Intracellular activity of fusidic acid against clinical isolates of *Staphylococcus aureus* of increasing MIC

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Abstract

Background:
Multiresistant *S. aureus* are of growing concern triggering the reassessment of older antibiotic classes. Fusidic acid is currently being reinvestigated for systemic administration, based on the low level of resistance observed in United States (Castanheira et al., AAC 2010, 54:3614-7), which is not the case in Europe (McLaws et al. AAC 2010 Dec 13. [Epub ahead of print]). Our aim was to examine the activity of fusidic acid against intracellular *S. aureus* of increasing levels of resistance to assess how extracellular and intracellular activities are related.

Methods:
MSSA ATCC 25923, MRSA ATCC 33591 (susceptible) and 3 clinical isolates of increasing MIC (see Figure) were used for (i) MIC determination (see values in the Figure); (ii) infection of human THP-1 macrophages. For studies with infected cells, phagocytosis was allowed for 1h at 37° C (after opsonization with human serum), extracellular bacteria were removed by washing and short term exposure to gentamicin, and cells were thereafter exposed for 24 h to a wide range of fusidic acid concentrations (from about 1/100 to 100 x the MIC) to obtain full concentration-effect relationships and determine the static concentrations (Cs), relative potencies (EC50) and maximal relative efficacies (Emax) (see Barcia-Macay et al, AAC 50:841-51).

Results:
The Figure shows that for all strains, dose-effect relationships could be modeled using a sigmoidal function (Hill equation; $R^2 > 0.902$). When data are plotted against weight concentrations (mg/L), curves align according to the MIC (increase in EC50 and in Cs). However, when data are plotted against multiples of the MIC, all curves become essentially indistinguishable, with non-significant differences in EC50, Cs and Emax ($P > 0.05$ [one-way ANOVA]).

Conclusions:
Contrary to what we recently observed with moxifloxacin, for which the intracellular activity is markedly decreased (with significant loss of Emax) once the MIC of the target MRSA exceeds 0.125 mg/L (Lemaire et al. JAC in press), the intracellular activity of fusidic acid is directly correlated to its extracellular activity as defined by the MIC. This shows that the intracellular growth of less susceptible intracellular *S. aureus* can still be controlled by fusidic acid providing its extracellular concentration is raised to reach an MIC/extracellular concentration ratio similar to that of the susceptible ones.

Background

Methicillin-Resistant *S. aureus* (MRSA) has become an increasing threat worldwide due to the emergence of highly virulent isolates and the ability of these organisms to acquire resistance mechanisms against whole classes of currently-used antibiotics by genome expansion. These issues have highlighted the need to developing novel antistaphylococcal agents but also to reassess older drugs acting on *S. aureus* by other mechanisms than those used by more recent molecules.

In this context, fusidic acid exhibits a low toxicity and potent anti-staphylococcal activity against recent MRSA isolates, irrespective of their resistance to others antibiotic classes.[1] We also showed that this compound actually displays significant activity towards the intracellular forms of fusidic acid-susceptible strains.[2]

Aims of the study

- To quantitatively assess the level of intracellular activity of fusidic acid towards clinically-relevant MRSA organisms with increasing MIC values in a pharmacodynamic model
- To use this information to attempt defining an intracellular susceptibility breakpoint

