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Title of the thesis: Role of sphingolipids in gut immunology: effect in Enterotoxigenic *E. coli* induced infectious diarrhoeal model in mice

ABSTRACT

Sphingosine-1-phosphate (S1P) is a bioactive pleiotropic phospholipid produced in cells, where it gets phosphorylated by two isozymes of sphingosine kinases, i.e., SPHK1 and SPHK2. Where SPHK-1 derived S1P acts through autocrine and paracrine pathways, S1P generated by SPHK-2 is generally restricted to the nuclear compartment. By Snp2 transporter proteins, S1P is transported extracellularly to bind to its five G protein-coupled receptors (S1PR1-5). The expression of S1PRs is largely heterogeneous and thus regulates diverse roles according to cell type. S1P is known to regulate innate immune response, cell survivability, vascular barrier maintenance, epithelial cell proliferation and migration, angiogenesis, etc. In inflammatory conditions, residential cells increase tissue S1P levels, creating a higher concentration gradient that attracts lymphocytes to the inflammatory site. This phenomenon has led to the development of S1P agonists and antagonists as drugs. FTY720, a pro-drug similar to S1P, targets S1P receptors through phosphorylation by Sphingosine kinase. Its binding to S1PR leads to receptor internalization and degradation, preventing the S1P signaling cascade. This thesis investigated the impact of S1P signaling inhibition on gut barrier function and immune response.

To witness that, we treated HT-29 colon epithelial cells with SPHK-1 inhibitor N-N-Dimethyl Sphingosine and noticed a decrease in Trans Epithelial Electrical Resistance of HT-29 monolayer in a trans well system as well as a downregulation of the Tight junctional protein, Occludin expression. siSPHK-1 transfected HT-29 cells showed a decreased expression of Claudin-5, Occludin, and Junction Adhesion Molecule-A. For the in vivo model, S1P functional antagonist FTY720 (3mg/kg body weight) was given to C57BL/6 mice for 14 consecutive days. FTY720-treated mice showed decreased expression of Occludin and Claudin-4 and increased Claudin-2 in the colon. We also investigated that FTY720 targets the mTOR-Akt pathway to downregulate the tight junctional protein synthesis. Leading to this increased gut permeability, we found an increased bacterial translocation to the liver; however, the colonic expression of the antimicrobial peptide Cathelicidin and Hepcidin significantly reduced. To investigate a leaky gut-induced inflammatory response, we examined and found an increased expression of pro-inflammatory TNF-α, IFN-γ, CXCL-1, and CXCL-2. FTY720 blocks S1PR1 receptors on T cells, which is essential for T cell migration, induced T–cell homing in MLN and Spleen; thus, we found no significant T cell infiltration but an increased infiltration of Neutrophils, Natural Killer cells, B cells and macrophage at the FTY720 treated colonic tissue. Histological analysis of the colon revealed architectural damage of colonic crypts due to immune cell infiltration, crypt hyperplasia, and loss of goblet cells. While working with the enteric infection model with Enterotoxigenic *E coli* (ETEC), we found that H10407 (LT+/ST+-ETEC) clinical isolate infected mice ileal tissues had an increased concentration of S1P. We chose probiotic and prebiotic-based therapy to suppress infection-induced inflammatory response over FTY720. The prophylactic administration of probiotics, prebiotics, and synbiotics (probiotic + prebiotic) was found to reduce ETEC colonization in the ileum and accelerate pathogen clearance. A reduced concentration of tissue S1P and low CD4+ T and CD8+ T cell population and reduced pro-inflammatory TNF-α, IFN-γ, IL-6, CXCL-1 and increased anti-inflammatory IL-10 was found in the treatment groups.

The study highlights that S1P signaling is vital for gut barrier maintenance, and its disruption leads to increased permeability, bacterial translocation, and inflammation. While FTY720 exacerbates gut dysfunction, probiotic/prebiotic therapy offers a promising alternative by restoring microbial balance, reducing inflammation, and enhancing pathogen clearance.

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