Influence of Phorbol Myristate Acetate (PMA) on Cellular Accumulation and Intracellular Activity of Antibiotics against against Hemin and Menadione Auxotrophic Small-Colony Variant (SCV) Mutants of Staphylococcus aureus in Human THP-1 cells

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*

Contact info:
F. Van Bambeke
francoise.vanbambeke@uclouvain.be

CHICAACGO 51st ICAAC ~ SEPT. 17-20, 2011 ~ CHICAGO

A1-1751

Abstract

BACKGROUND: SCVs of *S. aureus* cause persistent infections, which relates to their capacity to survive intracellularly. Using a model of infected THP-1 cells, we have compared the intracellular (IC) growth of an MRSA strain and its two stable menadione- and hemin-auxotrophic SCV mutants and assessed their IC susceptibility to antibiotics. Here, we examine the effect of activation of THP-1 monocytes on antibiotic (AB) uptake and IC activity.

METHODS: Activation: Incubation of THP-1 monocytes with PMA (200 ng/mL - 48 h). AB accumulation: Incubation of cells with AB for 2 h; cellular conc. measured by fluorimetry for moxifloxacin (MXF) and microbiological assays for rifampin (RIF) and gentamicin (GEN). Strains: MRSA COL (K7), its *\menD* mutant (K8) and its *\hemB* mutant (K9). IC activities: Change in CFU from the post-phagocytosis inoculum after 24 h incubation with AB (extracell. conc.: 0.01-100 X MIC); PD parameters calculated using Hill equation of dose-response).

RESULTS: Post-phagocytosis viable IC counts were reduced in activated cells (10-fold for K7, 30-fold for K8 and 20-fold for K9) but IC growth remained unaffected. The table shows that activation caused a 1.7 to 3.9 increase in AB accumulation and a 2- to 15-fold increase in AB potency (decrease in Cstat).

CONCLUSION: Activation of monocytes in macrophages enhances the potency of antibiotics against *S. aureus* and its SCVs, in direct relationship to accumulation.

Introduction

Staphylococcus aureus can produce subpopulations which are phenotypically very different from the parent strain. These naturally occurring subpopulations grow slowly, leading to colonies that are named "Small Colony Variants" (SCVs). The biochemical basis of this phenotypic abnormality is an auxotrophism for distinct growth factors such as menadione, hemin and/or thymidine (1). Related to their slow metabolism as well as to their propensity to persist and survive inside eucaryotic cells, SCVs are associated with persistent, recurrent and antibiotic-refractory infections (2-4).

We previously demonstrated that a menadione-dependent SCV shows slower growth intracellularly than an hemin-dependant strain in infected THP-1 cells. We also showed that this does not impair the efficacy of antibiotics (ICAAC 2010; abs. A1-678).

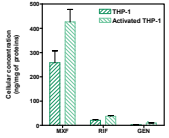
Due to metabolic and morphological similarities, the human monocytic cell line THP-1 can be differentiated to macrophages by the protein kinase C activators phorbol 12-myristate 13-acetate (5). These adherent THP-1 cells show remarkable phenotypic changes demonstrating their activated state, as an increased phagocytic activity (6) and an increase in the intracellular activity of antibiotics against intracellular bacteria, as demonstrated for fluoroquinolones and *Listeria monocytogenes* (7).

Aim of the study

The aim of this study was to compare unstimulated and phorbol-ester-differentiated THP-1 cells for the uptake of different antibiotics and for their activity against phagocytosed *S. aureus* and its SCVs mutants.

Results

Figure 1. Accumulation of antibiotics



Antibiotic	Accumulation ratio*
MXF	1.65
RIF	1.79
GEN	3.88

* Data expressed as the ratio of accumulation in activated vs. non-activated cells

