Synergy between bacterial Lde and macrophage MRP efflux pumps markedly reduces the activity of ciprofloxacin but not that of moxifloxacin against intracellular *Listeria monocytogenes*

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Background: CIP is a substrate for the Lde efflux pump in *L.m.* (AAC **47**:704) and for an MRP efflux pump in J774 macrophages (AAC **48**:2673). In contrast, MXF is not substrate for these pumps. We have studied the potential cooperation between Lde and MRP in reduction of CIP activity towards intracellular bacteria by using wild-type (EGD) and Lde-overexpressing (CLIP) *L.m.*, and wild-type (WT) and MRP-overexpressing (RS) macrophages.

Methods: MICs were determined without and with reserpine (R; inhibitor of Lde). Infection of macrophages by *L.m.* was performed as described (JAC 55:511). Infected cells were exposed for 24 h to 4.3 mg/L of CIP or 4 mg/L of MXF alone or combined with 20 mg/L of reserpine or 15 mM of probenecid (P; inhibitor of MRP).

Results: CIP intracellular activity was impaired by expression of Lde and by that of MRP. Reserpine and probenecid acted in synergy to restore, but only partially, CIP intracellular activity. MXF activity was not significantly affected by overexpression (as tested by ANOVA) of either of these pumps, neither by pump inhibitors.

condition	Difference log CFU from time 0			
	WT macrophages		RS macrophages	
	EGD	CLIP	EGD	CLIP
CIP	-1.4 ± 0.1	3.5 ± 0.1	2.0 ± 0.1	2.8 ± 0.3
CIP + R	-2.3 ± 0.4	1.2 ± 0.4	-0.9 ± 0.3	2.3 ± 0.1
CIP + P	-1.5 ± 0.2	1.2 ± 0.1	-0.2 ± 0.1	1.7 ± 0.1
CIP + R + P	-2.6 ± 0.2	-0.7 ± 0.3	-1.6 ± 0.1	0.7 ± 0.1
MXF	-2.8 ± 0.1	-2.4 ± 0.3	-2.8 ± 0.1	-2.5 ± 0.5
MXF + R + P	-2.8 ± 0.5	-1.7 ± 0.3	-2.1 ± 0.1	-2.1 ± 0.1
Control growth: 3-4 log				
MICs (-R/+R): CIP: 1.2/1.2 for EGD; 5/1 for CLIP; MXF: 0.6/0.6 for EGD; 0.5/0.25 for CLIP				

Conclusion: Lde is expressed in both extracellular and intracellular *L.m.* Cooperation with MRP transporter renders CIP ineffective against intracellular *L.m.* MXF retains full activity, not being a substrate of either of these transporters.

INTRODUCTION

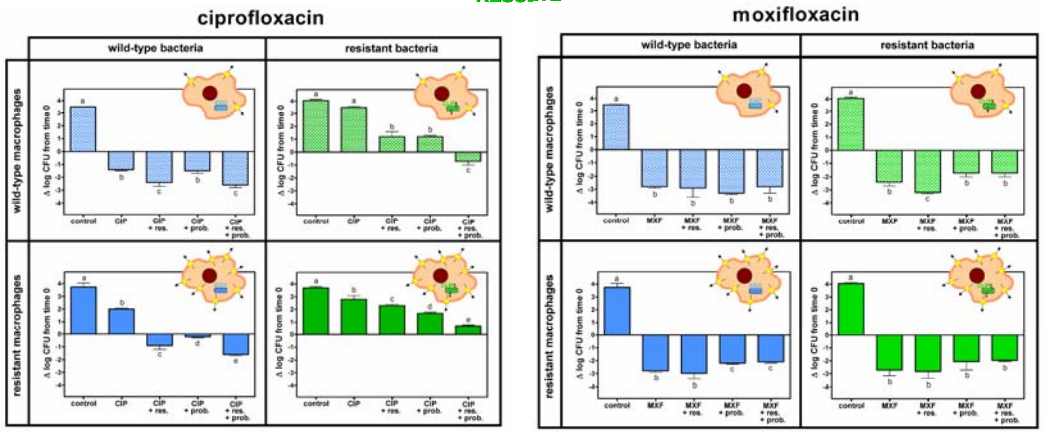
- Active efflux is an ubiquitous mechanism of resistance.
  - In bacteria, efflux pumps impair antibiotic activity by reducing their concentration in contact with the bacterial target [1].
  - In eucaryotic cells, efflux pumps reduce the accumulation of many drugs, including antibiotics. This may interfere with the activity of antibiotics against intracellular bacteria [2].
- Quinolone are well-established substrates of efflux pumps, both in bacteria and in eucaryotic cells, ciprofloxacin being a preferential substrate compared to moxifloxacin.
  - in *Listeria monocytogenes,* ciprofloxacin is effluxed by the Lde pump expressed in resistant strains of [3]. This pump is inhibitable by reserpine.
  - in J774 macrophages, ciprofloxacin, but not moxifloxacin, is effluxed by an MRP-like pump (over-expressed in cells made resistant to ciprofloxacin by chronic exposure to high concentrations of this drug [4]. This pump is inhibitable by probenecid.
- *Listeria monocytogenes* causes intracellular infections. In models of infected macrophages, quinolones are among the most active drugs [5].

