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Activities of Daptomycin (DAP), Vancomycin (VAN) and Linezolid (LDZ) alone or in combination with Fusidic acid (FUS) in an *in vitro* dynamic model of *Staphylococcus aureus* Biofilm

Wafi Siala,[1] Prabhavathi Fernandes,[2] Paul M. Tulkens,[1] and Françoise Van Bambeke[1]

[1] *Pharmacologie cellulaire et moléculaire,* Louvain Drug Research Institute, *Université catholique de Louvain*, Brussels, Belgium; [2] Cempra, Inc., Chapel Hill, NC, USA

Corresponding author:
F. Van Bambeke
Av. Mounier 73 B1.73.05
1200 Brussels, Belgium

Abstract

Objective: FUS is currently evaluated as an oral drug for the treatment of cSSSTI in which biofilms play a major role. We evaluated the activity of FUS alone or combined with other antistaphylococcal antibiotics (DAP, VAN, LZD in an *in vitro* pharmacodynamic model of staphylococcal biofilm using the *CDC reactor system,* exposing biofilms to shear forces and mimicking antibiotic pharmacokinetics.
Methods: Biofilms of *S. aureus* ATCC25923 were grown at 37°C on polycarbonate coupons inserted into rods contained in the CDC biofilm reactor using a starting inoculum of 10^5 CFU/ml. Preconditioning was achieved in TSB + 1% glucose and 2% NaCl by 6h batch incubation followed by 14h of continuous flow (11.6 mL/min). Antibiotics were then injected at concentrations corresponding to their human fC_{max}, with flow rates adapted to simulate their respective half-lives. Coupons were collected over time and washed in PBS. Bacteria were recovered by 3 alternating 60-s cycles of vortexing and sonication, and plated for CFU counting.
Results: FUS alone had no activity while VAN, LZD and DAP alone caused a minimal decrease in CFU (0.5-0.7 log). Combinations of FUS with DAP or LZD were highly synergistic, reaching 2.45 and 3.97 $\log_{10}$ CFU decrease compared to control, respectively. In contrast, combining FUS with VAN did not markedly improve activity on biofilms.
Conclusion: Combinations of FUS with DAP or LZD were the most effective against *S. aureus* biofilm in this pharmacodynamic model, warranting testing in vivo.

Introduction

Staphylococcus aureus is an important human pathogen causing chronic infections that are difficult to treat. Biofilms contribute to the persistence of infections, by protecting bacteria from the immune system and antimicrobial agents. We showed that many antibiotics are poorly active against biofilms [1], especially with clinical isolates from persistent infections [2].

Fusidic acid (FUS) may constitute a useful alternative for treatment of *S. aureus* infections (in regions with low resistance rates) but shows moderate activity when used alone against biofilms [1].

Aim and Pertinence of the Study

Since FUS is seldom used alone in chronic infections to avoid resistance selection, our aim was to evaluate bacterial killing of FUS against biofilms of *S. aureus* when combined with other antistaphylococcal antibiotics. To make the study pertinent to the future clinical use of such combinations, we used an *in vitro* pharmacodynamic model with the CDC reactor system that exposes biofilms to shear forces and mimics the antibiotic pharmacokinetics.

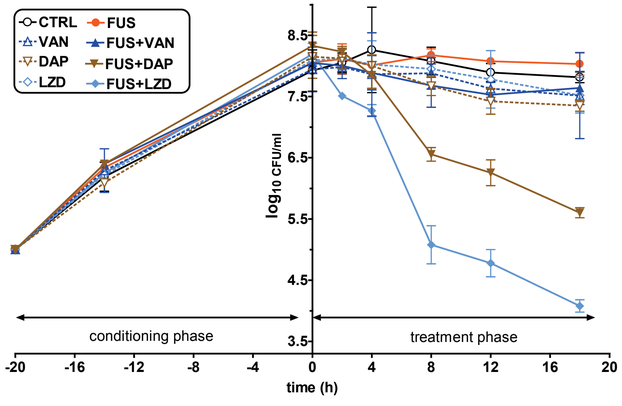
Results

Reduction in $\log_{10}$ CFU/ml within biofilms for antibiotics alone or FUS combined with VAN, DAP, LZD compared to untreated biofilm of ATCC25923 strain

