Research Article

SCISET
www.sciset.com

Journal of Nutrition, Dietetics & Nutraceuticals

2023
Vol. 1 No. 1: 104

Safeguarding Intestinal Integrity: Lignite Extract Supplement Shields Against Tight Junction Injury Caused by Glyphosate

John J Gildea[1], David A Roberts[2] and Zachary Bush[3]*

[1]Department of Pathology, University of Virginia, Charlottesville, Virginia
[2]Chief Public Health Officer, Biomic Sciences, LLC, Charlottesville, Virginia
[3]Director of Clinical Affairs, Revolution Health Center, Charlottesville, Virginia

Corresponding author:
Zachary Bush, Director of Clinical Affairs, Revolution Health Center, Charlottesville, Virginia. E-mail: mbush@gmail.com

Citation: Gildea JJ, Roberts DA, Bush Z. (2023) Safeguarding Intestinal Integrity: Lignite Extract Supplement Shields Against Tight Junction Injury Caused by Glyphosate. J Nutr Diet Nutraceuticals. Vol 1(1): 104.

Received: April 11, 2023; **Accepted:** April 30, 2023; **Published:** May 07, 2023

Abstract

Introduction: Disruption of tight junctions, critical for intestinal health, is linked to various inflammatory diseases, including inflammatory bowel syndrome. Glyphosate, known to inhibit tight junction function, poses a potential threat. This study aimed to evaluate the protective effects of a lignite extract dietary supplement derived from soil against glyphosate-induced tight junction impairment in cell cultures.

Methods: Small bowel (IEC-6) and colon epithelium (Caco-2) cells were subjected to stable Transepithelial Electrical Resistance (TEER) measurements. A control and a 20% concentration of the lignite extract supplement were applied to the cells overnight. Subsequently, the cells were exposed to glyphosate at 10 mg/ml. TEER was measured at 30 minutes. Immunofluorescent microscopy of the ZO-1 tight junction element was employed to assess tight junction expression before and after overnight incubation and following glyphosate exposure.

Results: TEER significantly increased with the lignite extract on IEC-6 (95%) and Caco-2 (35%) compared to the control. Glyphosate led to a substantial decrease in TEER in both IEC-6 (80%) and Caco-2 (76%) cells. The lignite extract effectively mitigated the glyphosate-mediated reduction in TEER.

Conclusion: The lignite extract supplement demonstrated the ability to counteract the glyphosate-induced decrease in TEER in both cell lines. Pending positive outcomes in clinical trials, RESTORE, the lignite extract supplement, holds promise in addressing intestinal sensitivities resulting from glyphosate-mediated tight junction disruption.

Keywords: Lignite, Transepithelial Electrical Resistance (TEER), Caco-2, IEC-6, Glyphosate, Tight junctions, Tetrahydrate, Intestinal permeability

Abbreviations

TEER: Transepithelial Electrical Resistance; ZO-1: Zonula Occludens 1; GALT: Gastrointestinal-Associated Lymphoid Tissue

Introduction

Intestinal barrier permeability, a critical aspect of tight junction function, plays a vital role in preventing the unregulated passage of foreign substances across epithelial cells. Tight junctions, present in various systems such as the digestive tract, act as barriers regulating water absorption, nutrient transport, and immune responses. These junctions comprise proteins like occludins, Junctional Adhesion Molecules (JAM), claudins, and Zonula Occludens (ZO), forming a paracellular space. Disruptions in tight junctions are associated with inflammatory diseases and certain cancers [1-4].

This study focuses on the impact of environmental toxin exposure, particularly through processed foods, on the epithelial tissue of the small and large intestines. Tight junction dysregulation leading to increased gut permeability is linked to inflammatory diseases

like ulcerative colitis and Crohn's Disease. Glyphosate, a widely used herbicide, has been implicated in disrupting tight junctions, potentially contributing to inflammatory conditions. This study investigates the protective effects of a lignite extract supplement on tight junctions, especially in the presence of glyphosate.

Glyphosate, a nonselective herbicide, inhibits the synthesis of essential amino acids in plants, affecting protein production and leading to plant death. Its extensive use has resulted in widespread environmental presence, raising concerns about its impact on human health. The study explores the potential of lignite extract, a soil-derived mineral supplement, to counteract glyphosate-induced disruptions in tight junctions, focusing on small bowel (IEC-6) and colon epithelium (Caco-2) cells.

With the decline in soil nutrient density due to modern agricultural practices, lignite extracts have gained attention for mineral supplementation. RESTORE, an alkaline liquid lignite-derived mineral supplement, aims to address nutrient deficiencies. The study evaluates how RESTORE influences tight junctions in the intestinal barrier, particularly under glyphosate exposure, using Transepithelial Electrical Resistance (TEER) measurements in epithelial cell models [5-11].

In summary, this investigation seeks to understand the impact of glyphosate on tight junctions and assess the potential protective role of lignite extract, providing insights into mitigating the effects of environmental toxins on intestinal health.

Methods

Cell Culture

Rat ileum epithelial (IEC-6) cell lines and human colorectal carcinoma (Caco-2) cell lines were procured from the American Type Culture Collection (ATCC). Cells were propagated in specific media according to manufacturer protocols.

Exposure to Glyphosate

Glyphosate (Sigma-Aldrich) freshly dissolved at 10 mg/ml concentration was introduced to the apical membrane, and Transepithelial Electrical Resistance (TEER) was measured after 30 minutes.

Exposure to Lignite Extract

Cell membranes were exposed to a lignite extract supplement (20% concentration) and purified water. RESTORE, comprising Terrahydrite™ lignite extract was used, with an alkaline pH (over 7.0). The supplement also contained a proprietary blend of ingredients, both inorganic and organic.

