Astabiotics: Antimicrobial Signal Transduction Activators

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The increasing prevalence of multi-drug resistant Gram-negative bacteria is a major public health concern. All available antibiotics are inhibitors of targets essential for virulence or growth. CpxRA is a highly conserved bacterial signal transduction system that responds to extracytoplasmic membrane stress. CpxA is a sensor with kinase and phosphatase activity; upon activation, CpxA donates a phosphate group to CpxR, activating a transcriptional response. Activation of CpxRA reduces the flow of protein traffic through the cytoplasmic membrane, dramatically reducing the expression of virulence determinants. Activation of CpxRA abolishes the virulence of *Salmonella* Typhimurium in mice. We found that activation of CpxRA crippled the ability of *Haemophilus ducreyi* to cause disease in experimentally infected human volunteers. Using an *Escherichia coli* reporter strain, we developed a high throughput screen to detect compounds that activate CpxRA. In a pilot screen of 36,000 compounds, we identified 1 class of compounds that shifts the equilibrium of CpxA to kinase activity, activating CpxR. Based on its potency, the calculated effective dose of the lead compound (a nitroindole) was 10 mg/kg. Female mice tolerated 100 mg/kg of the nitroindole given twice a day for 3 days. A CpxRA activating mutant constructed in uropathogenic *E. coli* (UPEC) was severely impaired in a murine urinary tract infection model; thus, activation of CpxRA is a valid treatment strategy for UPEC. However, when female mice were challenged with UPEC and treated with 100 mg/kg of the nitroindole using the schedule above, there were no differences in the recovered CFU in the urine, bladder, and kidney of sham and compound-treated mice. Future studies will include medicinal optimization of the nitroindole and identification and optimization other leads that activate CpxRA. Although our pilot test of efficacy was negative, astabiotics have great potential as broad-spectrum, adjunctive therapies to existing antimicrobials for treatment of Gram-negative infections.