P0317

Paper Poster Session II

Focus: Acinetobacter, Pseudomonas and other nonfermenters

DnpA, a putative de-*N*-acetylase involved in *Pseudomonas aeruginosa* persistence, reduces ciprofloxacin activity in an *in vitro* model of intracellular infection

S. Khandekar[1], V. Liebens[1], M. Fauvart[1], P.M. Tulkens[1], J. Michiels[1], F. Van Bambeke[1]

[1]*Université catholique de Louvain, Brussels, Belgium*

Objectives: Persisters are now recognized as a possible cause of antibiotic failure. The putative de-*N*-acetylase DnpA has been shown to increase persister levels in *P. aeruginosa,* conferring tolerance specifically to fluoroquinolones in broth (Liebens et al., Pathog Dis 2014, 71:39–54). *P. aeruginosa* is capable of invading epithelial and phagocytic cells. Intracellularly, the bacteria are less responsive to antibiotics, with a maximal efficacy only between 1 (beta-lactams) and 2.5 (fluoroquinolones) $\log_{10}$ CFU decrease from the post-phagocytosis inoculum (Buyck et al, AAC 2013, 57:2310-8), leaving an important intracellular bacterial load. Our objective was to assess a possible role of DnpA in this intracellular persistence of *P. aeruginosa* using ciprofloxacin as exemplative fluoroquinolone and meropenem as a representative antibiotic from another antipseudomonal class.

Methods: Strains: PAO1; a *dnpA* deletion mutant (Δ*dnpA*) and a *dnpA* overexpressing strain (generated in the Δ*dnpA* background). Susceptibility testing: MICs determined according to CLSI recommendations in cation-adjusted MHB. Intracellular infection and activity of antibiotics: these experiments were performed exactly as described (Buyck et al, AAC 2013, 57:2310-8), with activity expressed as change from the initial inoculum after 24 h of exposure to antibiotics. Data were used for fitting a concentration-response curve (Hill equation) to calculate the E_{max} and C_{static} pharmacodynamic parameters (see definitions in the Table).

Results: The Table shows the relevant pharmacodynamic parameters and MIC values. All strains showed similar MICs for each antibiotic. For ciprofloxacin, E_{max} was significantly larger (more negative value) for the ΔdnpA strain, while E_{max} was slightly lower (less negative value) and C_s was higher for the Δ*dnpa(dnpA)* overexpressing strain. For meropenem, no differences were observed among strains.

Conclusion: The expression of *dnpA* impairs the intracellular activity of ciprofloxacin but not of meropenem, suggesting it triggers persistence and fluoroquinolone tolerance intracellularly, extending this model to the observations previously made in broth. Targeting DnpA appears to be an appealing strategy to improve fluoroquinolone efficacy against intracellular *P. aeruginosa*.

Intracellular pharmacodynamic parameters and MIC values

	Ciprofloxacin (CIP)				**Meropenem (MEM)**			
Strain	E_{max}[a,b]	MIC[c]	C_{static}[d]	R^2	E_{max}[a,b]	MIC[c]	C_{static}[d]	R^2
PAO1	-2.34 ± 0.14 (A)	0.25	0.34	0.99	-0.69 ± 0.28 (A)	2	3.80	0.95
ΔdnpA	-3.94 ± 0.19 (B)	0.25	0.27	0.99	-1.31 ± 0.25 (A)	2	2.75	0.96
Δdnpa(dnpA)	-1.79 ± 0.42 (A)	0.5	1.71	0.92	-0.99 ± 0.24 (A)	4	5.62	0.97

[a] maximum decrease in log CFU compared to the post-phagocytosis inoculum for an infinitely high concentration in antibiotic [b] statistical analyses per column (ANOVA; Tukey post-hoc test): values with different letters in upper case are significantly different from one another [c] minimal inhibitory concentrations (in mg/L) [d] Concentration (in mg/L) resulting in no apparent bacterial growth (number of CFU identical to the post-phagocytosis inoculum)