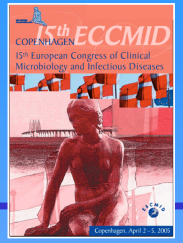

Ciprofloxacin (CIP) accumulation and intracellular activity against *L. monocytogenes* are reduced in ciprofloxacin-resistant macrophages due to increased efflux

M. Heremans, M.P. Mingeot-Leclercq, P.M. Tulkens, and F. Van Bambeke

Unité de Pharmacologie cellulaire et moléculaire, Université catholique de Louvain - Brussels - Belgium

Mailing address:
F. Van Bambeke
UCL 73.70 av. Mounier 73
1200 Brussels - Belgium
vanbambeke@facm.ucl.ac.be

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ABSTRACT

Objectives: CIP is substrate for an MRP efflux pump in J774 mouse macrophages (Michot et al, AAC 48:2673-82), which reduces its cellular accumulation. We have recently shown that CIP-"resistant" macrophages can be selected upon chronical exposure of these cells to CIP, in which CIP accumulation becomes negligible because of an increased activity and/or expression of the efflux transporter (ICAAC 2004, abstract A-1304). We have now examined whether this reduced accumulation affects CIP intracellular activity using a model of intracellular infection by *L. monocytogenes (L.m.)* and comparing resistant cells to the wild type parent cell line.

Methods: Infection was carried out using a bacteria:macrophage ratio of 7:1, according to the procedure described in Seral et al (JAC 51:1167-73). CFU/mg cell protein was determined after 5 h exposure to increasing concentrations of CIP +/-10 mM probenecid (inhibitor of MRP transporters). CIP cellular concentration was measured by fluorimetry (AAC 48:2673-82).

Results: In wild cells, an extracellular concentration corresponding to 3 X the MIC was sufficient to obtain a static effect, and this value rose to 30 X in CIP-resistant cells. This value was decreased to 1 X and 1.5 X the MIC, for wild and CIP-resistant cells respectively, in the presence of probenecid. In cells incubated with 20 mg/l CIP (10 X MIC), CIP accumulation was 4.8 ± 0.3 and 0.3 ± 0.1 in wild and CIP resistant cells, but increased to 21.3 ± 0.8 and 10.6 ± 0.5 in the presence of probenecid.

Conclusions: Increase in expression and/or activity of the CIP efflux transporter causes a reduction of the antibiotic efficacy against intracellular infection, which can be reversed upon inhibition of the efflux transporter.

INTRODUCTION

Active efflux is a general mean developed by cells for protection against invasion by diffusible molecules such as drugs (1).

Accordingly, active efflux from bacteria has been described as a wide-spread mechanism of resistance to antibiotics.

Eukaryotic cells also express multidrug efflux transporters, which are well known to confer resistance to anticancer agents. But these transporters present a very large substrate specificity. It is therefore not surprising that ciprofloxacin is actively transported out of J774 macrophages by pumps possessing MRP-like properties (2).

We have recently shown that chronic exposure of these macrophages to high ciprofloxacin concentrations selects a "resistant" phenotype, characterized by a drastic reduction in the cellular accumulation of ciprofloxacin, probably in relation with an increased expression/activity of the ciprofloxacin-transporter (3).

Quinolones are however considered as antibiotics of choice for the treatment of intracellular infection. Reduction in their cellular concentration as a consequence of active efflux from macrophages may make this activity suboptimal.

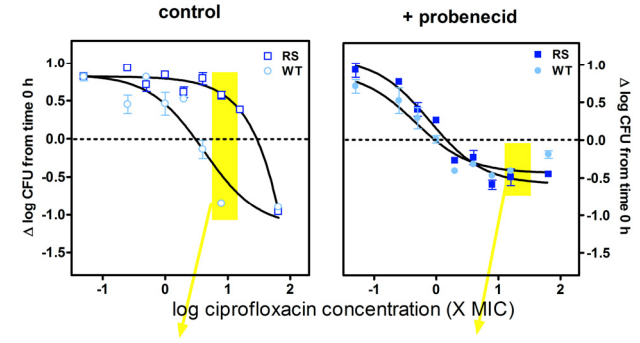
AIM OF THE STUDY

To evaluate the efficacy of ciprofloxacin

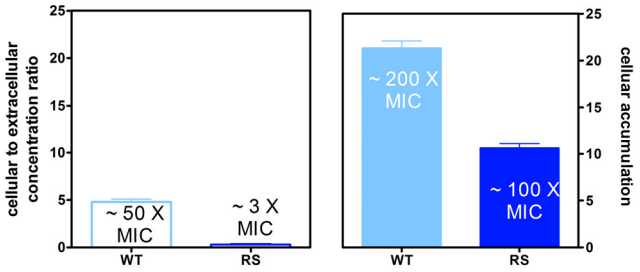
- against intracellular *Listeria monocytogenes*
- in wild-type macrophages and in resistant macrophages (overexpressing the ciprofloxacin transporter).

RESULTS

intracellular activity



cellular accumulation



Upper panels: intracellular activity of ciprofloxacin. J774 mouse macrophages were infected by *L. monocytogenes* and then exposed for 5 hours to ciprofloxacin at extracellular concentrations covering a large range of X MIC (MIC is 2 mg/L).
Lower panels: cellular accumulation of ciprofloxacin in J774 macrophages incubated with 20 mg/L ciprofloxacin (10 X MIC).

LEFT: control conditions (ciprofloxacin alone)
RIGHT: probenecid 10 mM was added during the incubation with the antibiotic

WT: wild-type macrophages; **RS**: ciprofloxacin-resistant macrophages

- in control conditions,
  - ciprofloxacin shows a static effect at 3 X MIC in wild-type cells but at 30 X MIC in resistant cells
  - ciprofloxacin cellular concentration is about 50 X MIC in wild-type cells but only 3 X MIC in resistant cells
- in the presence of probenecid,
  - ciprofloxacin shows a static effect at about 1 X MIC in both cell types
  - ciprofloxacin cellular concentration largely exceeds the MIC for both cells types

METHODS

- **selection of ciprofloxacin-resistant macrophages**: these cells were obtained by cultivating J774 macrophages for several months in the continuous presence of increasing concentrations of ciprofloxacin, so as to obtain cells growing normally with 68 mg/l ciprofloxacin (3).
- **intracellular infection** of J774 macrophages by *Listeria monocytogenes* was performed using an initial inoculum of 7 bacteria per macrophage, and following the procedure described by Seral *et al.* (4).
  We checked in preliminary experiments that probenecid did not influence the intracellular growth of the bacteria.
- **determination of cellular accumulation of ciprofloxacin:** cellular concentration of ciprofloxacin was measured by fluorimetric assay, as described by Michot *et al.* (2). Cellular accumulation was then calculated, considering that 1 mg cell protein in J774 macrophages corresponds to 3.08 µl cell volume (2).

CONCLUSIONS

- Ciprofloxacin shows a concentration-dependent activity against intracellular *Listeria monocytogenes*. The fact that its cellular concentration is drastically reduced in resistant cells therefore explains its lack of activity in this model.
- Inhibition of active efflux proves a useful strategy not only to restore intracellular activity in resistant cells, but also to make the drug active at lower extracellular concentrations in wild-type cells.
- Overexpression of efflux pumps would probably be more easily selected *in vivo* upon exposure to more toxic drugs, like anticancer agents. The present data therefore call for caution regarding the consequences of a potential cross-resistance with antibiotics for their efficacy against intracellular infections.

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