Induction of Fluoroquinolone (FQ) Efflux in *Streptococcus pneumoniae* by Subinhibitory Ciprofloxacin (CIP) Concentrations

L. Avrain,[1] N. Mesaros,[1] R. Vanhoof,[2] F. Van Bambeke,[1] and P.M. Tulkens.[1]

[1] Unité de pharmacologie cellulaire et moléculaire, Université catholique de Louvain, Brussels, Belgium

[2] Antibiotica resistentie en nosocomiale infecties, Instituut Pasteur, Brussels, Belgium

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Corresponding author :
Cellular and Molecular Pharmacology Unit – UCL 7370
1200 Brussels - Belgium
laetitia.avrain@facm.ucl.ac.be

ABSTRACT

Purpose: Efflux is an important mechanism of resistance towards FQ in *S. pneumoniae*. We have examined whether subinhibitory CIP concentrations favours emergence of this type of resistance.

Method: A clinical isolate of *S. pneumoniae* with a low expression of efflux pump, was exposed to half MIC of CIP, for 13 days followed by 21 days without antibiotic (reversion). MICs (arithmetic dilutions; agar medium) of CIP, levofloxacin (LVX), moxifloxacin (MXF), garenoxacin (GRN), and gemifloxacin (GEM) were then determined with and without reserpine (RES), a known inhibitor of PmrA pump.

Results: We observed a decreased susceptibility to CIP and LVX (partially antagonized by RES; reversible after 21 days without CIP), with no or only minor changes for MXF, GRN, and GEM.

Conclusion: Subinhibitory concentrations of CIP easily select for transient efflux overexpression in *S. pneumoniae* that also affects LVX.

AIM OF THE STUDY

- ✓ **to determine whether exposure of *S. pneumoniae* to sub-MIC concentrations of ciprofloxacin triggers the development of efflux-mediated resistance**
- ✓ **to evaluate the persistence of this type of resistance phenotype upon relief of ciprofloxacin pressure**

METHODS

Abbreviations: CIP, ciprofloxacin; LVX, levofloxacin; MXF, moxifloxacin; GRN, garenoxacin; GEM, gemifloxacin.

MICs determinations: MICs were determined by arithmetic dilutions in Mueller Hinton agar supplemented with 5% of Horse blood) with and without reserpine (RES), an inhibitor of pmrA.

Induction of resistance: *S. peumoniae* strains adjusted to 5.10^6 CFU/ml in Mueller Hinton broth supplemented with 10% of lysed horse blood, were exposed to increasing ciprofloxacin concentrations from 0.5 to 6 µg/ml for 24 h at 37°C. Bacteria growing at a CIP concentration equal to half the original MIC were collected and their MIC determined. Bacteria were then grown for 24 h in the presence of CIP at a concentration equal to half of this MIC. This was reapeated daily for 13 days.

Persistence: Bacteria growing by half the MIC observed at day 13 were incubated in the absence of CIP for 21 days.

CONCLUSION

- ✓ **Subinhibitory concentrations of CIP easily select efflux overexpression in *S. pneumoniae***
- ✓ **CIP and LVX are both affected by efflux mechanism**
- ✓ **Efflux co-operates with other resistance mechanism**
- ✓ **Efflux-mediated resistance is transient**

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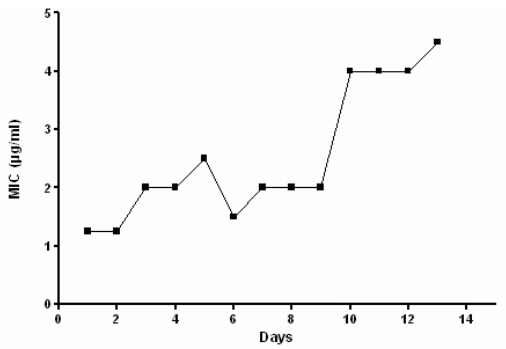
INTRODUCTION

- *Streptococcus pneumoniae* is the main bacterium causing community-acquired pneumonia.
- Resistance of *S. pneumoniae* by efflux is now well documented for fluoroquinolones (Brenwald at al., 1998; Gill et al., 1999), involving the inducible transporter pmrA. CIP is a substrate of this transporter.
- Suboptimal antibiotic treatment may trigger the development of efflux-mediated resistance to fluoroquinolones (Lamovskaya et al., 1999). We have examined here how exposure of *S. pneumoniae* to sub-MIC concentrations of CIP could trigger efflux of CIP, LVX, MXF, GAR, and GEM.

RESULTS

Typical increase in MIC of *S. pneumoniae* (SP 32 strain) towards CIP upon successive 24h incubations in the presence of CIP at concentrations equal to half the MIC observed each day



Characterization of the change in MIC with respect to the fluroquinolones and the addition of reserpine, during the induction in the presence of CIP and during the recovery period.

Days of exposure	MIC (mg.L^{-1})									
	CIP		LVX		MXF		GRN		GEM	
	-	+	-	+	-	+	-	+	-	+
0 (wild type)	1	0.5	0.75	0.75	0.15	0.15	0.05	0.05	0.04	0.03
3	1	0.5	0.75	0.75	0.15	0.1	0.05	0.05	0.04	0.03
10	4	1	1.5	1	0.2	0.2	0.1	0.1	0.06	0.04
13	4	1	1.5	1	0.2	0.2	0.1	0.1	0.06	0.04
13+2 days (rev.)	4	1	1.5	1	0.2	0.2	0.1	0.1	0.06	0.04
13+7 days (rev.)	1	0.5	0.75	0.75	0.15	0.1	0.05	0.05	0.04	0.03
13+21days (rev.)	1	0.5	0.75	0.75	0.15	0.15	0.05	0.05	0.04	0.02

- ➢ Within 10 days of exposure to CIP at half its MIC, the susceptibility of *S. pneumoniae* to CIP, LVX, MXF, GRN, and GEM was decreased by a factor of 4, 2, 1.33, 2 and 1.5, respectively.
- ➢ A reserpine effect was observed for CIP, LVX, and GEM (4-, 1.5-, and 1.5-fold decreases in MIC), indicating the intervention of a pmrA -like mechanism of efflux.
- ➢ Reserpine (10 mg/L), however, did not fully restore the original susceptibility, suggesting the occurrence of other mechanism(s) of resistance. This reserpine-independent decrease in susceptibility (1.33 to 2-fold) was seen for all 5 quinolones, suggesting target mutation.
- ➢ Both reserpine-dependent and -independent resistances receded after 7 days of culture in the absence of ciprofloxacin.

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