**Inhibition of *Helicobacter pylori* glycoprotein biosynthesis**

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The discovery of antibiotics and their ability to combat microbial infections revolutionized modern medicine. However, 90 years after the development of penicillin by Alexander Fleming, an emerging trend of antibiotic resistance has become apparent. To date, over 20,000 resistance genes have been identified, with 'superbug' species like *S. aureus* and tuberculosis pathogens displaying resistances to multiple drugs, reflecting increasing virulence and mortality rates.[1] Tp complicate matters, traditional broad-spectrum antibiotics are increasingly seen as insufficient as they indiscriminately harm beneficial bacteria which inhabit human microbiomes.

One particularly problematic, highly antimicrobial resistant bacterium is *Helicobacter pylori*. Upon entering its host, *H. pylori* colonizes the highly acidic environment of the stomach mucosa. Infected individuals are exposed to a flurry of toxins, damaging tissue and ultimately increasing risk of gastritis, gastric ulcers, and several stomach cancers.[2] *H. pylori* is ubiquitous – it causes approximately 5.2% of all cancers[3] – and it continues to develop new mechanisms of antimicrobial resistance, prompting the WHO to list *H. pylori* as a "high priority" target for the research and development of new antibiotics.[4]

One intriguing avenue of potential therapies is *H. pylori* cell surface glycoproteins. Glycoproteins are structures comprised of a protein and linked chains of sugars – called glycans – which together work to mediate interactions between cells and their environment. *H. pylor*i's surface glycans have been associated with the bacteria's ability to initial establish infection.[5] Bacterial glycoproteins are largely composed of characteristic, species-specific sugars. In fact, bacteria share remarkably few glycan sugars with mammalian cells.[6] Because of their exclusivity, *H. pylori* glycoproteins could be a highly selective target for combating infection. If the biosynthesis of these structures were halted or impaired, *H. pylori* could be inhibited without risking surrounding microbes or host cells.

This project set out to evaluate the bactericidal potential of molecules synthesized to selectively halt glycan and glycoprotein production in *H. pylori*. These molecules closely mimic normal sugars, but, by varying mechanisms, should diminish glycosylation. I hypothesized that glycan production will be diminished dependably and selectively upon introduction of inhibitors. By extension, once synthesized mimics are successfully introduced into cells, downstream functions of glycoproteins will be diminished, leading to decreased survivability, thereby decreasing the burden of *H. pylori* infection.[7] In turn, my hypothesis would prove the promise of the budding field of sugar-based therapeutics.

To test my hypothesis, I employed a technique called Metabolic Oligosaccharide Engineering (MOE), which allowed me to visualize glycans on the surface of cells. In conjunction with a key biochemical technique called Western blotting, I observed dramatic, dose-dependent ablation of glycoprotein profiles of *H. pylori* treated with inhibitors. These data corroborate preliminary observations of the efficacy of sugar-based therapeutics in combatting infection. In pursuit of further evidence of glycoprotein diminishment, I also experimented extensively with flow cytometry. I saw interesting results which conflicted with my initial hypothesis and Western blot results, which led me to explore several causes of this surprising trend. Although experimentation is ongoing, I discovered the methodology I used does not accurately depict glycoprotein expression and set out to optimize flow cytometry conditions for further experimentation.

In summary, I showed inhibitors affected *H. pylori* glycoproteins. I assayed a range of inhibitors and did preliminary work to determine the molecular mechanism of these potential therapeutics. In my yearlong honors project, I plan to determine the exact mechanism of action of inhibitors and obtain molecular-level evidence of truncated or dysfunctional glycoproteins. In pursuit of this goal, I have already purified samples for LCMS/MS mass analysis and prepared mammalian cell standards. Additionally, I will observe the effect of inhibitor treatment in human tissue. If no significant glycan impairment is observed, both my hypothesis and the viability of sugar therapeutics would be supported.


References

[1] Davies, J., and Davies, D. (2010) Origins and evolution of antibiotic resistance. *Microbiology and Molecular Biology Reviews* **74**, 417-433

[2] Marshall, B. J., McGechie, D. B., Rogers, P. A., and Glancy, R. J. (1985) Pyloric Campylobacter infection and gastroduodenal disease. *The Medical Journal of Australia* **142**, 439-444

[3] Plummer, M., Franceschi, S., Vignat, J., Forman, D., and de Martel, C. (2015) Global burden of gastric cancer attributable to Helicobacter pylori. *International Journal of Cancer* **136**, 487-490

[4] Tacconelli, E. a. M., N. . (2017) Global priority list of antibiotic-resistant bacteria to guide research, discovery, and development of new antibiotics. *World Health Organization.*

[5] Tra, V. N., and Dube, D. H. (2014) Glycans in pathogenicbacteria – potential for targeted covalent therapeutics and imaging agents. *Chem. Commun*., **50**, 4659−4673.

[6] Herget, S.; Toukach, P. V.; Ranzinger, R.; Hull, W. E.; Knirel, Y. A.; von der Lieth, C.W. (2008) Statistical analysis of the Bacterial Carbohydrate Structure Data Base (BCSDB): characteristics and diversity of bacterial carbohydrates in comparison with mammalian glycans. *BMC Struct. Biol.*, ***8***, 35.

[7] Gloster, T. M., and Vocadlo, D. J. (2012) Developing inhibitors of glycan processing enzymes as tools for enabling glycobiology. *Nature chemical biology* **8**, 683