and DAP were consistently lower when the agar dilution method was applied. Compared to DAP, FRI had a comparable spectrum of activity, but MIC_{90} values were nearly always 1 dilution step higher than those of DAP, except for *S. pyogenes* (4 dilution steps higher MICs of FRI).

Conclusions: FRI appears to be a promising new antimicrobial agent for the treatment of infections caused by Gram-positive bacteria including multi-resistant isolates.

Introduction

Friulimicin B (FRI) is a novel lipopeptide antibacterial agent, that exhibits potent activity against a variety of Gram-positive bacteria (**1-4**). Its chemical structure is shown in **Figure 1.** FRI shows a structural similarity to the lipodepsipeptide daptomycin (DAP), but its molecular mode of action is different (**5**). In contrast to the membrane interfering DAP, FRI inhibits late-stage cell wall synthesis (**6**, **7**).

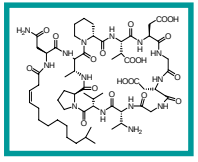
The present study was performed to evaluate the comparative *in vitro* activities of FRI and other antibacterial agents against a panel of clinical isolates of major aerobic Gram-positive bacteria susceptible or resistant to conventional antibacterial agents. Furthermore, the impact of the surfactant polysorbate 80 (0.002%) and human serum (50% v/v) on the MICs of FRI was investigated.

Methods

Bacterial strains

A total of 242 aerobic Gram-positive cocci collected from microbiology laboratories during various multi-centre studies conducted between 2001 and 2004 in Germany were tested: 30 *E. faecalis* (including six VRE), 25 *E. faecium* (including eight VRE), 52 *S. aureus* (25 MSSA, 27 MRSA), 22 *S. epidermidis* (11 MSSE, 11 MRSE), 56 *S. pneumoniae* (including 41 penicillin-non-susceptible and/or erythromycin-resistant strains), and 57 *S. pyogenes* (including 26 erythromycin-resistant strains).
All isolates were identified by routine laboratory methods and stored at -80°C until studied.

Fig. 1: Friulimicin B



Antibacterial agents

Antibacterial agents tested were
- friulimicin B,
- ampicillin,
- daptomycin (DAP),
- erythromycin,
- linezolid,
- oxacillin,
- penicillin, and
- vancomycin.

Determination of antimicrobial susceptibility

The CLSI broth microdilution procedure with geometric twofold serial dilutions in cation-adjusted Mueller-Hinton broth (CAMHB) purchased from Becton Dickinson (Heidelberg, Germany; BBL™ Cation Adjusted Mueller Hinton II Broth, lot no. 6317238, 20-25 mg Ca^{2+}/L) was used to determine MICs against all bacterial isolates (**8**). MIC plates were prepared in-house. For testing of FRI and DAP, the calcium concentration of the test solution was adjusted to 50 mg/L Ca^{2+}.
MICs of FRI and DAP for 24 isolates were also determined using the CLSI agar dilution method (**8**). Mueller Hinton agar purchased from Becton-Dickinson (Difco™ Mueller Hinton Agar, lot no. 6093202) was used as a nutrient medium. The calcium concentration of the test agar was adjusted to 50 mg/L Ca^{2+}. MICs of each strain were determined at least twice.
Furthermore, using the broth microdilution method, the effect of polysorbate 80 (0.002%) and human serum (50% v/v) on the MICs of friulimicin B was investigated against 25 isolates.
The accuracy of susceptibility testing was evaluated by MIC testing of four quality control organisms: *E. faecalis* ATCC 29212, *S. aureus* ATCC 29213, *S. aureus* ATCC 43300 (MRSA), and *S. pneumoniae* ATCC 49619 (**Table 1**).

Results

Comparative in vitro activity of friulimicin B using the broth microdilution method

Comparative activities of friulimicin B against the 242 aerobic Gram-positive cocci tested are shown in **Table 2.** Friulimicin B was consistently active against all pathogens, regardless of resistance to comparator agents. Overall, MIC values ranged from 0.5 to 8 mg/L.

