50 YEARS ICAAC | BOSTON | SEPTEMBER 12–15, 2010

A1-678

Pharmacodynamic Evaluation of the Intracellular Activity of Antibiotics against Hemin and Menadione-dependent Small-Colony Variants (SCV) of *Staphylococcus aureus* in a Model of THP-1 Macrophages

Contact info: F. Van Bambeke
Université catholique de Louvain
Pharmacologie cellulaire et moléculaire
Avenue E. Mounier 73, UCL7370
Brussels B-1200, Belgium
Email: francoise.vanbambeke@uclouvain.be

L. Garcia,[1] B. C. Kahl,[2] K. Becker,[2] R.A. Proctor,[3] S. Lemaire,[1] P.M. Tulkens,[1] F. Van Bambeke.[1]

[1]*Université catholique de Louvain, Brussels, Belgium;* [2]*Medical Microbiology, University Clinics Muenster, Muenster, Germany;* [3]*University of Wisconsin Med. School, Madison, WI*

Abstract

Background: SCVs of *S. aureus* can persist intracellularly, which might cause recurrent infections. We have compared the intracellular activity of antibiotics (ABs) against an MRSA strain and its two stable hemin and menadione dependent SCVs.

Methods: Strains: MRSA COL (K7), *menD* disruptant (K8), *hemB* disruptant (K9), *hemB* complemented strain (K10). MICs: measured by microdilution in MHB. Intracellular activities: determined at 24 h in human THP-1 macrophages over a wide range of extracellular concentrations; concentration-response curves used to calculate PD parameters.

Results: The amplitude of the response (Emin-Emax) was similar for K7 and K9 but smaller for K8, in relation to much lower Emin and slightly higher Emax. RIF, MXF and ORI reached an Emax > 1 log reduction against all strains and GEN, DOR and DAP against K8 only. DOR regained low activity against intracell. SCV of MRSA, as described for non-SCV strains. Static concentrations (extracell. conc. for which no change of intracellular inoculum is observed) were all close to the MICs. Dose-response curves for K10 matched those of K7.

Conclusion: The menadione-dependent SCV (K8) shows slower growth (lower Emin) intracellularly but this does not impair the efficacy of ABs, which display similar or higher Emax than for other strains. RIF, MXF and ORI were globally the more efficient ABs, as previously observed also for a thymidine-dependent SCV in the same model.

Introduction

Small-Colony Variants (SCV) of *Staphylococcus aureus* are a slow-growing subpopulation displaying different phenotypic characteristics and pathogenic traits such as slow growth, decreased pigment formation, altered expression of virulence genes, auxotrophism for distinct growth factors such as thymidine, hemin and/or menadione, and the ability to revert to the normal phenotype (1).

SCVs are associated with persistent, recurrent and antibiotic-refractory infections (1-3), related to their slow metabolism as well as to their propensity to persist and survive inside eucaryotic cells (3). We previously showed that a thymidine-dependent SCV was poorly susceptible to most antibiotics in a model of intracellular infection, with only oritavancin reaching a bactericidal effect after 24 hours of incubation (4). Yet, there is no data so far regarding the activity of antibiotics against hemin or menadione-dependent intracellular SCVs.

Hemin and menadione are two compounds involved in the synthesis of the electron carriers cytochrome and menaquinone. Hemin or menadione-dependent phenotypes are unstable, but stable in vitro mutants can be obtained (5) by inactivation of the *hemB* gene (one of the genes encoding enzymes of the porphyrin biosynthetic pathway) or of the *menD* gene (one of the genes required for menadione biosynthesis).

Aim of the study

The aim of this work was to examine the intracellular activity of seven antistaphylococcal drugs against the MRSA strain *S. aureus* COL, in comparison with its stable *menD* (menadione-dependent) and *hemB* (hemin-dependent) mutants with SCV phenotype and with a *hemB* complemented strain.

