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Dosing the magic bullets against intracellular bacteria : lessons from a comparison between beta-lactams and quinolones against *L. monocytogenes* and *S. aureus*

Dosing the magic bullets

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ABSTRACT

Background : Ehrlich' s principle of chemotherapy points to the necessity for an antibiotic to reach its target. This becomes still most critical when dealing with intracellular infections. We have thus examined the pharmacodynamic properties of a beta-lactam and a quinolone against the extracellular and intracellular forms (THP-1 human macrophages) of an infection by *L. monocytogenes* (L.m.; cytosol) and *S. aureus* (S.a.; phagolysosomes). Data are discussed in the context of the determination of drug accumulation levels.

Methods : THP-1 cells were infected with L.m or opsonized S.a., and exposed to antibiotics for up to 24 h. Experiments in broth were run in parallel. Activity was expressed as change in CFU number/ml (broth) or /mg cell protein. Moxifloxacin and ampicillin were assayed by fluorimetry, and oxacillin, by microbiological assay.

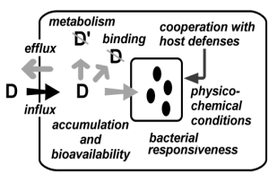
Results : The table shows the drug concentrations (in multiples of MIC) necessary to reach a static or cidal (-2 log) effect. Against L.m., ampicillin is more active intracellularly than in broth, even though its cell accumulation is ~1, whereas moxifloxacin, which accumulates 8-fold, is only as active as in broth. Against S.a., both types of drugs are less active intracellularly than in broth.

Antibiotic class	model	Concentration (X MIC) to reach a static / cidal effect			
		static	cidal	static	cidal
		L.m.		S.a.	
beta-lactam	Broth	10	> 100	0.5	5
	Cells	1	100	1	100
moxifloxacin	Broth	1	3	0.5	1
	Cells	1	3	0.8	3

Conclusions : Intracellular activity cannot be predicted on the simple basis of MIC and drug accumulation, probably due to the unpredictable influence of cell environment on antibiotic activity or bacterial responsiveness. Both beta-lactam and quinolone may prove useful for intracellular infections, provided sufficiently high concentrations are used.

INTRODUCTION

Reaching its target is the founding itself of the principle of chemotherapy enounced by Ehrlich. This apparently basic statement may become problematic when dealing with intracellular infections. In this case, indeed, the "Magic Bullet " used need to fulfill a series of pharmacokinetic and pharmacodynamic properties to be able to exert their pharmacological activity [1]. These include more importantly the capacity of the drug to rejoin the infected compartment at therapeutic concentration and to express its activity therein.



The importance for intracellular activity of time of exposure to the antibiotic or of drug concentration/MIC ratio, 2 parameters that can predict antibiotic efficacy extracellularly, is however not yet clearly established.

METHODS

All studies were performed using THP-1 human macrophages. These cells were infected by *L. monocytogenes* or *S. aureus* and exposed to antibiotics for up to 24 h, following the preocedure described by Carryn et al (L.m.) or Seral et al (S.a., with minor adaptations in this case for cells growing in suspension [2, 3]. Control cells were mainained in the continous presence of gentamicin at its MIC to prevent extracellular growth of bacteria released from died cells. Activity was calculated as the change in CFU/mg cell protein from the initial inoculum. Extracellular activity was determined in parallel by CFU counting.

Cellular concentration of ampicillin and moxifloxacin were determined by fluorimetry [4,5], that of oxacillin by microbiological assay using *Micrococcus luteus* ATCC9341 and Antibiotic Medium 2.

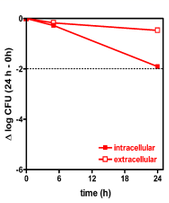
AIM OF THE STUDY

- To examine the influence of PK/PD parameters like time of exposure and concentration / MIC ratio on the activity of
  - beta-lactams, which are time-dependent antibiotics extracellularly and do not accumulate in cells
  - quinolones, which are concentration-dependent antibiotics extracellularly and do accumulate in the soluble fraction of cells,

  using in parallel a model of cytosolic *(L. monocytogenes*) and phagolysosomal infection (*S. aureus*).
- To compare extracellular and intracellular pharmacodynamics for these 2 classes of antibiotics.