In vitro activities of FRI and DAP using the agar dilution method

The *in vitro* activities of FRI and DAP were determined at least twice for five strains each of *S. aureus*, *S. epidermidis*, and *E. faecium* and *E. faecalis*. The results are shown in **Table 3**. MICs of both compounds were consistently lower than those determined with the broth microdilution method. Each compound inhibited all staphylococci at 1 mg/L and all enterococci at 4 mg/L.

Results

Impact of polysorbate 80 and human serum on the in vitro activity of FRI

Five strains each of *S. aureus*, *S. epidermidis*, *E. faecalis*, *E. faecium* and *S. pyogenes* were tested.
The effect of polysorbate 80, at a concentration of 0.002%, showed either no or a minor impact on the MICs of FRI (**Table 4**).
Sixteen and eight strains exhibited unchanged and one dilution step lower MICs, respectively, whereas there was one *S. epidermidis* strain with a two-fold increase in the MIC. The effect of normal human serum upon the activity of FRI against the 25 strains is also shown in **Table 4**. There was a decrease in activity in the presence of 50% (v/v) human serum against all strains, with MICs for 20 strains increasing more than fourfold.

Table 1: MICs of friulimicin B for quality control strains

Organism	MIC (mg/L)	
	Broth microdition	Agar dilution
E. faecalis ATCC 29212	8	4-8
S. aureus ATCC 29213	2-4	1
S. aureus ATCC 43300 (MRSA)	2	0.5-1
S. pneumoniae ATCC 49619	0.25-1	not tested

Table 2: In vitro activity of friulimicin B in comparison to other antimicrobial agents against 242 aerobic gram-positive bacterial isolates using the CLSI broth microdilution method

Organism (Number of strains tested)	Antimicrobial agent	MIC (mg/L)		
		Range	MIC_{50}	MIC_{90}
Staphylococcus aureus, methicillin-susceptible (25)	Friulimicin B	1-4	2	2
	Daptomycin	1-4	1	2
	Linezolid	1-4	2	2
	Vancomycin	0.5-2	1	1
Staphylococcus aureus, methicillin-resistant (27)	Friulimicin B	1-4	2	4
	Daptomycin	1-2	1	2
	Linezolid	1-4	2	4
	Vancomycin	0.5-2	1	2
Staphylococcus epidermidis, methicillin-susceptible (11)	Friulimicin B	1-2	2	2
	Daptomycin	1	1	1
	Linezolid	1-2	1	2
	Vancomycin	1-2	1	2
Staphylococcus epidermidis, methicillin-resistant (11)	Friulimicin B	2	2	2
	Daptomycin	1	1	1
	Linezolid	0.25-2	1	1
	Vancomycin	1-2	2	2
Enterococcus faecalis (30)	Friulimicin B	4-8	8	8
	Daptomycin	1-4	2	4
	Linezolid	1-4	2	2
	Vancomycin	1 - > 128	2	> 128
	Ampicillin	0.5-4	1	2
Enterococcus faecium (25)	Friulimicin B	2-8	4	8
	Daptomycin	2-8	4	8
	Linezolid	1-2	2	2
	Vancomycin	0.5 - > 512	1	> 128
	Ampicillin	2-128	64	128
Streptococcus pneumoniae (56)	Friulimicin B	0.5-4	1	2
	Daptomycin	0.5-1	0.5	1
	Linezolid	0.25-2	1	1
	Vancomycin	0.25-0.5	0.5	0.5
	Erythromycin	0.031- > 64	32	> 64
	Penicillin	≤ 0.016-4	0.063	2
Streptococcus pyogenes (57)	Friulimicin B	1-8	4	8
	Daptomycin	0.125-0.5	0.25	0.5
	Linezolid	0.5-2	1	2
	Vancomycin	0.25-1	0.5	0.5
	Erythromycin	0.031- > 64	0.063	> 64
	Penicillin	≤ 0.016	≤ 0.016	≤ 0.016

