Hypersensitization of *Helicobacter pylori* to antibiotics through perturbation of bacterial glycan armor

William Rackear, Class of 2022

Throughout my experience working in the Dube lab this summer, I spent my time manipulating the glycocalyx of the pathogenic bacteria, *Helicobacter pylori*, in an attempt to hypersensitize it to FDA approved antibiotics. Although these antibiotics have been very successful in the past, various strains of bacteria are developing antibiotic resistance. In fact, the Center for Disease Control estimates that there about 2.3 million new bacterial infections caused by antibiotic resistant bacteria each year in the United States.[1] As a result of the growing prevalence of antibiotic resistant bacterial infections, treatments will become more and more ineffective. With this in mind, new therapeutics are necessary to combat this growing issue.

One particular therapeutic target can be seen in the unique sugars, known as glycans, found on bacterial cells. Bacterial glycans are a target of interest as a result of the critical role they play in pathogenesis.[2] With this known role, we hypothesized that if we perturb the bacterial glycan structure, then the bacteria would become hypersensitized to antibiotics. My project for the summer focused on one particular strain of bacteria, *H. pylori*, which has unique glycans that can be altered by small molecule inhibitors.[3]

In order to test this working hypothesis, *H. pylori* were grown in the presence or absence of three established glycan altering agents: polymyxin B nonapeptide (PMBN), vancomycin, and Bac-diNAc-OBn (BnBac). These glycan altering agents perturbed different parts of the *H. pylori* glycocalyx. By growing the *H. pylori* in the presence or absence of these inhibitors, we had an untreated (control) condition and three experimental conditions. After the bacteria was grown and treated with glycan-altering agents, an Epsilometer test was performed to determine the minimum inhibitory concentration (MIC) of the FDA-approved antibiotic, levofloxacin. In each experimental condition, the MIC value was found to be lower than that of the control condition, signifying that a lower concentration of levofloxacin was needed to kill the *H. pylori* when treated with glycan altering agents. These data suggested that altering the glycan armor of *H. pylori* successfully hypersensitized it to levofloxacin and that this could be a viable strategy for fighting bacterial infections in the future. Of the three glycan altering agents, BnBac, which inhibited glycoprotein biosynthesis,[4] was the most effective as it resulted in a lower MIC value and substantially diminished bacterial growth. Considering these data, in the near future I plan on determining if these results can be recapitulated by interfering with glycoprotien biosysnthesis via genetic means and testing this hypothesis on antibiotic resistant isolates.

Faculty Mentor: Danielle H. Dube

Funded by the Student Faculty Research Grant Fellowship

References

1. *Antibiotic resistance threats in the United States, 2019*; Centers for Disease Control and Prevention.
2. Dube, D. H.; Champasa, K.; Wang, B., Chemical tools to discover and target bacterial glycoproteins. *Chem. Commun.* **2011,** *47* (1), 87-101.
3. Champasa, K., et al. Targeted Identification of Glycosylation Proteins in the Gastric Pathogen *Helicobacter pylori* (*Hp*). *Molecular & cellular proteomics: MCP* **2013** 12 (9), 2568-86.
4. Williams, D. A.; Pradhan, K.; Paul, A.; Olin, I. R.; Tuck, O. T.; Moulton, K. D.; Kulkarni, S. S.; Dube, D. H., Metabolic inhibitors of bacterial glycan biosynthesis. *Chem. Sci.* **2020,** *11* (7), 1761-1774.