Impact of *lpxM* deficiency on the drug sensitizing activity of the efflux pump inhibitor PAβN

S. Schuster[1], J.A. Bohnert[2], M. Vavra[1], J.W.A. Rossen[3], and W.V. Kern[1]

[1] Center for Infectious Diseases and Travel Medicine, University Hospital Freiburg, and Department of Medicine, Albert-Ludwigs-University, Freiburg, Germany
[2] Institute of Medical Microbiology, Greifswald University Hospital, Greifswald, Germany
[3] Department of Medical Microbiology, University of Groningen, University Medical Center Groningen, Groningen, the Netherlands
E-mail: sabine.schuster@uniklinik-freiburg.de

Background: PAβN (phenylalanyl arginyl β-naphthylamide) is an early agent shown to inhibit RND-type efflux pumps of Gram-negatives such as AcrAB-TolC from *Escherichia coli* [1]. However, an additional permeabilizing activity has been reported [1, 2]. Previously, we had selected an *E. coli* mutant (C5/1/17) showing partial resistance to the drug sensitizing activity of PAβN but without any mutations in *acrAB-tolC* [3]. In this study, we intended to elucidate the mode(s) of action of PAβN.
Methods: An *acrAB-tolC* overexpressing *E.coli* was subjected to error-prone PCR based in-vitro random mutagenesis targeting *acrB*. Mutants were selected in a single step on clarithromycin in the presence of PAβN. *acrB* alterations from mutants with confirmed PAβN resistance were reconstructed. Whole genome sequencing was applied in the case of lacking *acrAB-tolC* mutations associated with PAβN resistance. Phenotypic characterization was done by MIC testing with and without EPIs or membrane permeabilizers, and by dye accumulation and efflux assays.
Results: From targeted (*acrB*) mutagenesis, we obtained a mutant CP1 that, similar to C5/1/17 (obtained from serial selection), showed resistance to PAβN activity. We detected 4 mutations in *acrB* of CP1 (V129I**,** L270V, T495S, A873V), but their reconstruction within the parent did not reveal any association with PAβN resistance. However, this was maintained when replacing mutated *acrB* by wild-type *acrB* in CP1. By WGS sequencing we discovered loss-of-function mutations in *lpxM* of CP1 as well as of C5/1/17 (stop codon and frameshift, respectively). *lpxM* is coding for a myristoyl acyltransferase and its loss is known to result in pentaacylated lipid A. Interruption of *lpxM* within the parental strain confirmed its impact on PAβN activity. We found largest effects with drugs known to display high synergy with PAβN (8-16 fold decreased PAβN activity with novobiocin, rifamycines, macrolides). The permeability of the mutant for some agents appeared increased (nitrocefin, rifampin, poymyxin B nonapeptide), but only marginal for macrolides, rifaximine, colistin, and PAβN itself (≤ 2-fold, MIC parent / MIC mutant). Real-time efflux assays confirmed unimpaired AcrAB-TolC functionality. Interestingly, Δ*lpxM* was also associated with decreased drug sensitizing activity of colistin, but not of NMP and MBX2319.
Conclusions: We discovered a target of PAβN in the outer membrane proving an alternative mode of action that contributes to the drug sensitizing activity of this EPI.

References

[1] O. Lomovskaya, Antimicrob Agents Chemother. **45(1)**, 105-116 (2001)
[2] R.P. Lamers, PLoS One. **8(3)**, e60666 (2013)
[3] S. Schuster, Antimicrob Agents Chemother. **58(11)**, 6870-8 (2014)