AIM OF THE STUDY

To compare the intracellular activity of ciprofloxacin and moxifloxacin towards *Listeria monocytogenes*, using in parallel

- a wild-type strain (EGD), which does not express the quinolone efflux pump Lde, and a resistant strain (CLIP), which expresses the quinolone efflux pump Lde [3].
- wild-type macrophages, which express the quinolone efflux pump at a basal level, and ciprofloxacin-resistant macrophages, which overexpress the quinolone efflux pump [4].

RESULTS



Intracellular activity of ciprofloxacin (CIP) and moxifloxacin (MXF) towards *L. monocytogenes* wild-type (EGD; left panels) or resistant (CLIP; right panels) infecting wild-type macrophages (upper panels) or ciprofloxacin-resistant macrophages (lower panels). Infected cells were incubated during 24 h in the presence of the antibiotic alone (CIP: 4.3 mg/L; MXF: 4 mg/L [human C_{max}]) or together with 20 mg/L reserpine (res.), 15 mM probenecid (prob.) or their combination. Results are expressed as the change in the number of CFU/mg protein as compared to the initial inoculum. Data are means ± SD (n= 3). Statistical analysis (ANOVA): bars with different letters are significantly different from one another in each panel ($p < 0.05$).

quinolone	MIC (mg/L)			
	EGD		CLIP	
	Res. (-)	Res. (+)	Res. (-)	Res. (+)
CIP	1.2	1.0	5.0	1.0
MXF	0.6	0.6	0.5	0.25

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MATERIALS & METHODS

- **Bacteria:** we used the EGD strain (wild-type strain; received from P. Berche, *Service de microbiologie, Hôpital Necker- Enfants-malades*, Paris, France) and CLIP21369, a clinical isolate resistant to ciprofloxacin [3].
- **Extracellular activity:** MICs were determined by arithmetic dilutions in TSB, in the absence or in the presence of 20 mg/L reserpine.
- **Intracellular activity:** Intracellular infection was obtained by a 1 h incubation with bacteria (5 bacteria/ macrophage), extensive washing and reincubation in fresh medium containing the tested antibiotic, combined with pump inhibitors. Gentamicin (1x MIC) was added to controls to avoid extracellular contamination. CFU/mg cell protein were determined by plating cell lysates and measuring their protein content [4].

DATA DESCRIPTION

ciprofloxacin

- Towards EGD, CIP is less active in resistant macrophages.
- Towards CLIP, CIP is inactive in both cell lines.
- Towards EGD
  - and in wild-type macrophages, reserpine alone or combined with probenecid slightly improves CIP activity.
  - and in resistant macrophages, pump inhibitors have additive effects on CIP activity, making CIP as active as in wild-type macrophages in the absence of inhibitors.
- Towards CLIP,
  - reserpine and probenecid have additive effects on CIP activity in both cells lines;
  - however, even in the presence of the combined inhibitors, CIP is only static is wild-type macrophages and remains unable to prevent intracellular growth in resistant macrophages.

moxifloxacin

- MXF is highly active against both susceptible and resistant bacteria in both cell lines (2 to 3 log decrease from the initial inoculum).
- None of the pump inhibitors does markedly affect this activity.

CONCLUSIONS

- Bacterial (Lde) and macrophage (MRP-like) efflux pumps cooperate to reduce CIP intracellular activity against *Listeria monocytogenes*.
- Being no substrate for either of the pumps, MXF retains full activity in all cases.
- this study shows that screening for antibiotic efflux in both bacteria and eucaryotic cells may be important for a correct appraisal of the therapeutic response.