nature

https://doi.org/10.1038/s41586-019-1791-1

Supplementary information

A new antibiotic selectively kills Gram-negative pathogens

In the format provided by the authors and unedited

Yu Imai, Kirsten J. Meyer, Akira Iinishi, Quentin Favre-Godal, Robert Green, Sylvie Manuse, Mariaelena Caboni, Miho Mori, Samantha Niles, Meghan Ghiglieri, Chandrashekhar Honrao, Xiaoyu Ma, Jason J. Guo, Alexandros Makriyannis, Luis Linares-Otoya, Nils Böhringer, Zerlina G. Wuisan, Hundeep Kaur, Runrun Wu, André Mateus, Athanasios Typas, Mikhail M. Savitski, Josh L. Espinoza, Aubrie O'Rourke, Karen E. Nelson, Sebastian Hiller, Nicholas Noinaj, Till F. Schäberle, Anthony D'Onofrio & Kim Lewis

Supplementary Discussion

Nematophilic bacteria and antibiotics

We focus on nematophilic bacteria in search of antibiotics, since their lifestyle suggests the presence of compounds that may be of use to us. The *Photorhabdus* and *Xenorhabdus* bacteria inhabit nematodes, which suggests that the compounds they make are non-toxic. This begs the question of whether bacteria from animal microbiomes in general are a good source of antibiotics. The principle difference between these nematode symbionts and inhabitants of the gut of other organisms is that *Photorhabdus* and *Xenorhabdus* then enter into insect larvae and release antibiotics that should have properties we seek for compounds acting systemically: good penetration through tissues and in vivo stability (essentially good pharmacokinetics).

Stereochemistry of darobactin

Identification of the gene cluster responsible for the production of darobactin in the *P. khanii* genome suggested that all 7 amino acids of darobactin are in the L- configuration. The two chiral centers at the sites of cross linkages, C-17 and C-31 suggest four possible stereoisomers. Molecular modeling of darobactin showed that only isomer 17R, 31S fits the experimental ROESY data (Extended Data Fig. 1h and 2c) with the following observed key NOE correlations: H-1/H-4, H-17/H-7, H-17/H-24, H-31/H-23, H-30/H-21, and H-32/H-21.

Transcriptomic response to darobactin treatment

Darobactin treatment led to rapid changes in the transcriptome. Volcano plots of the differentially expressed genes (DEGs) highlight that within 15 min, the most significant was *degP* (Extended Data Fig. 7a), and this remained upregulated through the 1 h treatment period examined. DegP is the periplasmic chaperone canonically induced by the sigma E envelope stress response due to unfolded outer membrane proteins in the periplasm[1]. At 30 min the upregulation of *rpoE* and *rseA*, transcriptional regulators of the Sigma E response, and the downregulation of outer membrane proteins *ompF* and *ompA* was apparent (Extended Data Fig. 7b). At 60 min, *ompF* remained the most significantly downregulated gene, and the colanic acid/capsule operons of the Rcs stress response were highly upregulated (Extended Data Fig. 7c). A network analysis of the three time-points showed that 55 out of 204 DEGs were shared by 2 or more timepoints, and most of this overlap consisted of genes involved with the inner membrane, periplasm, outer membrane, or capsule (Extended Data Fig. 7d). A closer look at the DEGs found the presence of many canonical members of envelope stress regulons (Extended Data Fig. 7e). Along with *rpoE*, *rseA-C* operon, the small regulatory RNA *micL* of the Sigma E response was upregulated[2]. The RCS pathway was represented by transcriptional regulators *rcsA* and the small RNA *rprA*, and the corresponding upregulation of colanic acid/capsule operons and repression of flagellar genes[3]. Sigma E and Rcs were the major stress pathways represented in the DEGs, but one of the most highly upregulated genes was the periplasmic chaperone *spy*, usually associated with the Cpx and Bae pathways[2,4]. Further evidence of Cpx and Bae activation were found with upregulation of *cpxP* and *acrD*, respectively, and finally the phage shock pathway also showed activation with *psp* operon upregulation at 1 h[5]. The transcriptomic data highlights the level of overlap and crosstalk between stress pathways, and is consistent with the mechanism of darobactin occurring through inhibition of BAM function, leading to unfolded outer membrane proteins, a destabilized outer membrane, and envelope stress (O'Rourke, Beyhan, et al in submission).

Thermal proteome profiling with darobactin

Thermal proteome profiling in cell lysate[6,7] —which should reveal the direct targets of drugs[8]— did not show specific thermal stabilization of a protein in response to incubation with darobactin (10 min) (Extended Data Fig. 8b). The same experiment in living cells led to an upregulation of

Spy and DegP and a decreased abundance of outer membrane proteins (e.g., OmpC, OmpF, NanC, LamB) (Extended Data Fig. 8a,d), consistent with the activation of envelope stress pathways observed by transcriptomics (σE, Rcs, and Cpx (Extended Data Fig. 7)). We did not observe these effects when protein synthesis was inhibited with chloramphenicol prior to darobactin treatment (Extended Data Fig. 8c). Darobactin treatment caused an increased abundance and thermal destabilization of the BAM machinery in living cells (Extended Data Fig. 8d). The thermal destabilization is consistent with an increase of unassembled BAM machinery (Extended Data Fig. 8e), since we have previously shown that the melting behavior of these proteins is consistent with two populations being present: an assembled population that is resistant to heat-induced denaturation, and an unassembled population with a melting temperature between 60ºC (BamAB) and 70ºC (BamD)[6]. The increased levels of unassembled BAM perhaps contribute to the activation of envelope stress pathways. Given NMR experiments suggest darobactin does bind to BamA (Fig. 3c), the failure to observe thermal stabilization of BamA in the lysate experiments probably a consequence of the intrinsically high thermal stability of this protein when assembled in the BA complex and inserted in the outer membrane, which precludes it from being further stabilized.

References

1 Rhodius, V. A., Suh, W. C., Nonaka, G., West, J. & Gross, C. A. Conserved and variable functions of the sigmaE stress response in related genomes. *PLoS Biol* **4**, e2 (2006)

2 Grabowicz, M. & Silhavy, T. J. Envelope stress responses: an interconnected safety net. *Trends Biochem Sci* **42**, 232-242 (2017)

3 Majdalani, N. & Gottesman, S. The Rcs phosphorelay: a complex signal transduction system. *Annu Rev Microbiol* **59**, 379-405 (2005)

4 Raffa, R. G. & Raivio, T. L. A third envelope stress signal transduction pathway in Escherichia coli. *Mol Microbiol* **45**, 1599-1611 (2002)

5 Raivio, T. L. Everything old is new again: an update on current research on the Cpx envelope stress response. *Biochim Biophys Acta* **1843**, 1529-1541 (2014)

6 Mateus, A. *et al.* Thermal proteome profiling in bacteria: probing protein state in vivo. *Mol Syst Biol* **14**, e8242 (2018)

7 Becher, I. *et al.* Thermal profiling reveals phenylalanine hydroxylase as an off-target of panobinostat. *Nat Chem Biol* **12**, 908-910 (2016)

8 Mateus, A., Maatta, T. A. & Savitski, M. M. Thermal proteome profiling: unbiased assessment of protein state through heat-induced stability changes. *Proteome Sci* **15**, 13 (2016)