A-597

ICAAC 2012

52nd ICAAC – Sept. 9-12 - San Francisco

Activity of Antibiotics against a Normal Phenotype MRSA and its *menD* and *hemB* Mutants on Models of Bronchial Epithelial Cells Expressing Wild-Type, F508del or no CFTR

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

[1] Univ. catholique de Louvain, Brussels, Belgium; [2] Univ. Hospital Münster, Münster, Germany; [3] Univ. Wisconsin Med. School, Madison

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

ABSTRACT

BACKGROUND: Small Colony Variants (SCV) of *S. aureus* can persist in epithelial cells, which plays a role in persistence of infection, as seen in cystic fibrosis patients. We have studied the activity of antibiotics (AB) against a wild-type MRSA and its *menD* and *hemB* mutants in isogenic cells differing in their CFTR status.
METHODS: Strains: MRSA COL and its *menD* or *hemB* disruptants. Cells: CFBE41o- CFTR KO, or transduced by wild-type CFTR or F508del CFTR cDNA (Bebok et al, J. Physiol 2005, 569:601). MIC: microdilution. Intracellular activity: change in CFU from the post-phagocytosis inoculum after 24 h exposure to ABs at their human Cmax.
RESULTS: In control conditions, the *menD* SCV, and to some extent the *hemB* SCV, persisted better than WT COL in cells expressing a functional or a mutated CFTR. All ABs were more active against the *menD* SCV in all cell types (highest difference in CFTR cells), and to a lower extent against the *hemB* SCV in CFTR cells. Globally, MXF and RIF were the most active drugs. MEM was active intracellularly despite the MRSA character of the strains, as previously described (Lemaire et al., AAC 2007 51:1627).
CONCLUSION: ABs are more active against SCVs in cells expressing a functional CFTR than a mutated or no CFTR. This difference may contribute to the difficulty in eradicating them in cystic fibrosis patients.

INTRODUCTION & OBJECTIVES

- Small Colony Variants (SCVs) of *Staphylococcus aureus* are frequently isolated in cystic fibrosis patients and are associated with the recurrence of the infection [1]. They show a particular tropism for the intracellular medium, which may contribute to their persistence and to the difficulty of eradicating them [2].
- Cystic fibrosis (CF) is due to mutations in the gene encoding the transmembrane protein CFTR, which are accompanied by an alteration of several cellular functions. CFTR is also claimed to play a role in bacterial internalization [3].
- The aim of this study was to examine the activity of antibiotics against the intracellular forms of SCVs in bronchial epithelial cells differing by their CFTR status. To this effect we used in parallel
  - ⇨ The MRSA COL strains and its isogenic menadione- or hemin-dependent mutants;
  - ⇨ Isogenic bronchial epithelial cell lines expressing no CFTR, wild-type CFTR, or F508del CFTR (more frequent mutation in CF).

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

We thank Dr. J.P. Clancy (University of Alabama, Birmingham, AL) for the kind gift of CFBE41o- cell lines.
This work was supported by the French association *Mucoviscidose:ABCF.*

RESULTS

Human Cmax of used antibiotics and MICs against

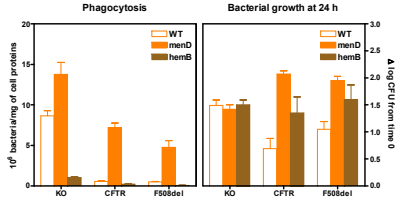
- **the parental strain (WT) with normal phenotype,**
- **the menadione-dependent (menD), and**
- **the hemin-dependent (hemB) mutants**

antibiotic	Cmax (mg/L)	MICs (mg/L)		
		WT	menD	hemB
RIF	18	0.02	0.02	0.02
MXF	4	0.03	0.125	0.125
GEN	18	0.25	1	0.5
VAN	50	1	1	1
MEM *	50	32	32	32