Results

Figure 1. Intracellular activity of antibiotics

Dose-response curves of antibiotics against the different isogenic strains of *S. aureus* phagocytized by THP-1 cells.
Cells were incubated with the antibiotic for 24 h at the concentrations (total drug) indicated on the abscissa.
The ordinate shows the change in the number of CFU per mg of cell protein as compared to the post-phagocytosis inoculum. All values are means standard deviations.
The horizontal line corresponds to an apparent static effect.

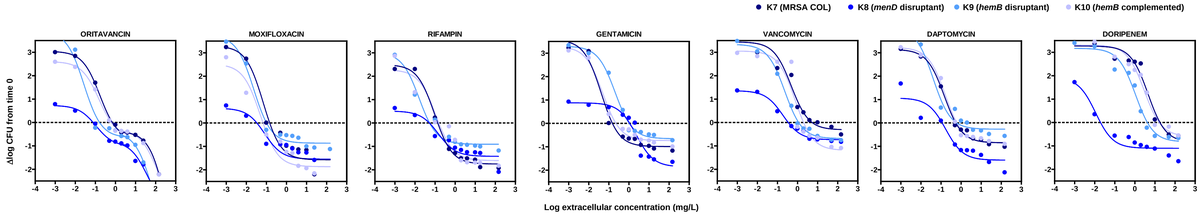

Table 1. Relative potency of antibiotics (µg/mL): MIC (extracellular) and static concentration (C_{stat}; intracellular)

AB	K7 (parent)		K8 (*menD* mutant)		K9 (*hemB* mutant)		K10 (*hemB* compl.)	
	MIC	C_{stat}	MIC	C_{stat}	MIC	C_{stat}	MIC	C_{stat}
ORI	0.25	0.71	0.03	0.08	0.03	0.17	0.25	0.60
MXF	0.03	0.14	0.125	0.03	0.125	0.10	0.03	0.07
RIF	0.03	0.13	0.03	0.05	0.03	0.05	0.03	0.15
GEN	0.125	0.15	2	1.05	1	1.05	0.25	0.23
VAN	2	5.21	1	0.34	2	1.14	2	2.00
DAP	0.5	0.56	0.5	0.11	0.25	0.60	1	0.60
DOR	16	17.30	32	0.02	16	4.10	16	15.97

C_{stat}: Extracellular concentration of antibiotic yielding no apparent change in CFU after 24 hours compared to the post-phagocytosis inoculum.

Table 2. Intracellular relative efficacy of antibiotics minimal (Emin), maximal (Emax) change in bacterial counts in dose-effects experiments

AB	K7 (parent)			K8 (*menD* mutant)			K9 (*hemB* mutant)			K10 (*hemB* compl.)		
	E_{min}	E_{max}	E_{min}-E_{max}	E_{min}	E_{max}	E_{min}-E_{max}	E_{min}	E_{max}	E_{min}-E_{max}	E_{min}	E_{max}	E_{min}-E_{max}
ORI	3.04	> - 2	> 5.04	0.73	> - 2	> 2.73	3.88	> - 2	> 5.88	2.60	> - 2	> 4.60
MXF	3.29	-1.58	4.87	0.63	-1.55	2.18	3.74	-0.86	4.60	2.52	-1.87	4.38
RIF	2.50	-1.75	4.25	0.51	-1.42	1.94	3,02	-0.91	3,93	2.28	-1.61	3.88
GEN	3.57	-0.99	4.56	0.88	-1.86	2.74	3.29	-0.66	3.95	3.29	-0.73	4.02
VAN	3.45	-0.31	3.76	1.36	-0.74	2.10	3.34	-0.69	4.03	3.06	-1.19	4.25
DAP	3.15	-0.84	3.99	1.07	-1.58	2.65	4.08	-0.27	4.35	3.23	-0.85	4.08
DOR	3.27	-0.84	4.11	1.93	-1.10	3.03	3.17	-0.83	4.00	3.31	-0.59	3.91

E_{min}: Max. increase in log CFU compared to initial inoculum for an infinitely low AB conc.;
E_{max}: Max. decrease in log CFU compared to initial inoculum for an infinitely high AB conc.;
$E_{min} - E_{max}$: amplitude of the antibacterial response.