Figure 2. Uptakeof bacteria by cells

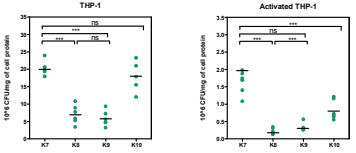


Figure 3. Intracellular growth of bacteria

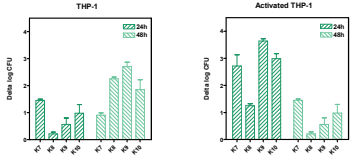
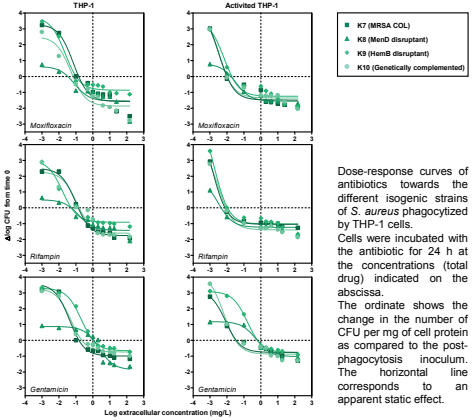


Figure 4. Intracellular activity of antibiotics



Dose-response curves of antibiotics towards 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. The horizontal line corresponds to an apparent static effect.

Figure 5. Intracellular potencies: Static concentration (Cs)

Antibiotic	Intracellular potency ratio (Cs)			
	K7	K8	K9	K10
RIF	0.06	0.08	0.20	0.28
MXF	0.07	0.51	0.19	0.15
GEN	0.23	0.55	0.62	0.13

Cs: Extracellular concentration of AB yielding no apparent change in CFU after 24 hours compared to the post-phagocytosis inoculum (intrapolated from dose-response curve); data expressed as the ratio of this value in activated vs. non-activated cells

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 (7 ,8).

Cells: Experiments were performed with human THP-1 cells (ATCC TIB-202, Manassas, VA), a myelomonocytic cell line displaying macrophage-like activity. Activation of THP-1 was obtained by the addition of 200 ng/mL of PMA during 48 hours.

Accumulation: Cells were incubated with an extracellular concentration equal to the Cmax measured in the serum of patients, for 2 hours, at 37°C. MXF was measured by fluorimetry; RIF and GEN by microbiological assay.

Cell infection and determination of the intracellular activities of antibiotics (9): 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 mg/L gentamicin (45min). Cells were then transferred to fresh medium supplemented with increasing concentrations of antibiotics (0.001- to 150 mg/L) 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

Accumulation
- Differentiation of monocytes into macrophages leads to an increased accumulation of antibiotics (1.7, 1.8 and 3.9-fold for RIF, MXF, and GEN, respectively);

Uptake by THP-1
- Internalization of each strain is reduced in activated THP-1 in comparison to non-activated cells (10 times for K7, 20 for K8 and K10, and 30 for K9);

Intracellular persistence
- After 24 hours of infection, intracellular counts were higher in activated cells as compared to non activated cells; yet, the menadione-dependent strain shows a slower growth as compared to the hemin-dependent mutant in activated macrophages, as previously observed in monocytes;
- After 48 hours, all strains persist to lower levels in activated cells as compared to non activated cells. This can be attributed to a better killing activity of macrophages in comparison to monocytes;

Intracellular activity of antibiotics
- Activation of monocytes in macrophages enhances the potency of antibiotics against *S. aureus* and its SCV forms (Cs decreased of 2 to 15 times). This could result from their higher cellular accumulation. In contrast, maximal efficacy is not affected.

References

(1) Proctor et al. 2006. *Nat Rev Microbiol.* 4:295–305; **(2)** Von Eiff. 2008. *Int J Antimicrob Agents.* 31:507-10; **(3)** Proctor et al. 1995. *Clin Infect Dis.* 20:95-102; **(4)** Proctor et al. 1994. *Infect Agents Dis.* 3:302-312; **(5)** Tsuchiya et al. 1992. *Cancer Res.* 42:530-6; **(6)** Tominga et al. 1998. *J Exp Med.* 198:99–11; **(7)** Van de Velde et al. 2008. *J Antimicrob Chemother.* 62:518-21; **(8)** Bates et al. 2003. *J Infect Dis.* 187(10):1654-1661; **(9)** Von Eiff et al. 1997. *J Bacteriol.* 179:4706-4712; **(10)** Barcia-Macay et al. 2006. *Antimicrob Agents Chemother.* 50:841-851;

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*.

This poster will be made available for download after the meeting at : http://www.facm.ucl.ac.be