Exploration of the Photosensitizer Chlorin-e4 for the Cyclooctyne-based Elimination of Helicobacter pylori

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The goal of this project is to explore a more effective approach in the elimination of the pathogenic bacteria Helicobacter pylori. Helicobacter pylori is a gram-negative bacteria that infects over 50% of the world’s population. H. pylori has been found to cause both duodenal and gastric ulcers and to be a precursor to stomach cancer. Infection is most commonly treated with a triple antibiotic therapy, but strains of H. pylori have evolved that are resistant to this therapy. As such, a new method of treating H. pylori infection is necessary.

One possible avenue for treating H. pylori is to use its natural sensitivity to light. H. pylori has a natural sensitivity to light due to the presence of light-sensitive compounds known as porphyrins, such as protoporphyrin IX (PpIX), within the cell. Ganz et al. has demonstrated that H. pylori death can be inflicted by exposure to high doses of blue light, which activates the PpIX in the bacterial cell. However, most hospitals do not have such high amounts of blue light available. Therefore, the Dube Lab has worked to increase light sensitivity by coupling additional photosensitizers to the bacterial cell membrane in an attempt to make the bacteria susceptible to lower energy white light. The therapeutic design for this project utilizes the photosensitizer Chlorin-e4, which has a very similar structure to that of PpIX, but absorbs longer wavelengths of light and has a stronger absorbance overall. These two qualities make Chlorin-e4 more sensitive to white light.

The therapeutic is coupled to the bacterial cell through the technique of metabolic oligosaccharide engineering (MOE). The process, which has been successfully utilized in the Dube Lab, involves the substitution of a naturally occurring sugar of H. pylori—N-acetylglucosamine (GlcNAc)—for a synthetic, azide-labeled sugar, peracetylated N-azidoacetylglucosamine (Ac₄GlcNAz). This results in surface glycoproteins labeled with the azide chemical tag. Azides, which are not naturally present in the human biological system, can then react with a compound known as a cyclooctyne. This reactivity gives us the ability to use a cyclooctyne to deliver compounds—such as Chlorin-e4—to the azide-labeled H. pylori.

Our therapeutic compound for delivery of Chlorin-e4 to the H. pylori cell has three main components: the cyclooctyne aza-dibenzo[cyclooctyne (DIBAC), the Chlorin-e4 photosensitizer, and a FLAG peptide to increase water solubility and enable detection via Western blot analysis. These three groups are linked together with the amino acid cysteine. Reaction of the azide group on Ac₄GlcNAz with the cyclooctyne DIBAC attaches the target compound to the bacterial cell, where the presence of the FLAG peptide allows us to evaluate the success of the coupling via western blot, and the presence of the Chlorin-e4 is hoped to inflict cell death when exposed to white light.

This summer I synthesized and purified the target compound, DIBAC-(Chlorin-e4)-Cysteine-FLAG, using high performance liquid chromatography (HPLC). Two different fractions collected on the HPLC showed presence of the target compound. My early data indicates that the target compound in both of these fractions binds to azide-labeled glycoproteins from live H. pylori. Following this finding, I ran further concentration experiments to minimize background reactivity of the compound with azide-free samples. Moving forward, I will assess the reactivity of the DIBAC-(Chlorin-e4)-Cysteine-FLAG probe with azide-labeled glycoproteins on live H. pylori cells, and will test the ability white light to kill H. pylori cells that have been covalently coupled to the target compound.

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References