Time	FUS [a]	VAN [a]		DAP [a]		LZD [a]	
(hours)	alone	alone	+ FUS	alone	+ FUS	alone	+ FUS
2	NE [b]	0.07 ± 0.02	0.05 ± 0.02	NE	NE	NE	0.54 ± 0.03
4	0.04 ± 0.01	0.19 ± 0.01	0.16 ± 0.01	0.05 ± 0.01	0.20 ± 0.03	0.02± 0.00	0.78 ± 0.17
8	NE	0.17 ± 0.05	0.37 ± 0.032	0.38 ± 0.09	1.49 ± 0.21	0.10 ± 0.00	2.97 ± 0.41
12	NE	0.42 ± 0.00	0.52 ± 0.16	0.63 ± 0.01	1.79 ± 0.22	0.27 ± 0.1	3.27 ± 0.4
18	0.02 ± 0.00	0.53 ± 0.01	0.41 ± 0.02	0.70 ± 0.10	2.45 ± 0.5 [c,*]	0.53 ± 0.02	3.97 ± 0.23 [c,**]

[a] Fusidic acid (FUS) MIC: 0.5 mg/L; fC_{max}: 35 mg/L; $t_{1/2}$: 6h
Vancomycin (VAN) MIC: 1 mg/L; fC_{max}: 20 mg/L; $t_{1/2}$: 6h
Daptomycin (DAP) MIC: 0.5 mg/L; fC_{max}: 9.8 mg/L; $t_{1/2}$: 8h
Linezolid (LZD) MIC: 1 mg/L; fC_{max}: 17 mg/L; $t_{1/2}$: 6h
[b] no effect (no reduction in inoculum observed as compared to control)
[c] statistical analysis at 18 h: * $p < 0.05$; ** $p < 0.01$ when comparing FUS alone with combination

Activity of FUS alone or combined with VAN, DAP, LZD against ATCC25923 biofilm in a pharmacodynamic model (see Table for concentration and half-lives)



- FUS alone was not active on biofilms in this model.
- The other antibiotics alone had reduced CFUs of only about 0.5 $\log_{10}$ at 18 h.
- Combining FUS with VAN did not markedly improve its activity on biofilms.
- In contrast, combining FUS with DAP or with LZD was highly synergistic, reducing bacterial counts by 2.5 and 4 $\log_{10}$ CFU, respectively.

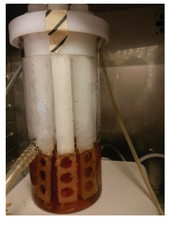
Acknowledgements and Conflicts of Interest

WS was a post-doctoral fellow of the program *Prospective Research for Brussels* (Innoviris). PF is CEO and a stockholder of Cempra Pharmaceuticals and the work was partly supported through a grant-in-aid from Cempra Pharmaceuticals.

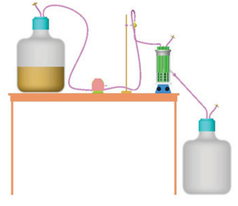
Materials and Methods

- Biofilms of *S. aureus* ATCC25923 were grown on polycarbonate coupons inserted into rods within the CDC biofilm reactor (BioSurfaces Technologies, Bozeman, MT).
- Biofilms were preconditioned during 20h as follows:
  a) 6h of incubation at 37°C of 10^5 cfu/mL in TSB + 1% glucose and 2% NaCl
  b) 14h of continuous flow at a rate of 11.6 ml/min.
- Antibiotics were added in the bioreactor at their fC_{max} with subsequent flow rate adapted to simulate the half-lives of the antibiotics.
- Coupons were aseptically removed at 0, 2, 4, 8, 12 and 18h and washed twice in PBS.
- Bacteria were recovered from biofilms by 3 alternating 60 sec cycles of vortexing and sonication.
- Samples were then serially diluted and plated onto TSA to allow colony counting.

a



b



a: **CDC reactor.** Biofilms are grown on 12.7 mm diameter coupons, suspended in the bulk fluid by coupon holders.

b: **Schematic view of the completely assembled reactor system [3].** A typical experiment would start with inoculation of the sterile reactor containing a nutrient solution and a period of batch operation with the effluent port clamped. Following the batch phase, the effluent port would be unclamped and a flow of a sterile fresh nutrient solution from the carboy, seen on the left, would start for a phase of continuous flow operation.

Conclusions and Future Directions

- Combinations of FUS with DAP or LZD were the most effective treatments against *S. aureus* biofilm in this model and should be evaluated in *in vivo* models.
- The mechanism of synergy shown here should be further investigated.

References

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