

Comparative Analysis of the Bactericidal Activities of Friulimicin B, Daptomycin, Tigecycline, and Vancomycin against Difficult to Treat Isolates of *S. aureus* and *S. pneumoniae*

S. SCHUBERT¹, A. DALHOFF¹, S. PELZER², H. LABISCHINSKI²

¹Univ. of Kiel, Kiel, Germany, ²Combinature Biopharm AG, Berlin, Germany

Contact information:
Dr. Axel Dalhoff
University-Hospital Schleswig-Holstein, Campus Kiel,
Institute for Infection Medicine
Brunswiker Str 4
D-24105 Kiel
Phone +49 (0) 202-2655236
Fax +49 (0) 202-2655297
E-mail: ADalhoff@T-Online.de

F1-1649

Revised Abstract

Background: Friulimicin B is a lipopeptide antibiotic with a novel mode of action; it is active against multidrug-resistant (MDR) Gram-positive bacteria including MRSA, VISA, and MDR-*S. pneumoniae*. We studied the bactericidal kill constants of friulimicin B (FRI) in comparison with daptomycin (DAP), vancomycin (VAN), and tigecycline (TIG) against *S. aureus* (Sa) and *S. pneumoniae* (Spn).

Methods: Six strains with defined resistances against methicillin, and/or VAN, linezolid, ciprofloxacin (Sa) or penicillin, macrolide, ciprofloxacin (Spn) and susceptible or resistant ATCC reference strains were used. All strains were grown in cation-adjusted MH-broth plus 0.002% Tween 80 + 50mg/l Ca²⁺ under batch-culture conditions and were exposed to multiples of their individual MICs (1, 4, 8, 16 times). Cultures were inoculated with approx 1X10⁸ CFU/ml. Samples were taken at 0, 1, 2, 4, 6, 8, 24h to determine CFUs. During the initial log-linear phase of CFU-decline single point kill rates and times needed for 3log- and 6log- kill were calculated. Drug-free cultures served as controls.

Results: Phenotypically, FRI and DAP exerted the most pronounced bactericidal effect against Sa and Spn. The single point kill rates of FRI and DAP increased over the entire concentration range. The bactericidal activity was independent of the resistance genotype and MDR-phenotype. FRI exerted a concentration-dependent 6log kill against Sa within 10-24h. Activity against Spn was not concentration dependent. DAP eliminated Sa more rapidly but was less active against Spn. TIG and VAN were only bacteriostatic.

Conclusions: The bactericidal activity of FRI against selected difficult to treat or MDR *S. aureus* and *S. pneumoniae* is independent of methicillin-, vancomycin-, linezolid-, quinolone-, penicillin-, or macrolide resistance. These characteristics may make FRI attractive for the therapy of infections in patients with critical illnesses.

Introduction

Friulimicin B (FRI, Fig. 1) is a natural compound produced by *Actinoplanes friulensis*; it belongs to a novel class of lipopeptide antibiotics. It has structural similarities with daptomycin (DAP) but has been shown to have a different mode of action [1,2].

FRI inhibits the late stage of cell wall synthesis producing a potent and broad spectrum activity against all relevant Gram positive pathogens, while no DAP cross resistance occurs. Thus it is destined for the treatment of infections caused by Gram-positive bacteria including but not limited to MRSA and VRE.

The aim of this study was to determine the *in vitro* bactericidal activity of FRI against a panel of Gram-positive bacteria susceptible or resistant to comparator drugs. This was done by determining time-kill curves with selected indicator strains with clinically relevant susceptibility/resistance patterns (i.e. β -lactam-, vancomycin-, linezolid-, and quinolone resistance). Thus, the strains studied - apart from the susceptible reference strains used as controls - represent a number of pathogens which are difficult to treat in the clinic.