Table 3: In vitro activity of friulimicin B and daptomycin against 20 aerobic bacterial isolates using the CLSI agar dilution method

Organism	Phenotype*	No. of tests	Mean MIC (mg/L)	
			Friulimicin B	Daptomycin
***Staphylococcus aureus* (n=5)**				
Staphylococcus aureus 710-1-22	MS	2	0.75	0.375
Staphylococcus aureus 710-1-23	MS	2	0.75	0.75
Staphylococcus aureus 710-1-11	MR	2	1.5	0.5
Staphylococcus aureus 710-1-49	MR	3	1	1
Staphylococcus aureus 710-1-78	MR	2	1	0.75
***Staphylococcus epidermidis* (n=5)**				
Staphylococcus epidermidis 720-1-34	MS	2	0.75	1
Staphylococcus epidermidis 720-1-40	MS	3	0.67	0.83
Staphylococcus epidermidis 720-1-42	MR	2	0.75	0.5
Staphylococcus epidermidis 720-1-2	MR	3	1	0.83
Staphylococcus epidermidis 720-1-1	MR	2	1.5	0.5
***Enterococcus faecalis* (n=5)**				
Enterococcus faecalis 810-1-36	VS	2	4	1
Enterococcus faecalis 810-1-6	VS	2	4	2
Enterococcus faecalis 810-1-28	VR	3	1.33	0.5
Enterococcus faecalis G10-65	VS	2	4	1
Enterococcus faecalis 810-1-65	VR	2	4	1
***Enterococcus faecium* (n=5)**				
Enterococcus faecium L7-10-70	VS	3	3.33	2.67
Enterococcus faecium L7-09-03	VS	3	2	4
Enterococcus faecium 820-1-4	VR	2	2	3
Enterococcus faecium 820-1-5	VR	2	2	3
Enterococcus faecium 820-1-14	VR	3	1	4

*MS, methicillin-susceptible; MR, methicillin-resistant; VS, vancomycin-susceptible; VR, vancomycin-resistant

Table 4: Effect polysorbate 80 (0.002%) and/or human serum (50% v/v) on broth microdilution MICs of friulimicin B

Organism	Phenotype*	MIC (mg/L)		
		Broth	Broth and polysorbate 80	Broth and human serum
***Staphylococcus aureus* (n=5)**				
Staphylococcus aureus 710-1-22	MS	2	1	8
Staphylococcus aureus 710-1-23	MS	4	2	16
***Staphylococcus aureus*, resistant to methicillin (n=3)**				
Staphylococcus aureus 710-1-11	MR	4	2	16
Staphylococcus aureus 710-1-49	MR	4	2	16
Staphylococcus aureus 710-1-78	MR	4	4	32
***Staphylococcus epidermidis*, susceptible to methicillin (n=2)**				
Staphylococcus epidermidis 720-1-34	MS	2	2	8
Staphylococcus epidermidis 720-1-40	MS	1	2	4
***Staphylococcus epidermidis*, resistant to methicillin (n=3)**				
Staphylococcus epidermidis 720-1-42	MR	2	2	8
Staphylococcus epidermidis 720-1-2	MR	4	2	8
Staphylococcus epidermidis 720-1-1	MR	2	2	16
***Enterococcus faecalis* (n=5)**				
Enterococcus faecalis 810-1-36	VS	4	4	32
Enterococcus faecalis 810-1-6	VS	8	8	32
Enterococcus faecalis 810-1-28	VR	4	2	32
Enterococcus faecalis 810-1-65	VR	8	4	32
Enterococcus faecalis 810-1-71	VR	8	8	32
***Enterococcus faecium* (n=5)**				
Enterococcus faecium L7-10-70	VS	4	4	32
Enterococcus faecium L7-09-03	VS	4	2	8
Enterococcus faecium 820-1-4	VR	4	4	16
Enterococcus faecium 820-1-5	VR	4	4	32
Enterococcus faecium 820-1-14	VR	4	4	16
***Streptococcus pyogenes* (n=5)**				
Streptococcus pyogenes 920-3-58	ES	1	1	4
Streptococcus pyogenes 920-2-9	ES	4	4	8
Streptococcus pyogenes 920-1-10	ER	4	4	8
Streptococcus pyogenes 920-1-16	ER	4	4	8
Streptococcus pyogenes 920-1-28	ER	1	1	4