All parameters were calculated from sigmoidal dose-response with Hill coefficient of 1 as shown in Figure 1.
For oritavancin, 2 successive sigmoidal curves were fitted to the data and maximal effect was not yet reached at the highest concentration tested.

Akcnowledgments: This research was supported by the Belgian *Fonds National de la Recherche Scientifique* and by the Belgian *Fonds pour la Recherche dans l'Industrie et l'Agriculture*.

Methods

Bacterial strains: *S. aureus* COL (K7; MRSA), its respective *menD* (K8) and *hemB* (K9) mutants and *hemB* complemented strain (K10) were used in this study. The *hemB and menD* mutants were constructed by allelic replacement with an *ermB* cassette-inactivated *hemB* gene and an *ermC* cassette-inactivated *menD* gene, respectively (5,6).

Susceptibility testings: MICs were measured by microdilution in Mueller Hinton Broth according to CLSI guidelines.

Cells: Experiments were performed with human THP-1 cells (ATCC TIB-202, Manassas, VA), a myelomonocytic cell line displaying macrophage-like activity.

Cell infection and determination of the intracellular activities of antibiotics (7): Phagocytosis was initiated at a bacteria per macrophage ratio of 10 (1h at 37 C), followed by elimination of non-phagocytosed bacteria by exposing the cells to 50 µg/mL gentamicin (45min). Cells were then transferred to fresh medium supplemented with increasing concentrations of antibiotics (0.001- to 150 µg/mL) for 24 hours. Results, expressed as the change in the intracellular inoculum at 24 h compared to time 0, were used to fit a Hill equation to allow determination of the values of two key pharmacological descriptors of antibiotic activity (static concentration and minimal and maximal relative efficacy).

Conclusions

SCV versus other strains

- Extracellularly, all drugs show similar intrinsic activity (MIC) against SCVs and parental strain.
- Intracellularly, the menadione-dependent SCV (K8) shows a slower growth (lower Emin). Yet, this does not impair the efficacy of antibiotics (similar or higher Emax than for other strains).

Antibiotic intracellular activity

- Oritavancin, moxifloxacin, and rifampin are the only drugs capable of reducing the intracellular counts by more than 1 log at 24 hours for all strains. These drugs prove also more efficient against a thymidine-dependent SCV in the same intracellular model (4).
- Oritavancin shows bimodal effects against all strains (2 successive zone of concentration-dependent activity, as already described against intracellular thymidine-dependent SCV [4]). This may denote the multiple modes of action of this drug (8).
- As previously described for other β-lactams and MRSA strains (9), doripenem regains activity intracellularly against the COL MRSA and its SCV variants as a consequence of the effect of the acidic pH of the phagolysosomes on PBP2a conformation (10).

Clinical implication

- Selection of antibiotics showing intracellular activity against SCVs may be important when dealing with infections for which SCVs play an important role, such as cystic fibrosis or persistent osteomyelitis, for example (1).

References

(1) Von Eiff. 2008. *Int J Antimicrob Agents*. 31:507-10; **(2)** Proctor et al. 1995. *Clin Infect Dis*. 20:95-102; **(3)** Proctor et al. 1994. *Infect Agents Dis*. 3:302-312; **(4)** Nguyen et al. 2009. *Antimicrob Agents Chemother*. 53:1434-1442; **(5)** Von Eiff et al. 2006. *J. Bacteriol.* 188:687-693; **(6)** Von Eiff et al. 1997. *J. Bacteriol.* 179:4706-4712; **(7)** Barcia-Macay et al. 2006. *Antimicrob Agents Chemother*. 50:841-851; **(8)** Van Bambeke et al. 2008. Trends Pharmacol Sci. 29:124-34; **(9)** Lemaire et al. 2007. *Antimicrob Agents Chemother*. 51:1627-1632; **(10)** Lemaire et al. 2007. *J Biol Chem.* 2008.283:12769-76.