Methods

Test strains and susceptibility to standard drugs

Time-kill experiments were performed with the following panel of 6 strains:
Staphylococcus aureus (Sa) ATCC 29213: Methicillin-susceptible (MSSA) wild type reference strain for resistance testing, (vancomycin-, linezolid- and ciprofloxacin-susceptible)
Staphylococcus aureus (Sa) ATCC 33593: Methicillin-resistant (MRSA) reference strain,
(vancomycin-, linezolid- and ciprofloxacin-susceptible)
Staphylococcus aureus (Sa) NRS 119: Methicillin-resistant isolate (MRSA), (vancomycin susceptible, linezolid-, and ciprofloxacin-resistant)
Staphylococcus aureus (Sa) VISA Mu 50: Vancomycin-intermediate (VISA), methicillin-resistant clinical isolate (vancomycin-intermediate, linezolid-susceptible, ciprofloxacin-resistant)
Streptococcus pneumoniae (Sp) ATCC 49619: Reference strain, clinical isolate, (penicillin-, vancomycin-, linezolid- and ciprofloxacin-susceptible)
Streptococcus pneumoniae (Sp) Bay 19397: Fluoroquinolone resistant laboratory strain (penicillin-resistant, vancomycin- and linezolid-susceptible, ciprofloxacin-resistant, *gyrA* mutation)

The strains were stored frozen at -80°C in a volume of 100 μ l.

Antibacterial agents tested

The agents tested in comparison with FRI (supplied by Combinature Biopharm AG, Berlin) were DAP (lot CDF 002/8, Novartis Pharma GmbH, Basel, CH), TIG (lot 24715, Wyeth-Lederly Pharma GmbH, Muenster, D), and VAN (lot 41354 TB 21, Hikma Pharma GmbH).

MIC determinations

MIC testing was done using a microdilution method according to CLSI (formerly NCCLS) guideline [3]. MICs were determined in Ca-supplemented (50mg/L) MH-broth plus 0.002% Tween 80 (CAMHB-50 + tween).

Time-Kill curve kinetics

Kill curve kinetics were done by using a slightly modified method according to CLSI (formerly NCCLS) guidelines [4] and as described by [5].

Mathematical models used for the calculation of kill-kinetics

The bactericidal effect was analyzed by using models as described recently by Schaper *et al* [6]. The basis is the assumption of a first-order exponential decrease with time of the number N of viable cells (CFU/ml) exposed to a drug at a certain concentration C (using multiples x of the MIC: C=x*MIC with x=1, 4, 8, 16, and 32). Single point kill rates (k) were calculated by $k = -(\ln(N/N_0))/t$; in addition, the concentrations needed for 3log kill (C3log) were calculated ($t_{\frac{1}{2}} = (\ln 2) / k$). For clinical microbiologists the more relevant time to obtain a decrease in the initial number of CFU from No to No/1,000 can be calculated by the more general equation $t_{\frac{1}{2}} = (\ln f) / k$ with f = 1,000.

Results and Discussion

MICs (Table 1)

In CAMHB-50 + tween the VISA-strain was inhibited by 0.5 mg/L of FRI, the two reference strains by 0.25 to 1.0 mg/L, and the MDR strain NRS119 by 0.5 mg/L. The two *S. pneumoniae* strains were inhibited by FRI concentrations of 1.0 and 0.5 mg/L. The MICs of the comparators were within the same range.

Results and Discussion

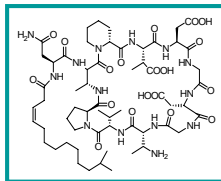


Fig. 1 Friulimicin B

Organism/Strain	MIC mg/L			
	FRI	DAP	VAN	TIG
Sa - MRSA ATCC 33593	0.5-1	0.5	1	0.5
Sa - MSSA ATCC 29213	0.25-0.5	0.5	1	0.5
Sa - VISA Mu 50	0.5	1	2	1-2
Sa - MRSA NRS 119	0.5	0.5	0.13	0.25
Sp - ATCC 49619	1	0.13	0.13	0.25
Sp - Bay 19397	0.5	0.5	0.5	4

Table 1 MIC values of compounds against the test strains (2 experiments)

Calculated single point kill rates (Table 2)

The single point kill rates calculated for FRI clearly demonstrate that the kill rates increase concentration dependently. The viable counts and thus the kill rates, too, of two strains only, *S. pneumoniae* ATCC 49619 and *S. pneumoniae* 19397 were independent of concentration. Likewise, the single point kill rates calculated for DAP increase concentration dependently for all strains tested. The VAN single point kill rates are small, thus indicating an almost bacteriostatic action in particular against *S. aureus*. TIG kill rates are small, with the exception of *S. pneumoniae* BAY 19397, and almost concentration independent.

