Activity of 9 antibiotics against intracellular forms of *S. pneumoniae*

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Abstract

Objectives: Although often considered as an extracellular organism only, *S. pneumoniae* has been shown to invade and survive within eukaryotic cells in vitro (Jonsson et al., JID 1985, 152:4-13 ; Peppoloni et al., Microbes infect 2010] 12:990-1001). Intracellular foci of *S. pneumoniae* have also been observed in patients with persistent streptococcal infections (Coates et al. Otolaryngol Head Neck Surg 2008, 138:778-81). We have set up an in vitro model of macrophages infected by *S. pneumoniae* in order to quantitatively assess and compare the activity of antibiotics against these intracellular forms.

Methods. *S. pneumoniae* ATCC 49619 and THP-1 myelomonocytic cells were used throughout. MICs were determined in cation-adjusted Mueller Hinton broth supplemented with 2.5 % lysed horse blood. Infection of THP-1 cells was performed with opsonized *S. pneumoniae* incubated for 2 h at 37°C at a 10:1 bacteria:cell ratio. Non-phagocytized bacteria were eliminated by incubation with gentamicin (50 mg/L; 1 h) and 4 successive washings with PBS. Infected cells were then transferred for 24 h in fresh culture medium containing antibiotic concentrations ranging from about 1/100 to 100 x the MIC (full concentration-dependent effects), collected, lysed and used for cfu counting. The change in cfu was plotted against antibiotic concentration and used for fitting a Hill equation to determine the static concentration (Cs) and the maximal relative efficacy (Emax) of each antibiotic (Barcia-Macay et al. AAC 2006 50:841-51).

Results. The table shows the MICs and the intracellular Cs and Emax of the antibiotics ordered by increasing maximal relative efficacy (increasingly negative Emax). Cs varied from values close to the MIC (LZD, MXF, DAP, Q-D) to large multiples of MIC (AZM, CEM-101, RIF), and Emax from -1 log_{10} (AMX) to -3 log_{10} (Q-D).

Antibiotic	MICs (mg/L)	Intracell. Activity (24 h)		
		Cs [a]		Emax [b] (CI)
		mg/L	x MIC	
Amoxicillin (AMX)	0.03	~ 0.32	~ 11	-1.03 (-1.50 to – 0.56)
Linezolid (LZD)	1	~ 2.09	~ 2	-1.41 (-2.19 to -0.62)
Azithromycin (AZM)	0.004	~ 0.23	~ 58	-1.85(-2.24 to -1.47)
Rifampicin (RIF)	0.002	~ 0.38	~ 190	-1.96 (-2.04 to -1.88)
Moxifloxacin (MXF)	0.125	~ 0.46	~ 3	-2.1 (-2.56 to -1.64)
Finafloxacin (FNX)	1	~ 1.13	~ 1	-2.21 (-2.82 to -1.59)
Solithromycin (CEM-101)	< 0.0001	~ 0.03	> 300	-2.25 (-2.71 to -1.79)
Daptomycin (DAP)	0.5	~ 1.86	~ 4	-2.4 (-3.42 to -1.39)
Quinupristin-dalfopristin (Q-D)	0.5	~ 0.52	~ 1	-3.11 (-3.58 to -2.64)

[a] concentration (in mg/L or in X MIC) resulting in no apparent bacterial growth

[b] relative maximal efficacy (decrease [in log_{10} scale] in the number of cfu from the post-phagocytosis inoculum, as extrapolated for infinitely large concentration of antibiotics; Hill equation, slope factor of 1) with 95 % confidence interval

Conclusions. This model shows that the intracellular activity of antibiotics can only partially be predicted from the determination of their MIC in broth, and that only one antibiotic (Q/D) yields a truly intracellular bactericidal effect (defined as 3 log_{10} cfu decrease). Further studies examining the subcellular localization of the phagocytized bacteria and of the antibiotics may help in rationalizing these observations.

Background and aim

While long considered as an extracellular organism only, *S. pneumoniae* is now increasingly recognized as being able to invade and survive within eukaryotic cells *in vitro*.[1-3] This intracellular niche may provide a territory where the bacterium is largely protected from the lethal action of immune defenses and antibiotics, and may account for therapeutic failures and relapses.

In this context, we have developed a model of *S. pneumoniae* infected-macrophages to examine the intracellular activity of commonly-used anti-streptococcal antibiotics using a pharmacological approach.

Methods

Cells. Experiments were performed with human THP-1 cells, a myelomonocytic cell line displaying macrophage-like activity.[4]

Bacterial strain and susceptibility testing. *S. pneumoniae* strain ATCC 49169 was used throughout. MICs determinations were made in Mueller Hinton broth supplemented with 2.5% lysed horse blood following the CLSI guidelines.

