47th ICAAC,
Chicago 2007

F1-1648

Activity of Friulimicin B Against Glycopeptide and Daptomycin Non-susceptible *S. aureus*

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Revised abstract

Background: Friulimicin B (FRI), an acidic cyclic lipopeptide intended for therapy of severe drug-R Gram-pos. infections, is structurally similar to daptomycin (DAP) with a different molecular target. We tested FRI, DAP, vancomycin (VAN), teicoplanin (TEC), quinupristin-dalfopristin (Q/D), linezolid (LIN) against a spectrum of 19 mostly difficult to treat *S. aureus* strains and one DAPR VRE.

Methods: Three DAPR clinical strains (1 from Hershey which developed from MRSA to VISA while the patient was on VAN) had DAP MICs 4-8 µg/ml and 6 lab DAPR clones obtained by multistep R selection had DAP MICs ≥16 µg/ml. VAN-non-S strains were 3 VRSA and 2 VISA. MIC was by CLSI agar dilution (+ Ca $^{2+}$ for FRI, DAP). Time-kills were in CAMHB (CLSI), final inocula 5 x 10^5 to 5 x 10^6 cfu/ml. In addition a DAPR clinical *E. faecium* isolate was included in the analysis.

Results: MICs (µg/ml) against the 5 VAN-S MRSAs were: FRI, DAP, VAN, TEC, Q/D all 0.5-1 µg/ml, LIN 4 µg/ml. MICs (µg/ml) against 9 DAPR strains were 4-16 (FRI); 2-16 (VAN); 2-32 (TEC); 0.12-0.5 (Q/D); 1-4 (LIN). Against defined VISA strains, FRI and DAP MICs (µg/ml) were 8 and 4, resp., and against VRSA were 2-4 and 0.5-1, resp. VAN MICs were 4->32 µg/ml, TEC MICs 4->32 against VISA and VRSA. MICs of Q/D and LIN against VISA and VRSA were 0.25-0.5, 2-4. resp. Time-kills (MIC/2xMIC/4xMIC) showed that FRI and DAP were bactericidal (99.9% killing) at 4 x MIC after 24 h against 7/9 and 6/7 DAPR isolates, respectively, and against all 5 VAN-non-S strains. VAN and TEC were cidal against 1 isolate each. TEC, Q/D and linezolid were static against all strains. MICs (µg/ml) against the DAPR *E. faecium* isolate were 2 (FRM); 16 (DAP); >64 (VAN); 16 (TEC); 1 (Q/D); 1 (LIN). No killing at all was observed for TEC, Q/D, and LIN. DAP was bactericidal at 8 x MIC (128 µg/ml), a clinically non-relevant concentration.

Conclusions: FRI and DAP were very active against VRSA (0.5 - 4 µg/ml); MICs against DAPR- and VISA strains were 8-16, 4-8 µg/ml, resp. FRI was cidal against 7/9 of DAPR and reduced the CFU of the DAPR *E. faecium* isolate by 99% after 24 hrs at 16 µg/ml (8 x MIC).

Introduction

FRI (Fig. 1) is a novel lipopeptide antibiotic that is produced by *Actinoplanes friuliensis*. FRI is structurally similar to the lipodepsipeptide DAP, but has a distinct molecular mode of action. It displays good *in vitro* activity against a range of important Gram-positive pathogens such as staphylococci, enterococci and pneumococci[1-3], including multi-resistant strains.

Recent reports have described staphylococcal and enterococcal clinical strains with reduced sensitivity vs. DAP, and , in the case of VISA strains, a correlation between DAP, VAN and other high molecular weight antibiotics was observed[4-6]. Here we report on the susceptibility of such strains vs. FRI and other antibiotics using MIC and time kill-experiments.

Methods

Bacterial strains:
Organisms tested comprised 9 strains pre-qualified as DAP resistant (3 clinical, the remainder obtained by prior resistance selection studies), 2 pre-qualified as vancomycin-intermediate strains (VISA) (including one isolated in our hospital which developed vancomycin and DAP resistance after sequential treatment with both of these drugs[6]), 3 vancomycin resistant strains (VRSA) (including the Hershey strain) and 5 vancomycin susceptible methicillin-resistant strains (MRSA). The DAP resistant VRE strain *E. faecium* J4026[7] was kindly provided by J. H. Jorgensen, University of Texas. FRI was obtained from Combinature Biopharm AG. Berlin, Germany and other drugs from their respective manufacturers.

