Beta-lactams are bactericidal against intracellular forms of *S. aureus* in a 24-h human macrophages model (THP-1 cells)

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Background : Beta- lactams do not accumulate in eucaryotic cells and are usually reported as being poorly active in short-term incubation intracellular infection models. These, however, neglect the fact that etaB-lactams are time-dependent antibiotics. We have examined the intracellular and extracellular activities of 4 penicillins against *S.. aureus* over a 24-h period, using clinically-meaninful extracellular concentrations 1X MIC, 10X MIC, Cmax).

Methods: *S. aureus* ATCC 25923 (devoted of resitance mechanisms) was used throughout. Antibiotic efficacy towards extracellular (medium) and intracellular bacteria (post-phagocytosis) was determined by CFU counting.

Results:

			Change in log CFU from time 0 [a]			
			extracellular		intracellular	
AB	Ce (mg/L)	X MIC	3 h	24 h	3 h	24 h
None			0.6	3.0	0.1 [b]	1.1 [b]
NAF	0.25	1	- 0.7	- 2.9	0.0	- 0.8
	2.5	10	- 0.9	- 3.2	- 0.1	- 0.9
	40 [c]	160	- 1.3	- 3.8	- 0.2	- 1.3
Pen V	0.015	1	- 1.1	- 3.6	0.1	- 0.8
	0.15	10	- 1.2	- 3.7	- 0.1	- 0.9
	6.3 [c]	420	- 1.5	- 5.0	- 0.2	- 1.2
AMP	0.06	1	- 0.8	- 3.1	0.0	- 0.8
	0.6	10	- 1.0	- 3.6	- 0.1	- 0.9
	48 [c]	800	- 1.5	- 4.9	- 0.3	- 1.5
OXA	0.25	1	- 1.2	- 1.7	0.1	- 0.1
	2.5	10	- 1.6	- 2.8	- 0.1	- 0.5
	63 [c]	500	- 2.7	- 6.0	- 0.6	- 2.1

[a] Means of triplicates (SD < 0.3 log); [b] gentamicin 0.5 mg/L (1 X MIC) in controls only; [c] typical Cmax in humans

Extracellular activities were all time- and concentration-dependent. Intracellularly, all 4 penicillins were static at 3-h but caused a marked decrease in the post-phagocytosis inoculum at 24 h. This effect was also concentration-dependent, especially for AMPI and OXA

Conclusion: Beta-lactams show killing activities against intracellular *S. aureus*, provided time is taken into account. However, these activities remain lower than the extracellular ones.

INTRODUCTION

S. aureus is one of the main agents of infections in humans, often causing recurrent infections. These may be partly due to the opportunistic intracellular character of *S. aureus*, which is able to survive and multiply inside the host cells (1). Intracellularly indeed, bacteria can escape humoral host defenses and are partly protected from the action of antibiotics.

Beta-lactams are often considered as antibiotics of choice for *S. aureus* infections because of the possibility to select molecules with a narrow spectrum and because of their high therapeutic index. They are, however, not considered active against intracellular infections because they do not accumulate to high levels in eucaryotic cells. Yet, intracellular models usually do not take into account the fact that beta-lactams are antibiotics for which activity develops on a time-dependent manner.

METHODS

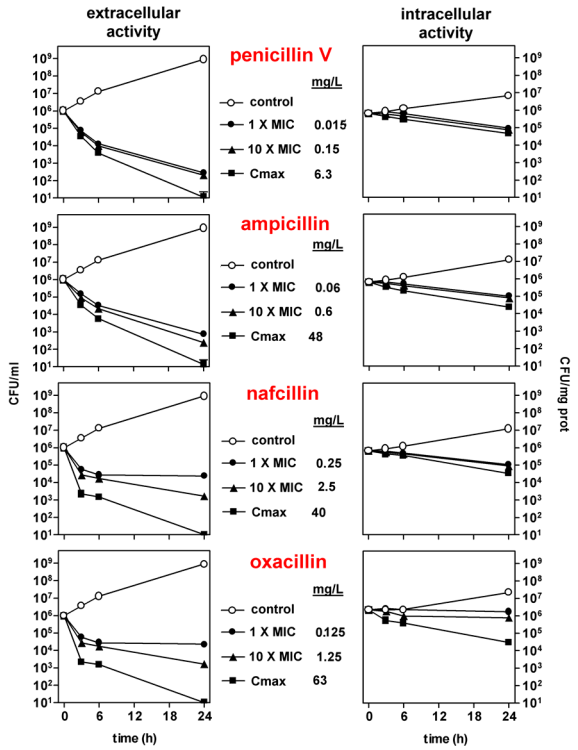
- Extracellular activity was evaluated by CFU counting after a 24 h incubation in culture medium.
- Intracellular activity: Infection of THP-1(2) was obtained by a 1 h incubation with serum-opsonized *S. aureus* ATCC 25493 (4 bacteria/cell), washing with 50 mg/L gentamicin and reincubation in fresh medium 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

AIM OF THE STUDY

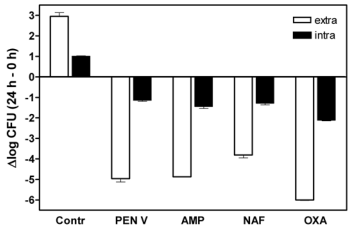
To compare the activity of 4 penicillins:
- penicillin V (PEN V)
- ampicillin (AMP)
- nafcillin (NAF)
- oxacillin (OXA)

- against the extracellular and intracellular forms of *S. aureus* (culture medium; THP-1 macrophages)
- using a range of therapeutically meaningful concentrations (from MIC to the human Cmax)
- over a 24-h incubation time

Extracellular and intracellular activity of Beta-lactams against *S. aureus:* time and concentration effects

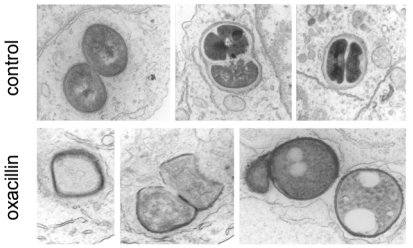


Bactericidal activity at 24 h and Cmax



comparison between extra- and intra-cellular activities

Demonstration of intracellular killing in electron microscopy



CONCLUSIONS

- Extracellularly, all tested penicillins show a time- and concentration-dependent bactericidal activity.
- Intracellularly, all penicillins were static at 3 h but slightly bactericidal at 24 h. This effect was concentration-dependent for ampicillin and oxacillin.

⇨ Despite their lack of accumulation, beta-lactams show a significant intracellular activity in cells exposed to high concentrations for prolonged times, suggesting that these antibiotics have a sufficient access to the infected compartment(s).

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