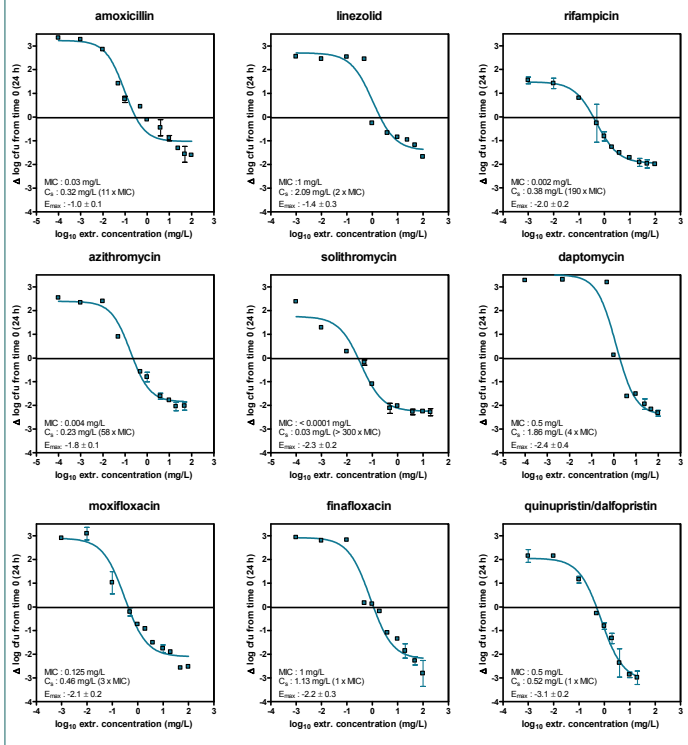
Cell infection and determination of the intracellular activities of antibiotics. Opsonization of bacteria was performed in cell culture medium containing 10 % human serum (45 min). Phagocytosis was initiated at a bacteria per macrophage ratio of 10, followed by elimination of non-phagocytised bacteria by exposing the cells to 50 mg/L gentamicin (30-45 min). Cells were then transferred to fresh cell culture medium supplemented with increasing concentrations of antibiotics.

Results. These are expressed as the change in the intracellular inoculum at 24 h compared to time 0. The data were used to fit a Hill equation to allow determining the values of two key pharmacological descriptors of antibiotic activity (see ref. 5 for details), namely:

- the relative maximal efficacy (E_{max}), corresponding to the decrease in the number of cfu from the original, post-phagocytosis inoculum, as extrapolated from an infinitely large concentration of antibiotics;
- the static concentration (C_s), corresponding to the concentration of antibiotic yielding no apparent bacterial growth.

Results

Intracellular activities of antibiotics



Dose-response curves of the 9 antibiotics tested towards *S. pneumoniae* phagocytized by human THP-1 macrophages. Antibiotic activities were determined after 24 h incubation in the presence of increasing concentrations of antibiotics, and the results (means ± standard deviations of three independent determinations) are expressed as the change in cfu compared to the initial, post-phagocytosis inoculum (time 0 h) (solitromycin: CEM101; quinupristin/dalfopristin: SYNERCID)

Intracellular activities differ markedly Between antibiotics, with :

1. static concentrations (C_s) at values close to the MIC for several antibiotics (linezolid, moxifloxacin and finafloxacin, quinupristin-dalfopristin) but at large multiples for others (rifampicin, azithromycin and solithromycin)
2. relative maximal efficacies (E_{max}) varying between -1 to -1.4 log_{10} (amoxicillin, linezolid) to -1.8 to 2.4 log_{10} (rifampicin, azithromycin, solithromycin, moxifloxacin, finafloxacin, daptomycin) and -3.1 for quinupristin/ dalfopristin.

Conclusions

- There is no apparent correlation between the known cellular accumulation of antibiotics (azithromycin and solithromycin > moxifloxacin > daptomycin) and their maximal relative efficacies against intracellular *S. pneumoniae*.
- amoxicllin eradicates most poorly intracellular S. pneumoniae in this model, whereas only quinupristin-dalfopristin yields a true bactericidal effect (- 3 log cfu).

References

(1) Ichakawa et al, *Proc Natl Acad Sci USA* (2000), 97:9659-9664
(2) Song et al, *Genomics* (2009), 93:72-82
(3) Song et al, *Can J Microbiol* (2008), 54:189-200
(4) Tsuchiya *et al*, *Intern. J Cancer* (1980), 26: 171-176
(5) Barcia-Macay et al, *Antimicrob Agents Chemother.* (2006),50:841-51.

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