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DnpA, a putative de-*N*-acetylase, increases intracellular persistence upon fluoroquinolone exposure in *Pseudomonas aeruginosa*

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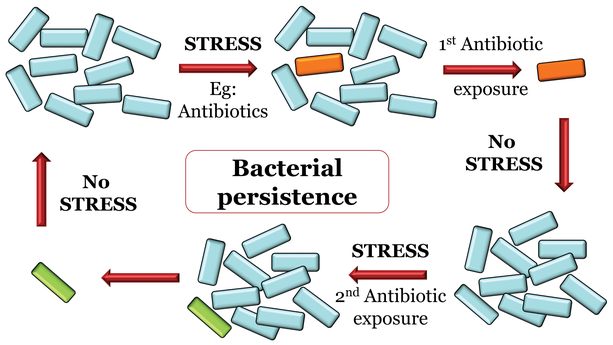
Introduction

Bacterial persisters are characterized as genetically drug susceptible quiescent organisms that

- Transiently survive exposure to a given antibiotic
- Revive under specific conditions

Persisters are now recognized as a major cause of antibiotic treatment failure.



The opportunistic human pathogen *Pseudomonas aeruginosa* is capable of invading epithelial and phagocytic cells. Intracellularly, the bacteria are less responsive to antibiotics[1].

The putative de-*N*-acetylase DnpA has been shown to increase persister levels in *P. aeruginosa*, conferring tolerance specifically to fluoroquinolones in broth[2].

A possible role of persisters in recalcitrance of intracellular *P. aeruginosa* to antimicrobial agents has not yet been documented.

Objectives

To assess the putative role of DnpA in the poor responsiveness of intracellular *P. aeruginosa* to antibiotics, using:

- **Fluoroquinolones:** Ciprofloxacin, Levofloxacin, Moxifloxacin
- **Beta-lactams:** Meropenem
- **Aminoglycosides**: Amikacin

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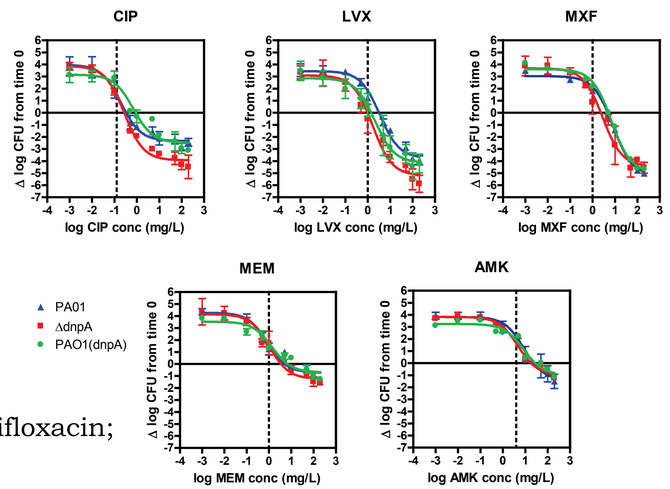
Results

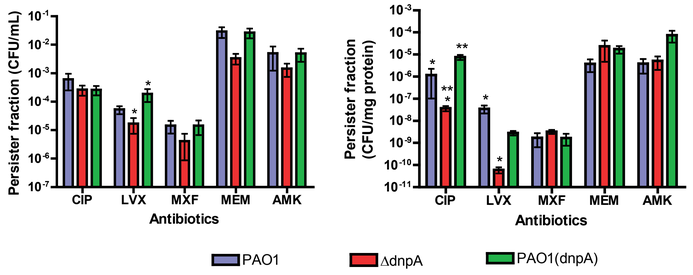
Concentration-response curves of antibiotics against intracellular *P. aeruginosa* strains.

The graphs show the changes in the number of CFU per milligram of cell protein in THP-1 cells after 24 h of incubation at increasing extracellular concentrations of antibiotic. Higher E_{max} (more negative) value indicates less persisters

Data are means ± standard deviations (n=3). Vertical dotted line indicates minimum inhibitory concentration value in broth. Horizontal solid line indicates bacteriostatic effect.

(CIP: Ciprofloxacin; LVX: Levofloxacin; MXF: Moxifloxacin; MEM: Meropenem; AMK: Amikacin)





Relative persister fractions of extra- and intracellular *P. aeruginosa* strains.