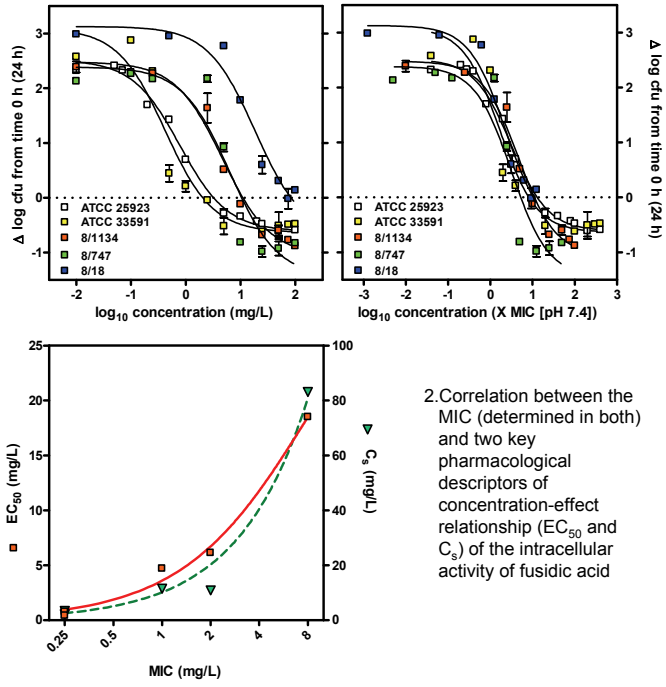
Methods

- **Bacterial strains and susceptibility testing.**
  MSSA ATCC 25923 (0.25 mg/L), HA-MRSA ATCC 33591 (0.25 mg/L), and 3 Belgian MRSA isolates (1, 2 and 8 mg/L). MIC were determined by microdilution in MH broth.
- **Cells**
  THP-1 cells (ATCC TIB-202), a human myelomonocytic cell line displaying macrophage-like activity.[3,4]
- **Cell infection**
  Infection was performed as described previously.[5-6] Briefly, phagocytosis of opsonized bacteria was allowed to take place during 1 h using a 4:1 bacteria:macrophage ratio. Extracellular bacteria were removed by a short term incubation with 50 mg/L gentamicin and 3 successive washings with PBS. Starting bacterial inoculum typically ranged between ~ 1 to 2.5 x10^6 CFU/mg prot.
- **Assessment of intracellular activity**
  Infected cells were exposed to antibiotic concentrations ranging from about 0.001 to 1,000 the MIC and the change in the inoculum observed after 24 h compared to the post-phagocytsois value (time 0 h).
- **Curve fitting analysis.**
  Data were used to fit a Hill equation (sigmoid; slope factor of 1), allowing to calculate 3 key pharmacological descriptors of antibiotic efficacy, namely

- the relative maximal efficacy (E_{max} [in $\log_{10}$ units], the change in the number of cfu for an infinitely large concentration of antibiotic);
- the relative potency (EC_{50}, i.e. the concentration of antibiotic yielding a reduction of the bacterial inoculum halfway between E_{min} [cfu increase at an infinitely low antibiotic concentration]) and E_{max});
- and the static concentrations (C_s, i.e. the concentration of antibiotic causing no apparent change compared to the original inoculum).

Results

Intracellular activity of fusidic acid



1. Dose-response curves of fusidic acid towards the intracellular forms of S. *aureus* phagocytized by human THP-1 macrophages.

   The ordinate shows the change in cfu (means ± SD; 3 independent determinations) per mg of cell protein compared to the initial, post-phagocytosis inoculum after 24 h incubation in the presence of the antibiotic. The abscissa shows the antibiotic concentration (total drug) expressed as mg/L (left-hand panel) or in MIC mutiples (right-hand panel; MICs of fusidic acid: ATCC 25923, 0.25 mg/L; ATCC 33591, 0.25 mg/L; 8/1134, 1 mg/L; 8/747, 2 mg/L; 8/18, 8 mg/L). The dotted line indicates a static effect (no apparent change from the original inoculum).

2. Correlation between the MIC (determined in both) and two key pharmacological descriptors of concentration-effect relationship (EC_{50} and C_s) of the intracellular activity of fusidic acid

References

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Conclusions

- The present study confirms that fusidic acid is active against intracellular *S.aureus* with not significant difference between MSSA and HA-MRSA if displaying the same MIC.
- The intracellular activity of fusidic acid is dependent from the MIC (measured in broth) with a marked decrease of the pharmacological parameters examining the concentration-relationship of the response (EC_{50}, C_s) once the MIC is > 2 mg/L. Conversely, E_{max} seems unaffected (with the strains investigated).
- This suggests an intracellular breakpoint at 2 mg/L, which is close to the EUCAST breakpoints for systemic infections ($S \leq 1$ mg/L - $R > 1$mg/L)

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