Friulimicin B (f = 1-16)	Multiple of MIC			
	1	4	8	16
Sa ATCC 33593	1.61	1.91	1.96	2.11
Sa ATCC 29213	1.42	1.20	1.67	1.91
Sa VISA Mu50	1.32	1.54	1.40	1.62
Sa NRS 119	0.43	0.55	0.66	0.71
Sp ATCC 49619	1.14	1.01	0.86	0.87
Sp Bay 19397	1.48	1.19	1.23	1.33

Multiple of MIC	Friulimicin B			
	1	4	8	16
Sa ATCC 33593	4.20	3.61	3.53	3.27
Sa ATCC 29213	4.86	5.76	4.39	3.61
Sa VISA Mu50	5.22	4.48	4.93	4.26
Sa NRS 119	16.06	12.50	10.45	9.72
Sp ATCC 49619	6.05	6.83	8.02	7.93
Sp Bay 19397	4.66	5.79	5.61	5.18

Table 2 Calculated Single point kill rates

Table 3 Time (hours) for a 3log reduction in viability

Calculated periods of time needed for 3 log kill (Table 3)

The times needed for a reduction of viable counts of the four *S. aureus* test strains by 3log titres decrease with increasing concentrations of FRI; the times needed for a reduction of *S. pneumoniae* by 3 log titres are independent from the FRI concentration. Likewise, the times needed for a reduction of viable counts of all six indicator organisms by 3log titres decrease with increasing DAP concentrations. However, the times needed for 3log kill of the four *S. aureus* test strains at 1xMICs of FRI and DAP, respectively, are significantly different. FRI at 1x MIC reduced the viable counts of *S. aureus* much more rapidly than DAP. VAN and TIG reduced the viable counts of the 6 indicator organisms by 3log titres very slowly, if at all.

Summary

The bactericidal activity of FRI was concentration-dependent against these difficult to treat or MDR *S. aureus* and *S. pneumoniae* and is independent from methicillin-, vancomycin-, linezolid-, quinolone-, penicillin-, or macrolide resistance. At a concentration of 16 x MIC of FRI a regrowth of the test strains, except *S. aureus* ATCC 33593, was prevented. Therefore, resistance to FRI cannot develop under these test conditions. DAP also had a concentration dependent bactericidal effect. The DAP kill rates were higher than those for FRI, indicating a stronger bactericidal effect BUT the 2 *S. pneumoniae* strains were not eliminated, so that in principle development of resistance may occur. The bactericidal activities of FRI and DAP, differed significantly at 1xMIC. FRI exhibited a stronger bactericidal effect against *S. aureus* at low drug concentrations than DAP did. The time needed for a reduction of viable counts of *S. aureus* by 3 orders of magnitude (except the multi-resistant *S. aureus* NRS 119) were 2- 3 times smaller upon exposure to FRI than following an exposure to DAP.

Conclusions

- The concentration-dependent bactericidal activity of FRI against selected difficult to treat or MDR *S. aureus* and *S. pneumoniae* is independent of methicillin-, vancomycin-, linezolid-, quinolone-, penicillin-, or macrolide resistance
- The pronounced bactericidal activity of FRI as opposed to the moderate activity of DAP at low drug concentrations may be of clinical relevance. Both agents are relatively highly protein bound. Therefore, free and thus antibacterially active drug concentrations may be close to the MICs of the relevant pathogens
- FRI appears to be a promising new antimicrobial agent for the treatment of infections caused by Gram-positive organisms, including isolates that are resistant to currently available drugs

Literature

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