***Drug Discovery and E. coli HeptosyltransferaseI - How basic science research might change our understanding of antibiotic mechanism of action***

A clinically relevant inhibitor for Heptosyltransferase I (HepI) has been sought for many years because of its critical role in the biosynthesis of lipopolysaccharides on bacterial cell surfaces. While many labs have discovered or designed novel small molecule inhibitors, these compounds lacked the bioavailability and potency necessary for therapeutic use. Extensive characterization of the HepI protein has provided valuable insight into the dynamic motions necessary for catalysis that could be targeted for inhibition. Structural inspection of Kdo2-lipid A suggested aminoglycoside antibiotics as potential inhibitors for HepI. Multiple aminoglycosides have been experimentally validated to be first-in-class nanomolar inhibitors, with the best inhibitor demonstrating a Ki of 600 +/- 90 nM. Detailed kinetic analyses were performed to determine the mechanism of inhibition while circular dichroism spectroscopy, intrinsic tryptophan fluorescence, docking, and molecular dynamics simulations were used to corroborate kinetic experimental findings. While aminoglycosides have long been described as potent antibiotics targeting bacterial ribosomes’ protein synthesis leading to disruption of the stability of bacterial cell membranes, more recently researchers have shown that they only modestly impact protein production. Our research suggests an alternative and novel mechanism of action of aminoglycosides in the inhibition of HepI, which directly leads to modification of LPS production in vivo. This finding could change our understanding of how aminoglycoside antibiotics function, with interruption of LPS biosynthesis being an additional and important mechanism of aminoglycoside action. Further research to discern the microbiological impact of aminoglycosides on cells is warranted, as inhibition of the ribosome may not be the sole and primary mechanism of action. The inhibition of HepI by aminoglycosides may dramatically alter strategies to modify the structure of aminoglycosides to improve the efficacy of HepI inhibition while minimizing undesired clinical side effects when administering these compounds to fighting bacterial infections.