Antibiotic Transport and Resistance in Gram-negative Bacteria

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ABSTRACT

The permeability barrier of Gram-negative cell envelopes is a major obstacle in the discovery and development of new antibiotics. In particular, the synergistic interaction between the low permeability of the outer membrane and active multidrug efflux makes the task all the more difficult. In our work, we use isogenic strains of *Escherichia coli* with controlled permeability barriers, with different influx and/or efflux capacities, to evaluate the specific contribution of each barrier to drug accumulation and activity. For the first part of this talk, I will illustrate this approach with a recent study on fluoroquinolones, which target the bacterial cytoplasm, to perform Structure to Intracellular Concentration and Activity Relationship (SICAR) analyses. On one hand, the outer membrane of *E. coli* contains about 200,000 copies of OmpF and OmpC porins per cell, which are responsible for the nonspecific diffusion of small polar compounds. Consequently, several antibiotics are active against this species. On the other hand, the inner membrane of *E. coli* contains several multidrug efflux pumps that differ in their structures and mechanisms. Among them, the transmembrane AcrAB-TolC complex is the major efflux pump responsible for the intrinsic resistance of *E. coli* towards a wide variety of antimicrobials. Noteworthy, porin modifications and/or AcrAB overexpression have a significant impact on the emergence of multidrug resistance in clinical strains. Antibacterial activities were examined by using standard minimal inhibitory concentration and resazurin-based real time viability assays. Fluorimetry allowed quantification of intracellular drug concentrations and determination of SICAR coefficients to measure the porin-mediated influx and efflux efficiencies among series of chemically related antibiotics. Together, ratios of drug accumulation and susceptibility were used to rank the importance of antibiotic properties in relation with permeability barriers. Importantly, these experimental data also reflect (favorable or unfavorable) molecular interactions between in transit antibiotics and membrane transporters, which can be confirmed by molecular modelling and molecular dynamics simulations. For the second part of this talk, I will present future lines of research linking several beamlines at SOLEIL. With DISCO, we will broaden our approach to analyze the contribution of permeability barriers from other bacterial species, including clinically problematic species and potential biological weapons, and address restoration of antibiotic accumulation in the presence of efflux pump inhibitors. With PROXIMA 1, we will work to elucidate the molecular bases of antibiotic translocation through OmpF and OmpC porins with the generation of co-crystals. We will also work to report *in situ* efflux pump structure and assembly using cryo-electrotomography at the next TOMO XMous. Ultimately, all these data will help to establish practical rules to maximize influx and minimize efflux for optimal antibacterial drug activities.

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