Persisters obtained are expressed as CFU/mL for extracellular assay, and as CFU/mg protein for intracellular assay. Antibiotics were used at a concentration of 100x MIC (except for AMK which was used at 50x MIC for intracellular assay).

* indicates difference is significant, while ** indicates the difference is highly significant (One-way ANOVA and Tukey post-hoc test).

- All strains showed similar MIC values for the respective antibiotics
- ΔdnpA showed significantly lower levels of
  - Intracellular persisters for CIP and LVX
  - Extracellular persisters for LVX
- CIP did not affect extracellular persisters
- MXF, MEM and AMK did not significantly alter persister fraction between the strains

Extra- and intracellular pharmacodynamic parameters

Antibiotic	CIP			LVX			MXF			MEM			AMK		
Strain	E_{max}	MIC	C_s	E_{max}	MIC	C_s	E_{max}	MIC	C_s	E_{max}	MIC	C_s	E_{max}	MIC	C_s
PAO1	-2.34 ± 0.17	0.25	0.34	-3.76 ± 0.22	1	3.18	-5.23 ± 0.25	1	4.7	-0.73 ± 0.30	2	5	-1.44 ± 0.20	4	23.9
ΔdnpA	-3.94 ± 0.27	0.25	0.27	-5.20 ± 0.35	1	0.98	-4.45 ± 0.25	1	2.3	-1.24 ± 0.22	2	3.4	-1.01 ± 0.16	4	18.9
PAO1 (dnpA)	-2.49 ± 0.37	0.5	0.88	-4.12 ± 0.25	1	1.43	-4.99 ± 0.25	2	5.1	-0.71 ± 0.30	4	6.8	-0.99 ± 0.19	4	27.1

E_{max} : maximal decrease in inoculum (in $\log_{10}$ units) compared to the post-phagocytosis inoculum as extrapolated for an infinitely large antibiotic concentration,
MIC: minimal inhibitory concentration in broth (mg/L)
C_s : static concentration i.e., the extracellular concentration (mg/L) resulting in no apparent bacterial growth (number of CFU identical to the post-phagocytosis inoculum)

Materials & Methods

Intracellular infection assay

Bacterial growth (18-20 h, 37°C) → **Opsonization** (1 h, 37°C) 9 mL RPMI-1640 + 1 mL human serum → **Phagocytosis** (2 h, 37°C, 5% CO_2) 10 bacteria/THP-1 cell → **Killing extracellular bacteria** (1 h, 37°C) 100 mg/L gentamicin → **Incubation** (24 h, 37°C, 5% CO_2) Increasing concentration of antibiotic → **Cell harvesting, lysis, CFU counting & cell protein content determination**

Bacterial strains

- *P. aeruginosa* PAO1: wildtype
- ΔdnpA: Deletion of *dnpA*
- PAO1(dnpA): Overexpression of *dnpA*

Persistence assay

Bacterial growth (18-24 h, 37°C) → **1:200 dilution** (18-24 h, 37°C) → **Culture + 100x MIC antibiotic / H_2O control** (5 h, 37°C) → **CFU counting** → $\frac{\text{CFU after antibiotic}}{\text{CFU after } H_2O}$ **Persister fraction**

Conclusions

✓Deletion of *dnpA*:

- ✓Decreases extra- and intracellular persister formation upon certain fluoroquinolone exposure, and
- ✓Does not affect beta-lactam or aminoglycoside activity in broth and in cells.

✓Overexpression of *dnpA* differentially affects extracellular and intracellular activity of antibiotics.

✓The results extend the previously described observations of extracellular model.

Discussion

- Expression of *dnpA* might trigger intracellular persistence and fluoroquinolone tolerance, especially for CIP and LVX.
- Only fluoroquinolone-related persister formation is affected, suggesting an interference with the activity of these antibiotics that needs to be further explored.
- The difference in extra- and intracellular activities of antibiotics suggests a more intricate intracellular role of DnpA.
- Targeting DnpA appears to be an appealing strategy to improve fluoroquinolone efficacy against intracellular *P. aeruginosa*.

References

1. Buyck *et al*, AAC 2013, 57:2310-8
2. Liebens *et al.*, Pathog Dis 2014, 71:39–54

Acknowledgements

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