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New preclinical data on adjunctive antibacterial therapies

In vitro enhancement of Gram-positive antibacterial compounds by recently described efflux pump inhibitors in MDR and comparison with the membrane permeabilizer PMBN

T. Schweigger[1], M. Vavra[1], L. Greim-Kuczewski[1], N. Specht[1], S. Schuster[1], W.V. Kern[1]

[1]*Division of Infectious Diseases- University Hospital, Freiburg, Germany*

Objectives

Bacterial multidrug resistance (MDR) is increasing among potentially pathogenic gram-negative bacteria and is often associated with reduced membrane permeability or enhanced efflux. Efflux pump inhibitors (EPIs) or membrane permeabilizers (MPs) could sensitize gram-negative bacteria for enhanced activity of a variety of anti-gram-positive compounds such as macrolides or oxazolidinones and others. Using well-characterized reference strains and an international collection of MDR E. coli clinical isolates we evaluated the sensitizing effect of new putative EPIs including MBX2319 and the phytochemicals lanatoside C, diadzein and protocatechiuc acid by assaying fold-reductions of the MICs of a panel of various test (levofloxacin, moxifloxacin and novobiocin, tetracycline, minocycline and tigecycline, linezolid, erythromycin, clarithromycin and azithromycin, clindamycin, chloramphenicol, rifampicin and rifaximin, oxacillin and cefuroxime) and control (gentamicin, streptomycin) compounds.

Methods

MBX2319, a novel pyranopyridine EPI (Oppermann et al *AAC*2014), was obtained from T.J. Oppermann (Microbiotix, Worcester, Mass./USA). The phytochemicals thought to interact with AcrB residues in close proximity to binding site 1 according to modeling and recently shown to exhibit in *E.coli* synergistic activity with carbenicillin and/or levofloxacin (Aparna et al *PLoSONE*2014) were from Sigma. We initially screened for intrinsic inhibitory activity of the EPIs to define subinhibitory concentrations suitable in MIC reduction studies. Control EPIs were NMP (100 µg/mL) and PAßN (25 µg/mL). Polymyxin B nonapeptide (PMBN, at 1 µg/mL) was used as MP. Synergistic activity was defined as ≥4-fold MIC reduction. Tests were done at least in duplicate. Test strains included 33 clinical isolates from various parts of the world that showed multidrug resistance (to 3°generation cephalosporins, fluoroquinolones, trimethoprim-sulfamethoxazole and tetracycline) and had different levels of *acrB* and OmpC/OmpF expression. All test strains showed ≥4-fold reduction of linezolid (up to 32-fold) and minocycline (up to 96-fold) MICs with NMP.

Results

MBX2319 was used at a final concentration of 25 µmol at which there was no bacterial growth inhibition. Higher concentrations could not be tested due to solubility problems. At this concentration the compound was synergistic (maximal 8-fold MIC reduction) with minocycline in 16 clinical isolates, with linezolid in 12 isolates, and with tetracycline in 9 isolates. The synergy tests with other drugs generally yielded poorer results. MBX2319 was inactive in Δ*tolC* and Δ*acrB* test strains. The phytochemicals were tested in concentrations up to 128 µg/mL with and without addition of Mg. None of them showed synergy with any of the test drugs (including levofloxacin). PMBN reduced the test drug MICs ≥4-fold in all strains for all macrolides/clindamyin and novobiocin, and in most strains for tetracycline/minocycline, linezolid, and rifampicin/rifaximin. PMBN activity correlated well with PAßN but not with NMP activity.

Conclusion

The recently discovered EPIs showed no or limited activity in MDR *E.coli* clinical isolates. Screening for more potent EPIs is urgently needed.

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