Comparative Activity of 12 Antibiotics Used at Clinically-meaningful Extracellular Concentration Against *Staphylococcus aureus* in Broth and in Human THP-1 Macrophages

Poster #A-1174

M. Barcia-Macay, C. Seral, M.P. Mingeot-Leclercq, P.M. Tulkens, ***F. Van Bambeke***

Pharmacologie Cellulaire et Moléculaire, Université catholique de Louvain - Brussels - Belgium

F. Van Bambeke
Pharmacologie cellulaire et moléculaire
UCL 73.70 av. Mounier 73
1200 Brussels - Belgium
vanbambeke@facm.ucl.ac.be

ABSTRACT

Background:
The recurrent character of *S. aureus* infections is ascribed to its persistence in intracellular niches. A 24 h model of *S. aureus*-infected human THP-1 macrophages was set-up to assess and compare the activity of ABs against extracellular and intracellular bacteria.

Methods:
THP-1 were infected with serum-opsonized *S.aureus* ATCC 25923 and exposed to ABs at a concentration corresponding to their Cmax in patients.

Results:

AB	MIC (mg/L)	AB concentration (mg/L)	Activity (log change in cfu in 24h) broth	macrophages
none			2.98 ± 0.21	0.89 ± 0.05*
OXA	0.125	6	- 3.02 ± 0.56	- 1.39 ± 0.05
RIF	0.03	4	- 2.94 ± 0.03	- 0.88 ± 0.02
GEN	0.5	18	< - 4.5	- 0.98 ± 0.01
AZI	0.5	0.5	- 0.5 ± 0.01	- 0.17 ± 0.00
TEL	0.06	2	- 1.84 ± 0.02	- 0.31 ± 0.04
VAN	1.0	50	- 2.92 ± 0.23	- 0.88 ± 0.02
TEC	0.25	100	- 3.00 ± 0.17	- 0.83 ± 0.01
ORI [a]	0.25	25	< - 4.5	- 2.88 ± 0.01
LVX	0.125	4	- 4.48 ± 0.05	- 1.43 ± 0.04
CIP	0.125	4.3	- 3.21 ± 0.10	- 1.60 ± 0.02
GAR [b]	0.06	4	- 3.81 ± 0.12	- 1.88 ± 0.04
MXF	0.06	4	- 4.57 ± 0.05	- 1.91 ± 0.00

* addition of gentamicin (1 x MIC) required to prevent extracellular growth in controls only;
[a] oritavancin, [b] garenoxacin (BMS284756)
All ABs except AZI were bactericidal in broth. Activity was considerably decreased intracellularly with only ORI showing a marked bactericidal effect ([a] 2 log reduction in CFU).

Conclusion:
ABs commonly recommended in *S. aureus* infections (OXA, RIF, GEN, VAN) are poorly active against intracellular forms (inappropriate cellular bioavailability or expression of activity ?). ORI, and to some extent MXF or GAR show more efficacy and could be further assessed in appropriate *in vivo* models.

INTRODUCTION

S. aureus is responsible for recurrent infections, which are attributed to its opportunistic intracellular character (1). Intracellular forms are indeed protected not only from humoral host defenses but also from the action of antibiotics.

The selection of appropriate antibiotics to eradicate intracellular infections remains indeed challenging, because antibiotic activity is usually evaluated against extracellular bacteria.

Models of intracellular infection need therefore to be developed, which are as relevant as possible of in vivo situations. In this respect, THP-1 macrophages are considered as a valuable cell line to mimic human monocytes, while presenting the additional advantage of a more homogeneous population (2).

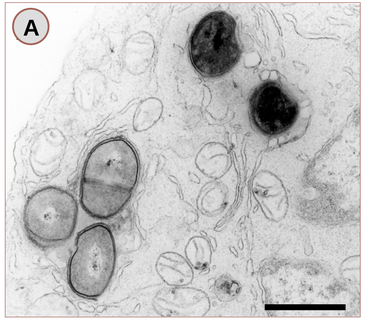
AIM OF THE STUDY

- To develop a model of 24 - h infection of THP-1 human macrophages by *S. aureus.*
- To compare the extracellular and intracellular activity of antibiotics in broth and in infected THP-1 macrophages using a concentration mimicking the Cmax reached in patients undergoing therapy with conventional dosages.