Susceptibility tests:
MICs were predetermined by macrodilution in cation-adjusted Mueller-Hinton broth (BBL Microbiology Systems, Cockeysville, MD) according to standard methodology. DAP and FRI susceptibility testing was in MH-broth adjusted to 50 µg/ml of calcium per standard methodology and glycopeptide MICs were read after 24 h. All strains were tested by the time-kill method with each compound alone as described previously[8].

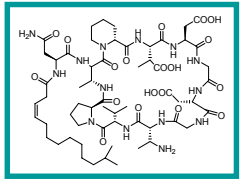


Fig. 1: Friulimicin B

Results

MICs (µg/ml) against the 9 DAP resistant isolates (Table 1) were 4-16 FRI; 2-16 vancomycin; 2-32 teicoplanin; 0.125-0.5 quinupristin/dalfopristin; 1-4 linezolid. Against VISA strains, FRI and DAP MICs (µg/ml) were both 4-8, and against VRSA they were 2-4 and 0.5-1, respectively. For the *E. faecium* VRE isolate FRI and DAP MICs were 2 and 16 µg/ml, respectively. Vancomycin MICs were 4->64 µg/ml and teicoplanin MICs were 4->32 against VISA, VRSA, and VRE. MICs of quinupristin/dalfopristin and linezolid against VISA and VRSA were 0.25-0.5 and 2-4 respectively. Based on these results, 11 out of the 19 *S. aureus* strains would have to be qualified as DAP resistant, 4 as VISA and 6 as VRSA. It should be realized that the classification of glycopeptide non-susceptible *S. aureus* strains is still in a state of flux and method-dependent, with a possible one dilution MIC difference potentially changing the classification of specific strains according to the method used.

Time-kill studies (Fig. 2, Table 2) on 14 strains for FRI and 12 for DAP (same strains but without strains Mut3 and Mut5) showed that FRI and DAP were bactericidal at 4 x MIC after 24 h against 7/9 and 6/7 isolates reported previously as DAP resistant isolates, respectively, and against all 5 vancomycin-non-susceptible strains.

Results

Strain	Pre-qualified resistotype	Determined resistotype	FRI	DAP	VAN	TEC	Q/D	LIN
SA212	MRSA	VSSA	1	0.5	1	0.5	1	4
SA238	MRSA	VSSA	0.5	1	0.5	0.5	0.5	4
SA487	MRSA	VSSA	0.5	0.5	1	0.5	0.5	4
SA490	MRSA	VSSA	1	1	1	0.5	0.5	4
SA495	MRSA	VSSA	1	0.5	1	1	0.5	4
SA506	MRSA, VISA	VISA, DAPR	8	4	4	8	0.25	4
SA507	MRSA, VISA	VISA, DAPR	4	4	4	4	0.5	2
SA509	MRSA, VRSA	VRSA	2	1	16	32	0.5	4
SA510	MRSA, VRSA	VRSA	4	0.5	32	16	0.5	4
SA512	MRSA, VRSA	VRSA	2	0.5	>32	>32	0.5	4
SA555	MRSA, DAPR	VISA, DAPR	8	4	8	32	0.125	4
SA560	MRSA, DAPR	VSSA, DAPR	8	2	2	4	0.125	4
SA562	MRSA, DAPR	VSSA, DAPR	8	2	2	2	0.125	4
SA Mut1	MRSA, DAPR	VRSA, DAPR	16	32	16	32	0.25	1
SA Mut3	MRSA, DAPR	VISA, DAPR	16	>32	8	8	0.5	4
SA Mut4	MRSA, DAPR	VSSA, DAPR	4	64	2	2	0.25	2
SA Mut5	MRSA, DAPR	VSSA, DAPR	8	64	2	8	0.125	4
SA Mut8	MRSA, DAPR	VRSA, DAPR	16	16	16	32	0.5	2
SA Mut9	MRSA, DAPR	VRSA, DAPR	16	32	16	16	0.5	1
EFJ4026	VRE, DAPR	VRE, DAPR	2	16	>64	16	1	1

Breakpoints used: vancomycin (S, I, R) ≤ 2, 4-8, ≥ 16, daptomycin (S, R) ≤1, >1 (for *S. aureus*).

