Abstract

Redox Molecule Protection of Tight Junctions

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New Earth Dynamics

The evaluation and treatment of chronic disease stemming from intestinal epithelial barrier or tight junction dysfunction results in billions of dollars in healthcare expenditures in the United States annually. Damage to the tight junction can occur through various mechanisms including lipopolysaccarides from infections such as cholera and *C. difficile*, or from environmental toxins such as glyphosate, as well as naturally occurring components of our food, such as gluten. Intestinal epithelial barrier dysfunction is increasingly recognized as an early step in the pathogenesis of many acute and chronic inflammatory diseases including cholera, inflammatory bowel disease (Crohn's Disease and ulcerative colitis), asthma, autoimmune disease, type 2 diabetes, cardiovascular disease, Alzheimer's dementia, and Parkinson's disease. The impact on human health is profound: 3-5 million cases of cholera occur each year worldwide, resulting in over 100,000 fatalities, mostly among children. Four million people suffer from inflammatory bowel disease worldwide, including 1.4 million people in the U.S., and one in three children born after year 2000 are expected to be diabetic.

The objective of this multi-phase SBIR proposal is to complete development and validation of a fulvic acid derivative and soil mineral compound that is prepared to go into large scale production and distribution as the dietary supplement RESTORE. Early clinical data from our multidisciplinary team has demonstrated that RESTORE prevents an increase in tight junction permeability and the resulting systemic inflammation that leading to the various clinical syndromes that can occur. Our preliminary basic science data has demonstrated upregulation of the dipeptidylpeptidase IV (DPP4) enzyme pathway as the potential mechanism by which RESTORE preserves transepithelial electrical resistance (TEER) across tight junctions in the small and large intestinal epithelium and by increasing cell surface expression levels.

We have established methodology to evaluate reactive oxygen species and reactive nitrogen species production as well as rates of apoptosis in cell culture treated with varying concentrations of RESTORE in cell lines of proximal renal tubule, small bowel epithelium, and colon epithelium. Second, we have developed a measure of the functional permeability of the tight junction integrity by TEER measurement across *in-vitro* small and large bowel epithelial membranes. Structural changes to the tight junction pre- and post-treatment will be performed via electron microscopy. Lastly, the biochemical pathway of RESTORE will be established by performing immunoflourescent assays in small bowel and colonic epithelium to establish zonulin and DPP4 levels with glyphosate challenge. There are currently no supplements on the market proven to provide protection against tight junction dysfunction.