White Paper

RESTORE’s Repair and Protection of Tight Junctions from Glyphosate

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Introduction

The potential public health impact and commercial value of a nutritional supplement capable of restoring the frontline of defense against chronic inflammatory and autoimmune diseases is significant. The clinical result of tight junction or 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 [1]. Over 4 million people suffer from inflammatory bowel disease worldwide, including 1.4 million people in the United States. It is estimated that 3-5 million cases of cholera occur each year around the world, resulting in over 100,000 fatalities. Unlike other diseases resulting tight junction dysfunction, cholera can result in death within hours if left untreated.

Inflammatory stress is a unifying pathogenesis of chronic disease in the developed world. We are now witnessing epidemic levels of many of these diseases including type 2 diabetes and spectrum disorders in children, and Alzheimer's dementia in our adult population [1, 2]. Dysregulation of the immune system and unchecked oxidative stress occur as the cellular coping mechanisms of the anti-oxidant system are overwhelmed or depleted. Over the last three decades anti-oxidants have gotten much attention in their roll in modulating the immune system response; they have been touted as anti-cancer agents and dubbed the foundation of the anti-inflammatory mechanism. However, in recent years there is growing evidence that the anti-oxidants – especially the nutritional anti-oxidant sources (e.g. blueberries) are not the primary mediator of inflammation. Instead, redox biochemistry is increasingly recognized as the fundamental communication network of cellular protection and repair. Endogenous sources of these redox molecules are produced by bacteria in the gut in the form of carbon-based fulvic molecules, and intracellular reactive oxygen species (ROS) and reactive nitrogen species (RNS) are produced by vascular endothelium and mitochondria [3, 4].

The anatomical and functional arrangement of the gastrointestinal tract regulates passage of micro- and macro-molecules between the environment and the host through transcellular transport (micromolecules) and paracellular diffusion (macromolecules) via modulation of the intercellular tight junctions. To prevent harm to the host and reduce inflammation, the paracellular pathway minimizes antigen presentation and toxin exposure of the gut-associated lymphoid tissue that abuts the bowel epithelium. Epithelial cells are tightly bound together to minimize the passage of large molecules between adjacent cells. These tight junctions, also called zonula occludens, form a physical barrier throughout the digestive tract that is critical to health.

Zonulin is a serine protease with structure similarities with epidermal growth factor that modulates the polymerization of actin filaments that compose the tight junction; zonulin has been established as the primary mediator of tight junction permeability. For many years the zonulin pathway has been utilized in pharmaceuticals to allow delivery of drugs, macromolecules, or vaccines that normally would not be absorbed through the gastrointestinal mucosal barrier under normal physiologic conditions. Cholera exemplifies the most severe example of pathologic tight junction dysfunction – the zonula occludens
toxin (Zot) that is produced by this bacteria mimics the function of intrinsic zonulin, and its over production during cholera infection leads to sufficient damage to the tight junction to allow for reverse transit of macromolecules into the gut lumen producing a self-perpetuating osmotic diarrhea as water is pulled back into the lumen resulting in the life threatening watery diarrhea. Twenty percent of these cases do not respond successfully to the standard treatment with oral rehydration salts.

In the developed world, the unintentional chronic stimulation of zonulin-mediated intestinal permeability from food elements such as gluten and pesticides such as glyphosate compromises the tight junctions and leads to unregulated absorption of organic and inorganic material. This results in marked increase in oxidative stress and antigen presentation in both the intestinal submucosa as well as the liver which can cause intestinal (celiac sprue) and extraintestinal (Hashimoto’s thyroiditis, rheumatoid arthritis) autoimmune disorders in genetically susceptible individuals. This new model suggests that autoimmune processes may be able to be prevented and arrested if the regulation of intestinal antigen presentation is restored. For those populations not predisposed to autoimmunity, the chronic antigen presentation resulting from tight junction permeability can lead to systemic immune activation, oxidative stress, and end organ damage including renal and vascular diseases, osteoarthritis, and type 2 diabetes.

Our translational research and the commercialization of a stable, soil-derived redox supplement that safely modulates the zonulin-dependent intestinal permeability has the potential to impact many aspects of public health and a variety of translational research fields.

Results

RESTORE’s Protection

RESTORE was found to protect the gut wall from glyphosate insult. A study was performed using this methodology where Transepithelial electric resistance (TEER) was measured in 24 well transwell plates using the epithelial Volt-ohmmeter fitted with a planar electrode. Cells were seeded and incubated until a stable TEER was measured three days in a row.

RESTORE at a 20% concentration, glyphosate at 10 mg/ml (sigma), or both RESTORE (20% concentration) and then glyphosate (10 mg/ml, sigma) was added to the apical membrane compartment and TEER measured at the 30 minute time point. RESTORE increased the TEER in both IEC6 (95%) and Caco-2 (35%). Glyphosate dramatically decreased the TEER in both Caco-2 (76%) and IEC6 (80%) cells. RESTORE blocked this glyphosate dependent decrease in TEER in both cell lines (Figure 1a and b) resulting in TEER that was 296% higher in Caco-2 (p<0.05, n=4) and 474% higher in IEC6 (p<0.05, n=4).

Figure 1a. Average TEER in Caco-2 (large bowel) cells & 1b. IEC6 (small bowel) cells
**RESTORE’s Repair**

RESTORE was also found to repair the gut wall after glyphosate insult. A similar study was performed using this methodology where TEER was measured in 24 well transwell plates using the epithelial Voltohmmeter fitted with a planar electrode. Cells were seeded and incubated until a stable TEER was measured three days in a row. RESTORE at 20% concentration, glyphosate at 10 mg/ml (sigma), or both glyphosate (10 mg/ml, sigma) and RESTORE (20% concentration) was added to the apical membrane compartment and TEER measured at the 30 minute time point.

RESTORE alone increased the TEER in IEC6 (111%). Glyphosate dramatically decreased the TEER in both Caco-2 (83%) and IEC6 (66%) cells. RESTORE repaired this glyphosate dependent decrease in TEER in both cell lines (Figures 2a and 2b) resulting in TEER that was 414% higher in Caco2 (p<0.05, n=4) and 315% higher in IEC6 (p<0.05, n=4).

![Figure 2a. Average TEER in Caco2 (large bowel) cells & 2b. IEC6 (small bowel) cells](image)

**References**