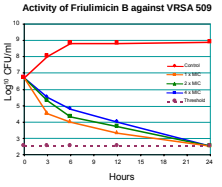
Table 1: MICs (µg/ml) of all agents against strains tested

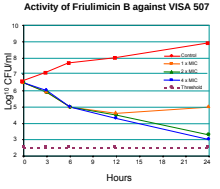
Drug	3 h			6 h			12 h			24 h		
% killing	90[a]	99[a]	99.9[a]	90[a]	99[a]	99.9[a]	90[a]	99[a]	99.9[a]	90[a]	99[a]	99.9[a]
Friulimicin B												
4 x MIC	5[b]	1	0	12	4	0	13	12	7	13	13	12
2 x MIC	4	1	0	11	4	0	13	11	3	13	13	11
MIC	2	1	0	10	3	0	12	6	2	12	6	6
Daptomycin												
4 x MIC	10	4	2	12	10	8	12	12	12	12	12	12
2 x MIC	7	3	1	11	9	6	12	12	9	12	12	11
MIC	4	0	0	10	7	2	12	9	6	10	7	4
Vancomycin												
4 x MIC	0	0	0	0	0	0	5	0	0	7	5	1
2 x MIC	0	0	0	0	0	0	5	0	0	8	5	1
MIC	0	0	0	0	0	0	4	0	0	7	4	1
Teicoplanin												
4 x MIC	1	0	0	1	1	0	6	2	1	10	2	1
2 x MIC	1	0	0	1	1	0	6	2	1	10	2	1
MIC	1	0	0	1	0	0	3	1	0	9	0	0
Q/D												
4 x MIC	2	0	0	5	0	0	7	2	0	10	2	0
2 x MIC	0	0	0	4	0	0	7	2	0	9	2	0
MIC	0	0	0	1	0	0	6	1	0	5	1	0
Linezolid												
4 x MIC	0	0	0	0	0	0	3	0	0	7	1	0
2 x MIC	0	0	0	0	0	0	2	0	0	6	1	0
MIC	0	0	0	0	0	0	1	0	0	2	1	0

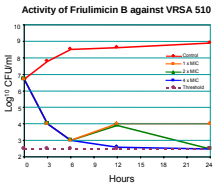
a: % killing, b: No. of strains killed

Please note that 14 *S. aureus* strains were tested against friulimicin B and 12 strains (same strain set, but without strains Mut3 and Mut5) in case of daptomycin

Table 2: Results of time-kill experiments







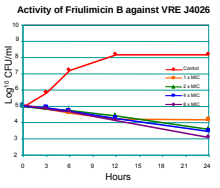


Fig. 2: Selected kill curves

Vancomycin and teicoplanin were bactericidal against 1 isolate each. Teicoplanin, quinupristin/dalfopristin and linezolid were bacteriostatic against all isolates.

The current study shows that FRI MICs fell in at least 3 distinct categories when compared to DAP:

i) Classical MRSA strains (marked in red in Table 1) with low FRI MICs, essentially identical to those of DAP

ii) VISA and other strains with reduced vancomycin sensitivity in which, as a rule all high molecular weight compounds show reduced susceptibility (marked in yellow). The mechanism behind this peculiar phenotype (tentatively named HISA for intermediate susceptibility vs. high-molecular weight inhibitors against *S. aureus*) could be reduced diffusion through thickened cell walls[9-11]

iii) Strains with strongly reduced sensitivity to either DAP or FRI, but no cross-resistance, as expected for compounds of distinct mode of action (marked in blue)

Conclusions

- Standard MRSA strains have good susceptibility to friulimicin B and to daptomycin.
- Strains ("HISA") with reduced susceptibility to all high molecular weight inhibitors irrespective of molecular mode of action were observed
- The mechanism behind HISA could be thickened cell walls as first described by Hiramatsu, Cui, and co-workers
- Kill kinetics of friulimicin B and daptomycin were similar, relative to MICs
- Friulimicin B at 8 x MIC led to a 24 hrs CFU reduction by 99% for a daptomycin resistant VRE isolate

Literature

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