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Oligosaccharides, Antioxidants, Amino Acids, and Pufas Each had Different Effects on Heat- And Hypoxia-Induced Epithelial Injury in the Caco-2/Ht-29 Co-Culture Model

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Abstract

When cells in the intestinal epithelium are subjected to heat and hypoxia, compromising the epithelial integrity of the epithelium, a (heat) stress response is triggered. It appears that several categories of nutritional components have beneficial effects on maintaining the integrity of the intestinal epithelium when it is stressed. When Caco-2/HT-29 cells were exposed to heat or hypoxia, the expression of claudin-3 and zonula occludens-1 (ZO-1) was prevented from decreasing by non-digestible oligosaccharides (GOS), fructo-oligosaccharides (FOS), chitosan oligosaccharides (COS), antioxidants (-lipoic acid (ALA), resveratrol (RES), amino acids By reducing MDA production caused by heat or hypoxia, COS, RES, and EPA demonstrated anti-oxidative stress effects, while Arg further increased HSP-70 expression caused by heat or hypoxia. 4) Several nutritional components may be interesting subjects for future clinical trials and in vivo studies on heat stress and hypoxia-related gastrointestinal disorders, based on the findings of this study. These conditions include heat stress and hypoxia.

Keywords: Hypoxia; Heat stress; Epithelial integrity; Nutritional components; Tight junction

Introduction

Changes in diet, the internal environment, and exposure to stressors like toxins and infectious agents have an impact on the homeostasis of the human gastrointestinal (GI) tract. When participating in challenging activities, which are significant sources of stress, competitors and others are more likely to develop various digestive issues. Due to local hypoxic conditions and heat stress, redistribution of blood from the intestine to the extremities and elevated body temperature during strenuous exercise, such as marathon running and cycling, may alter the integrity of the intestinal epithelial barrier [**1**].

The essential structures that guarantee the intestinal barrier's integrity are junctional complexes between adjacent intestinal epithelial cells. A collection of proteins from the tight junction (TJ), adherens junction (AJ), and desmosome all control paracellular transport, stability, and epithelial tightness of this barrier. In a co-culture model utilizing two human colonic epithelial cell lines, Caco-2 and HT-29, our previous research demonstrated that expression of the TJ protein significantly decreased after two hours of exposure to heat (40 or 42 °C) and hypoxia (5% oxygen), while expression of the AJ protein E-cadherin was elevated. After hypoxia and heat treatment, epithelial permeability increased and trans-epithelial electrical resistance (TEER) decreased. The increased permeation of luminal antigens, endotoxins, and bacteria into the local tissues and blood circulation is facilitated by leaky or dysfunctional intestinal epithelial tight junction barriers, which may outcome in severe local and systemic inflammatory conditions. HS-induced intestinal injury and associated disorders may be alleviated by any treatment that restores epithelial integrity or prevents the abnormal expression of TJ/AJ proteins [**2-5**].

Some of the nutritional supplements that have the potential to effectively treat and prevent intestinal disorders brought on by HS include antioxidants, non-digestible oligosaccharides, polyunsaturated fatty acids (PUFAs), and amino acids. These supplements alter immune responses as well as stress resilience pathways, in addition to restoring the expression of TJ/AJ proteins. In HS-exposed Caco-2 cells and chickens, our group demonstrated that galacto-oligosaccharides (GOS) and -lipoic acid (ALA) modulate heat shock protein (HSP)-70 expression, the primary regulator of the HS response.

Integrity of the intestinal epithelial barrier [6]

Paracellular permeability increases and epithelial TJ protein expression decreases in the small intestine epithelium in response to heat and hypoxia. TJ proteins include claudins, occludins, and zonula occludens (ZO). Claudins and Occludins interact with one another on their extracellular sides to facilitate junction assembly, while the ZO family supports the cell's structural integrity. When E-cadherin, the most important cadherin on the epithelial surface, links up with E-cadherin on the cell next to it, AJ formation takes place. When epithelial TJ/AJ structures are destroyed and dissociated, there is increased leakage of luminal toxins or bacteria into the bloodstream. TJ/AJ protein breakdown must be reduced or prevented in order for epithelial cells to better adapt to heat and hypoxia [**7-9**]. As previously demonstrated, Caco-2 cells supported and maintained intestinal homeostasis under heat stress by utilizing GOS, the antioxidant ALA, and the amino acid Arg.

Discussion

An in vitro co-culture model and two human colonic cell lines, Caco-2 and HT-29, were used to measure intestinal barrier function, oxidative stress, and heat shock responses in this study to see how nine nutritional components affected heat/hypoxia-induced intestinal

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epithelial injury. Non-digestible oligosaccharides GOS, FOS, and COS, antioxidants ALA and RES, amino acids Glu and Arg, and PUFAs DHA and EPA may be potential candidates to support intestinal homeostasis and prevent HS/hypoxia-induced alterations in the integrity of the intestinal epithelial barrier, according to our review of potential promising nutritional intervention strategies [**10**].

To replicate the human intestine, our co-culture model uses 90% Caco-2 cells and 10% HT-29 cells. Goblet cells and other specialized secretory cells in the intestinal epithelium are represented by the mucus-producing HT-29 cells. Goblet cells, which make up 10–20 percent of the intestinal cells in the epithelium, form the mucosal layer, a crucial physical and chemical barrier that keeps the intestinal epithelium intact. Caco-2 cells, human intestinal absorptive enterocytes, form columnar monolayers that are well-polarized and resemble a closed intestinal epithelial barrier. The best Caco-2 has been the subject of numerous studies: HT-29 ratio for in vitro studies that mimic the anatomy and physiology of the intestinal in vivo model. It is thought that the most physiologically relevant ratios are those between 9:1 and 7:3 (Caco-2/HT-29), where TEER, a measurement of the monolayer's barrier properties, reaches values that are very similar to those found in the human intestine. 25% of HT-29 cells significantly decreased the TEER values of the Caco-2 monolayer and increased paracellular permeability, whereas 10% of HT-29 cells had no effect on epithelial integrity, in contrast to Caco-2 cells alone. All of the proteins and genes associated with mucins, TJ/AJs, heat shock, and oxidative stress were present in the 9:1 Caco-2/HT-29 monolayer. Therefore, this Caco-2/HT-29 co-culture model can be utilized to investigate how the intestinal barrier is affected by heat and/or hypoxia.

Conclusion

By maintaining TEER values, decreasing paracellular LY permeability, and increasing tight junction protein expression, the non-digestible oligosaccharides, particularly GOS and FOS, the antioxidant ALA, and the PUFA EPA were able to protect Caco-2/HT29 cells from heat/hypoxia-induced intestinal injury. Amino acid Arg behaved more like a "double-edged sword" because its beneficial effect on intestinal barrier function (TEER) was only limited to its physiological level. Higher concentrations further enhanced the heat/hypoxia-induced increase in HSP-70 expression. In addition to their barrier-preserving properties, the antioxidants RES and EPA demonstrated antioxidative activity under heat and hypoxia. The amino acid Glu and the PUFA DHA were less effective at preventing heat or hypoxia-induced intestinal damage in this particular Caco-2/HT-29 co-culture model. Non-digestible oligosaccharides (like GOS, FOS), antioxidants (like ALA, RES), and polyunsaturated fatty acids (like DHA) may be an intriguing preventive (or therapeutic) strategy for combating heat/hypoxia-induced intestinal injury. This may be a significant area of future in vivo and clinical research.

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