Abstract

Title of the thesis:

"Multidimensional Approaches in Developing a Vaccine against Circulating Strains of *Helicobacter pylori*"

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Helicobacter pylori is a major health concern worldwide, particularly in developing nations, affecting nearly half of the global population. Classified as a class I pathogen by the WHO, it is a significant contributor to various gastric diseases, including chronic gastritis, ulcers and gastric cancer. Current diagnostic methods are costly and invasive, making them inaccessible to many individuals. Additionally, the lack of an effective vaccine means that antimicrobial therapies are the primary treatment option, leading to the development of antimicrobial resistance (AMR). In this present study, initially we screened strains isolated from patients suffering from various gastric diseases. Phenotypic and genotypic features of these indigenous strains with putative virulence features allowed selection of the immunogen strains. Selected strains were then evaluated for two types of vaccine platforms: (i) Outer membrane vesicles (OMVs) based and (ii) Nanocurcumin-induced Bacterial Ghosts (CurBGs) based against circulating strains of *H. pylori*. Outer membrane vesicles (OMVs) are protein-rich microvesicles secreted by gram-negative bacteria, with the potential to stimulate an immune response. On the other hand, nanoformulated curcumin disrupts bacterial cell membranes, resulting in the formation of empty bacterial envelopes known as Bacterial Ghosts (CurBGs). Dynamic Light Scattering (DLS) and Transmission Electron Microscopy (TEM) were used to characterize both OMVs and CurBGs. Proteomic analysis revealed the OMVs laden with a wide range of somatic antigens. Conversely, nanocurcumin formulation led to the development of curcumin nanoparticles less than 100nm in size and subsequent treatment to bacteria induced bacterial ghost cells devoid of any inclusion bodies. In-vitro cytotoxicity assay with both the immunogens using murine macrophage cells (RAW 264.7) resulted in significantly low toxicity. Three doses of successive oral immunization using C57BL/6 mice on the 0th, 14th, and 28th day showed a significant increase in serum and secretory antibody titers against outer membrane proteins (OMPs) and whole cell lysate (WCL) of the virulent wild-type strain. Serum antibodies from vaccinated animals showed excellent bactericidal activity and mitigate motility and mucin penetration ability. Ex vivo analysis of harvested spleen cells re-stimulated with respective antigens showed a steep spike in the inflammatory cytokine profile in immunized animals compared to non-immunized animals. In addition, we established an intra-gastric surgical model whereby the stomach was exposed surgically to inject the bacterial inoculums directly into the gastric environment leading to the development of an active infection in less time. In terms of protective efficacy study, histopathological observation showed a significant improvement in gastric architecture in immunized animals, which was further supported by the reduction of colonization. Therefore, this model can be extremely useful in prophylactic, therapeutic, or pathogenesis study. In conclusion, both CurBGs and OMVs have shown to be better immunogens, eliciting a more pronounced immune response in mice compared to the non-immunized, indicating the prospects as potential vaccine candidates.

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