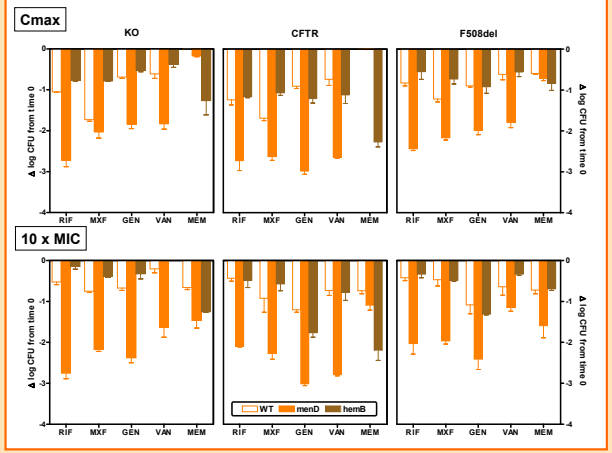
* See poster A596 for data examining β-lactam activity against intracellular SCV MRSA

Phagocytosis (left) and intracellular survival (right) of the parental strain (WT), its *menD*- and *hemB*- mutants in cells

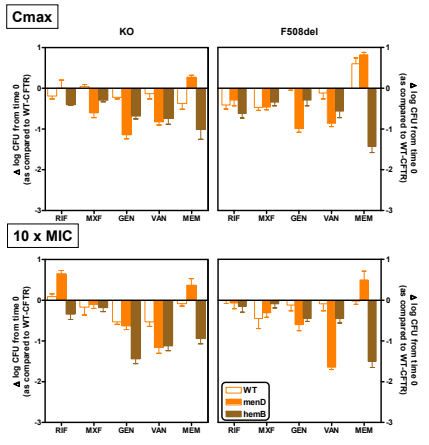
- **with no expression of CFTR (KO)**
- **expressing WT CTF (WT)**
- **expressing del508F CFTR (del508F)**



Antibiotic activity against the COL strain and its SCV mutants after 24 h incubation with antibiotics at their human Cmax (top) or at 10 X their MIC (bottom) in the three cells types.



Difference between antibiotic activity as measured in cells expressing WT CFTR and in cells KO for CFTR (left) or expressing F508del CFTR (right).



CONCLUSIONS

- Internalization is more efficient in KO cells for all strains, but the *menD* mutant shows improved phagocytosis in all cell types.
- Intracellular survival is higher for SCVs than for the parental strain in all cell types, in accordance with their known intracellular tropism [2].
- Antibiotic activity is higher against the *menD* SCV than against the other strains in all cell types, suggesting that, as previously observed in phagocytic cells, the intracellular fate of this mutant as well as its pattern of susceptibility to antibiotics inside the cells differs from those of the *hemB* mutant or of the parental strain [4].
- Antibiotic activity is reduced in KO or F508del cells vs. cells expressing a functional CFTR, which may contribute to explain persistence in CF patients. This does not apply to meropenem, possibly due to specific effects of oxidant stress (see poster A596).

METHODS

- **Bacterial strains and susceptibility testing:** strain COL (wild-type, HA-MRSA); its *menD* and *hemB* SCV mutants [5,6]. MICs determined following CLSI recommendations in CA-MHB.
- **Cells:** CFBE41o− bronchial epithelial cells KO for CFTR, stably transduced with wild-type (WT) or and F508del CFTR (F508del) [7].
- **Intracellular activity:** 2 h internalization with an initial inoculum of $1x10^8$ CFU/ml [8]; elimination of extracellular bacteria using high concentrations of gentamicin [9]. Incubation with antibiotics during 24 h at their human Cmax or 10 x MIC. CFU counting; data expressed as change form the post-phagocytosis inoculum, using cell protein content for normalization.

REFERENCES

1. Proctor *et al.* Nat Rev Microbiol 2006; 4, 295-305.
2. Tuchscherr *et al.* J Infect Dis 2010; 202,1031-40.
3. Mitchell *et al.* Infect Immun 2011; 79, 3541-51
4. Garcia *et al.* Antimicrob Ag Chemother 2012; 56, 3700-11
5. Bates *et al.* J. Infect. Dis 2003; 187, 1654-61.
6. Von Eiff *et al.* J. Bacteriol 1997; 179, 4706-12
7. Bebok *et al.* J Physiol 2005; 569, 601-15
8. Mempel *et al.* Br J Dermatol 2002; 146, 943-51.
9. Lemaire *et al.* Antimicrob Ag Chemother 2008; 52, 2797-805