RESULTS

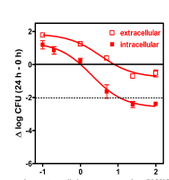
Listeria monocytogenes



AMPICILLIN

cellular to extracellular concentration ratio ~ 1

- time-dependent bactericidal activity extracellularly and intracellularly
- higher activity intracellularly than extracellularly



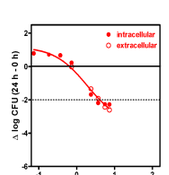
- static effect at 10 X MIC extracellularly and 1 X MIC intracellularly
- cidal effect (- 2 log) at 100 X MIC intracellularly, not reached extracellularly

Upper panel: Ce = human Cmax = 50 mg/L
Lower panel: time = 24 h; MIC = 0.4 mg/L

MOXIFLOXACIN

cellular to extracellular concentration ratio ~ 8

- time-dependent bactericidal activity extracellularly and intracellularly
- similar activity intracellularly than extracellularly



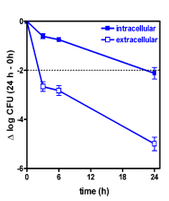
- static effect at 1 X MIC
- cidal effect (- 2 log) at 3 X MIC

Upper panel: Ce = human Cmax = 4 mg/L
Lower panel: time = 24 h; MIC = 0.6 mg/L

BETA-LACTAM

QUINOLONE

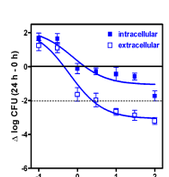
Staphylococcus aureus



OXACILLIN

cellular to extracellular concentration ratio < 4 (below the limit of sensitivity of the assay)

- time-dependent bactericidal activity extracellularly and intracellularly
- lower activity intracellularly than extracellularly



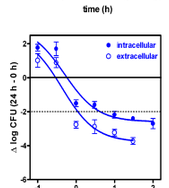
- static effect at 0.5 X MIC extracellularly and 1 X MIC intracellularly
- cidal effect (- 2 log) at 5 X MIC extracellularly and 100 X MIC intracellularly

Upper panel: Ce = human Cmax = 63 mg/L
Lower panel: time = 24 h; MIC = 0.125 mg/L

MOXIFLOXACIN

cellular to extracellular concentration ratio ~ 8

- time-dependent bactericidal activity extracellularly and intracellularly
- lower activity intracellularly than extracellularly



- static effect at 0.5 X MIC extracellularly and 0.8 X MIC intracellularly
- cidal effect (- 2 log) at 1 X MIC extracellularly and 3 X MIC intracellularly

Upper panel: Ce = human Cmax = 4 mg/L
Lower panel: time = 24 h; MIC = 0.06 mg/L

CONCLUSIONS

- Activities of both the beta-lactam and the quinolone studied develop on a time- and concentration-dependent manner against extracellular and intracellular forms of infection.
- Against *L. monocytogenes*,
  - the beta-lactam is more active intracellularly than extracellularly, despite its lack of accumulation
  - the quinolone is as active, but not more active intracellularly, eventhough it accumulates in cells
- Against *S. aureus*, both the beta-lactam and the quinolone are less active intracellularly, but can eventually reach a similar effect despite their difference in accumulation level.

➔Intracellular activity cannot be predicted on the simple basis of MIC value and antibiotic accumulation level.
The influence of cellular environment on both antibiotic activity and bacterial responsiveness needs to be further explored.

➔Both the beta-lactams and the quinolone appear potentially useful for eradicating intracellular infection, provided sufficient concentration (> 10-100 X MIC) and time of exposure can be obtained.

➔This study may constitute a first attempt in an approach aiming to determine intracellular breakpoints for antibiotics.

REFERENCES

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4. Carryn et al (2002) AAC 46: 2095-2103
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