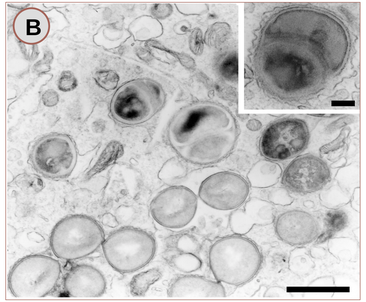
RESULTS

Electron microscopy: intracellular infection of THP-1 *by S. aureus*

1 h of incubation



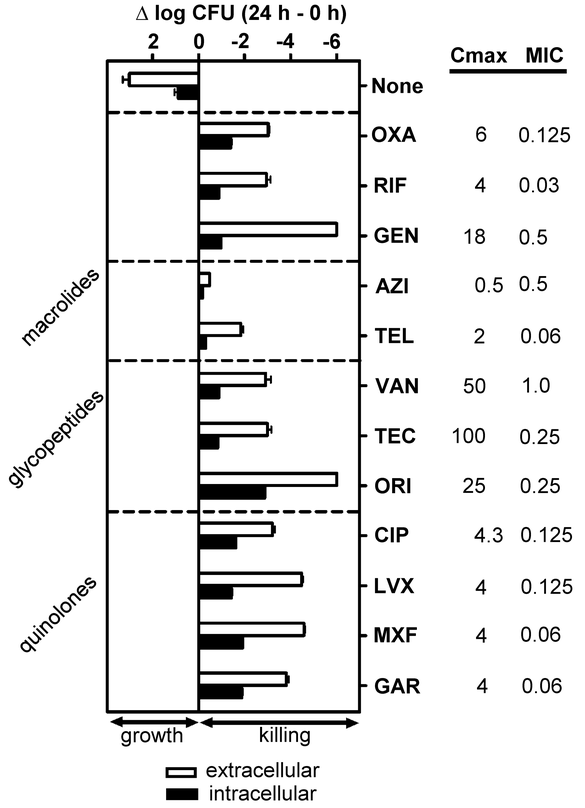
24 h of incubation



bars:
1 µm (inset : 200 nm)

RESULTS (Cont'd)

Extracellular and intracellular activity of antibiotics against *S. aureus*
(24 h incubation with antibiotics at their Cmax in human serum)



METHODS

- THP-1 cells were cultured in RPMI medium supplemented with 10 % foetal calf serum and 2mM L-glutamine, in a 5 % CO_2 atmosphere. They were used at a density of 0.5 10^6/ml.
- Infection of THP-1 was obtained by a 1 h incubation with serum-opsonized *S. aureus* ATCC 25923 (4 bacteria/cell), washing with 50 mg/L gentamicin and reincubation in fresh medium containing either the tested antibiotic or 0.5 mg/L gentamicin (control). This method is based on ref (3).
- CFU/mg cell protein were determined by plating cell lysates obtained after 24 h.
- Extracellular activity was evaluated by CFU counting after a 24 h incubation in MHB.

CONCLUSIONS

Model :

- Intracellular infection by *S. aureus* in an actively multiplying form was successfully obtained over a 24 h period.
- Infected THP-1 could therefore be used to evaluate the intracellular activity of antibiotics, including those characterized by a time-dependent activity or requiring bacterial growth to exert their activity.

Antibiotic activity :

- All antibiotics but azithromycin are bactericidal in broth.
- The activity of all antibiotics is lower intracellularly than extracellularly, probably due to inappropriate cellular bioavailability and/or expression of activity.
- Oritavancin, and to a lesser extent moxifloxacin and garenoxacin, show the best activity against both extra- and intra-cellular forms, and could therefore be evaluated in appropriate animal models.

REFERENCES

1. Waldvogel, F. A. (2000). *Staphylococcus aureus*. In *Principles and practice of infectious diseases*, 5th (Mandell, G. L., Bennett, J. E., & Dolin, R., Eds), pp. 2069-2092. Churchill Livingstone, Philadelphia, Pen.
2. Auwerx, J. (1991) The human leukemia cell line, THP-1: a multifacetted model for the study of monocyte-macrophage differentiation. Experientia 47, 22-31
3. Seral, C., Van Bambeke, F. & Tulkens, P. M. (2003). Quantitative analysis of gentamicin, azithromycin, telithromycin, ciprofloxacin, moxifloxacin, and oritavancin (LY333328) activities against intracellular *Staphylococcus aureus* in mouse J774 macrophage. Antimicrob.Agents Chemother. 47, 2283-2292.