

Restoration of Colonic Barrier Protein Expression following Lipopolysaccharide and Cytokine-Induced Inflammation in Human Colon Organoids

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Introduction: Aquamin is a mineral complex derived from mineralized remains of marine red algae. It is rich in calcium, magnesium and seventy-two minerals and trace elements. Recent studies with Aquamin have demonstrated that it improves gut barrier structure and function in colon organoids (3D tissue culture) derived from colon biopsies of healthy subjects. Colonic barrier dysfunction is a feature of inflammatory bowel diseases such as ulcerative colitis and Crohn's disease. Since barrier dysfunction could be a result of toxic insult or inflammatory attacks on epithelial cells that line the colon, strengthening the colonic barrier is critical. Through experimentation in organoids derived from normal colon tissue, these studies will allow us to determine how the pro-inflammatory environment modulates barrier structure proteins and inflammation-related proteins in the colon and to determine if, and to what extent, treatment with Aquamin can mitigate these effects.

Methods: We carried out studies using healthy human colon derived organoids (n=5) that were exposed to a mix of pro-inflammatory cytokines and lipopolysaccharides (LPS) in the absence and presence of Aquamin. Organoids were also cultured in a control medium (LWRN25%) with and without Aquamin. After 7 days in culture, tissue samples were assessed for a proteomic profile by tandem mass tag (TMT) mass-spectrometry. Pathway analyses were conducted using UniProt and Reactome databases.

Results: On proteomic screen, LPS/cytokines stimulation caused an upregulation of proinflammatory moieties. Aquamin upregulated anti-inflammatory and antimicrobial proteins and increased the expression of proteins involved in barrier structure. LPS/cytokines and Aquamin alone altered 92 and 91 proteins, respectively, while Aquamin treatment in the presence of LPS/cytokines altered 145 proteins with a 1.8-fold-change cutoff. Twenty-six proteins were commonly altered by both Aquamin alone and Aquamin in the presence of LPS/cytokines. These included several keratins, filaggrin-2, trefoil factor 2, protocadherin-1, olfactomedin-4, cadherin-17, desmoglein-2 and glutathione S-transferase A1. The above proteins were all upregulated by Aquamin alone and in the presence of LPS/cytokines and downregulated by LPS/cytokines alone. *Interferon Signaling*, *Cytokine Signaling in Immune system*, *ER-Phagosome pathway* and *TCR signaling* were the top LPS/cytokine-induced upregulated pathways. In contrast, Aquamin (alone and with LPS/cytokines) upregulated *formation of the cornified envelope*, *keratinization*, and *Vitamin D (calciferol) metabolism*; it downregulated *MyD88 deficiency (TLR2/4)*, *IRAK4 deficiency (TLR2/4)*, and *regulation of TLR by endogenous ligand* pathways.

Conclusion: These data imply that Aquamin can attenuate the inflammatory effects induced by LPS/cytokines. Further investigation may elucidate the beneficial role of Aquamin in mitigating inflammatory bowel diseases and improving the colonic barrier.