*MS, methicillin-susceptible; MR. methicillin-resistant; VS, vancomycin-susceptible; VR, vancomycin-resistant; ES, erythromycin-susceptible; ER, erythromycin-resistant

Conclusions

- Overall, the spectrum of activity of FRI resembles that of DAP.
- Using the broth microdilution method, MICs of FRI were nearly always one dilution step higher than those of DAP, with MIC_{90} values of FRI for staphylococci, enterococci and streptococci ranging between 2 and 8 mg/L.
- Using the agar dilution method we found a trend towards lower MICs compared to the broth microdilution method and the differences in the MICs between FRI and DAP were less striking.
- Polysorbate 80 at a concentration of 0.002% had either no or a minor effect on the MICs of FRI.
- In the presence of 50% (v/v) human serum, a decrease in activity of FRI against all strains was observed.
- Activity of FRI was very uniform independent of resistance phenotype against marketed antibiotics.
- FRI appears to be a promising new antimicrobial agent for the treatment of infections caused by Gram-positive organisms including isolates that possess resistances to currently available drugs.

Literature

[1] Bemer P, Juvin ME, Bryskier A, Drugeon H. In vitro activities of a new lipopeptide, HMR 1043, against susceptible and resistant gram-positive isolates. Antimicrob Agents Chemother 2003;47:3025-9.
[2] Schaumann R, Adler D, Pelzer S, Labischinski H, Rodloff AC. Activity of friulimicin and five other antimicrobial agents against 179 gram-positive obligatory anaerobic bacteria. 47th ICAAC, Chicago 2007, Poster No. F1-1644
[3] McGhee P, Bogdanovic T , Credito K, Labischinski H, Appelbaum PC. Activity of friulimicin against glycopeptide and daptomycin non-susceptible *S .aureus*. 47th ICAAC, Chicago 2007, Poster No. F1-1648
[4] Schubert S, Dahlhoff A, Pelzer S, Labischinski H. Comparative Analysis of the bactericidal activities of friulimicin, daptomycin, tigecycline, and vancomycin against difficult to treat Isolates of *S. aureus* and *S. pneumoniae*. 47th ICAAC, Chicago 2007, Poster No. F1-1649
[5] Zuehlke D, Voigt B, Hecker M, Jordan S, Mascher T, Pelzer S, Labischinski H. Distinct mode of action of the lipopeptide antibiotic friulimicin and the lipodepsipeptide daptomycin: a proteomic study. 47th ICAAC, Chicago 2007 Poster No. F1-1641
[6] Silverman JA, Perlmutter NG, Shapiro HM. Correlation of daptomycin bactericidal activity amd membrane depolarization in *Staphylococcus aureus*. Antimicrob Agents Chemother 2003; 47: 2538-2544
[7] Schneider T, Gries K, Wiedemann I, Pelzer S, Labischinski H, Sahl HG. Friulimicin Inhibits Cell Wall Biosynthesis through Complex Formation with Bactoprenol-Phosphate. 47th ICAAC, Chicago 2007 Poster No. F1-1640
[8] National Committee for Clinical Laboratory Standards (NCCLS). Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically. Approved Standard - 6th Edition. M7-A6. National Committee for Clinical Laboratory Standards, Wayne, Pa., 2003.