Uptake mechanisms and biological response of Gram-negative bacteria to siderophore – natural product conjugates

Lukas Pinkert, Helmholtz Centre for Infection Research, Braunschweig, Germany

Yi-Hui Lai, Helmholtz Centre for Infection Research, Braunschweig, Germany

Carsten Peukert, Helmholtz Centre for Infection Research, Braunschweig, Germany

Mark Brönstrup, Helmholtz Centre for Infection Research, Braunschweig, Germany

Multidrug resistance in bacteria becomes a serious challenge in the clinic, so it is exigent to seek novel and effective antibiotic treatment options. Antibiotics conjugated to siderophores are actively imported to the pathogen by hijacking the siderophore transport system, and thereby reach enhanced intracellular accumulation, which is an important contributor to efficacy. However, the underlying transport mechanism and the bacterial response to exposure to siderophore drugs is poorly understood. In this study, we designed and synthesized several novel siderophore-drug conjugates and verified their enhanced antibacterial potency compared to the unconjugated compounds. One of the scaffolds, named LP-600, is based on a non-natural siderophore motif. LP-600 displays high activity against both laboratory and uropathogenic *E. coli*. We identified three outer membrane receptors that contribute to the uptake of LP-600 using real-time PCR and lambda-red recombination genome editing methods. Surprisingly, *E. coli* shows a paradoxical re-growth upon treatment of LP-600 at concentrations 16x higher MIC, a phenomenon coined the 'Eagle-effect' in other antibiotics. To pinpoint proteins and metabolites involved in the Eagle effect, a combined transcriptome and a metabolome analysis was conducted. We have also raised and genome-sequenced resistant mutants against LP-600 and present two proteins conferring resistance. Taken together, our study unveils the potential to enhance the antibiotic activity of natural products by a conjugation approach.

1) Ferreira, Kevin; Hu, Hai-Yu; Fetz, Verena; Prochnow, Hans; Rais, Bushra; Mueller, Peter P.; Broenstrup, Mark *Angew. Chem. Int. Ed.* **2017**, *56*, 8272-8276.

2) Klahn, Philipp; Brönstrup, Mark. *Nat. Prod. Rep.,* **2017**, *34*, 832 – 885.