Interferon-gamma (IFN-γ) Potentiates the Intracellular Activity of Rifampin (RIF) towards Intracellular Staphylococcus aureus in Human THP-1 Macrophages.

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Abstract (revised)

Background. . Treatment of severe staphylococcal infections remains challenging, probably in relation with the ability of this bacterium to survive intracellularly. We showed previously that the intracellular activity of many antibiotics, including rifampin, is considerably lower than expected when compared to the extracellular one (CMI 2008, 14:766-77), suggesting that novel alternatives are needed to help eradicating intracellular *S. aureus* foci. Our aim was to assess the potential use of IFN-γ when combined with rifampin in a validated model of intracellular infection (AAC 2006, 50:841-51).

Methods. Bacteria: *S. aureus* strain ATCC 25923 (MIC of RIF: 0.01 mg/L [MHB; microdilution]). Intracellular activity of RIF: measured as the change in cfu observed after 24 h incubation of cells with increas. concentr. of RIF (0.05-20 mg/L) compared to the post-phagocytosis inoculum (= control response). Activity of increasing conc. of IFN-γ (0.01-5 µg/L): measured as the increase in killing effects for each selected RIF conc. (IFN-γ added 24 h prior to infection and maintained throughout phagocytosis and exposure to RIF).

Results. Results were shown in the Figure. At all RIF concentrations, IFN-γ showed additive effect (gain of about 2 $\log_{10}$ cfu decrease at the highest conc. tested).

Conclusions. IFN-γ improves the activity of rifampin against intracellular *S. aureus* in this *in vitro* model, which may justify further *in vivo* studies.

Background and aims

***S. aureus* can cause severe, recurrent and difficult-to-treat infections such as endocarditis, osteomyelitis and complicated skin infections. This may be ascribed to the ability of this bacterium to survive and multiply intracellularly within eukaryotic cells,[1-2] which confer to the bacterium an intracellular niche against the lethal action of antibiotics and immune defenses.**

Because the activity of antibiotics (including rifampin) remains only limited towards the intracellular forms of S. *aureus* ([3-4]), we now aimed at examining the potential use of IFN-γ when combined to rifampin in a validated model of intracellular infection.[3]

Methods

Bacterial strains, susceptibility testings and dose-kill studies in acellular media.

We used *S. aureus* strain ATCC 25923 for all experiments. MICs (microdilution) and dose-kill studies in acellular media were determined in MH broth following the methods described previously [3-4].

Cells and cell infection.

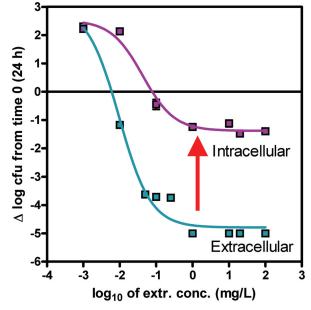
THP-1 cells (ATCC TIB-202), a human myelomonocytic cell line displaying macrophage-like activity, was used in our experiments. Infection was performed as described previously.[3-4] Briefly, phagocytosis of opsonized bacteria was allowed to take place during 1 h using a 4:1 bacteria:macrophage ratio. 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 antibiotic activity.

Extracellular (MH broth) and intracellular (THP-1) bacteria were exposed to increasing concentrations of antibiotics and the change in the inoculum was observed after 24 h compared to the initial inoculum (time 0 h)[3-4]. Data from the Dose-responses curves were used to fit a sigmoidal function (Hill function)[3-4].

Results

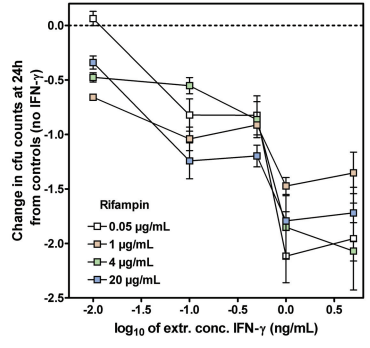
A) Comparative activity of rifampin towards the extracellular and intracellular forms of *S. aureus*



Influence of increasing concentrations of rifampicin (0.001 to 100 µg/mL) on the extracellular (MH broth) and intracellular (THP-1) activity of rifampin towards *S. aureus* strain ATCC 25923. Each point is the mean ± SD of three independent determinations.

Rifampicin acts only poorly against the intracellular forms of *S. aureus*, as compared to the extracellular ones

B) Effect of increasing conc. of IFN-γ on the activity of rifampicin towards the intracellular forms of *S. aureus*



Influence of increasing conc. of IFN-γ (0.01 to 5 ng/mL) on the activity of rifampin towards the intracellular forms of *S. aureus*, as determined for each conc. indicated in the graph. Results shown are the differences between values without and with IFN-γ. Each point is the mean ± SD of three independent determinations.

We observed an enhanced intracellular activity of rifampin when combined to IFN-γ suggestive of an additive effect

Conclusions

- **We observed a decreased activity of rifampicin towards the intracellular forms of *S. aureus*, as compared to the extracellular ones.**
- **When combined to rifampicin, IFN-γ (1-5 ng/mL) markedly increases the intracellular activity of the antibiotic, suggestive of an additive effect. This may need to be further explored in *in vivo* studies.**

This poster will be made available for download after the meeting: http://www.facm.ucl.ac.be/posters.htm

References

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