Transepithelial Electrical Resistance (TEER)

TEER, an indicator of tight junction permeability, was measured in IEC-6 and Caco-2 cells using 24-well transwell plates. After three days of incubation for stable TEER, media with or without lignite extract was added, and measurements were taken at the 30-minute time point after introducing glyphosate.

Zona Occludens Protein 1 Immunofluorescence Microscopy

Following TEER measurements, cells were fixed and permeabilized, then blocked and incubated with an anti-ZO-1 monoclonal antibody. Fluorescent labeling was done using Alexa 488 labeled donkey anti-mouse IgG. Nuclei were stained by Hoechst. Imaging was performed with a Zeiss Axiovert automated 6D fluorescent microscope.

Statistics

Experiments were conducted in four replicates, and mean values ± standard error from the mean are presented. P-values were determined using t-tests between groups [12-14].

Results

Effect of Glyphosate on TEER

Glyphosate caused a significant 76% decrease in TEER ($p<0.001$) after 30 minutes. The lignite extract supplement increased TEER by 93% ($p=0.002$). When lignite and glyphosate were combined, lignite prevented the glyphosate-induced decrease in TEER ($p=0.414$) (Figures 1 and 2)[15].

Caco-2 Cells (Large Bowel)

Glyphosate led to an 80% reduction in TEER ($p<0.001$) after 30 minutes. The lignite extract supplement increased TEER by 35% ($p=0.004$). Combining lignite and glyphosate showed lignite preventing the glyphosate-induced decrease in TEER ($p=0.736$).

Effect on Tight Junction Integrity (ZO-1)

Examining ZO-1 of the tight junction by immunofluorescence microscopy in IEC-6 cells revealed:

- In control cells, ZO-1 showed interspersed gaps between cells.
- After incubation with lignite extract for two hours, there was an increase in ZO-1 abundance between cells.
- Glyphosate decreased the consistency of ZO-1 localization between cells.
- Lignite extract prevented the loss of ZO-1 localization induced by glyphosate.

Discussion

Glyphosate and Tight Junction Dysregulation

Glyphosate, introduced in 1976, is widely used in agriculture and daily environments. This study reveals that even low quantities (20 ppm) found in grocery store foods can rapidly disrupt the intestinal tight junction barrier in both small intestine and

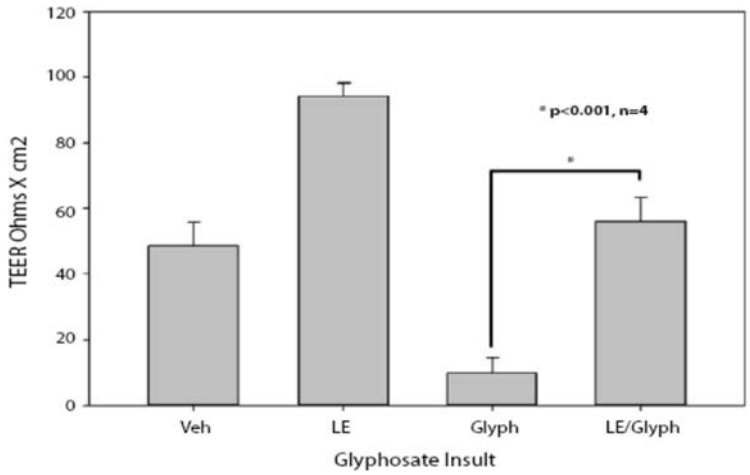

Figure 1: Average TEER in IEC-6 (small bowel) cells. Effects of glyphosate (10 mg/ml) and lignite extract (LE) (20 vol/ vol concentration in media) on the transepithelial electrical resistance (TEER) of IEC-6 monolayers. Data are presented for one experiment, with four replicates. Results are written as mean ± standard error (*Represents a TEER value that is statistically significantly different from the TEER of cells with glyphosate and those with both glyphosate and LE ($p<0.001$)).

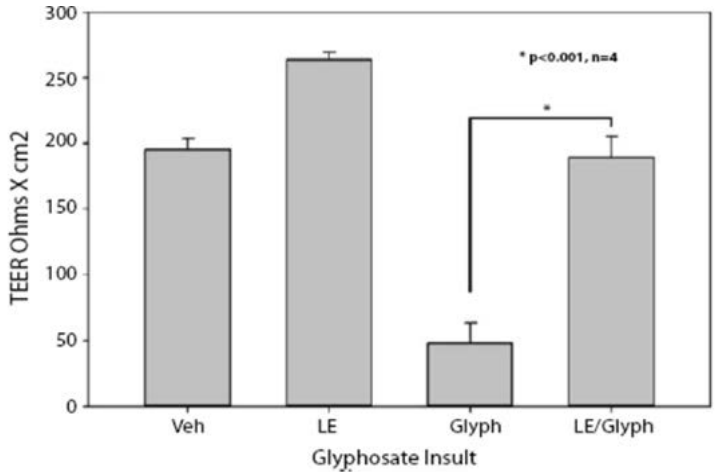

Figure 2: Average TEER in Caco-2 (large bowel) cells. Effects of glyphosate (10 mg/ml) and lignite extract (20 vol/ vol concentrations in media) on the transepithelial electrical resistance (TEER) of Caco-2 monolayers. Data are presented for one experiment, with four replicates. Results are written as mean ± standard error (*Represents a TEER value that is statistically significantly different from the TEER of cells with glyphosate and those with both glyphosate and lignite extract ($p<0.001$)).

colon epithelial membranes. The mechanism of tight junction dysregulation by glyphosate is not fully understood, but it may involve pathways similar to how gluten disrupts tight junctions through the upregulation of zonulin and the Zonulin Occludins Toxin (ZOT) pathway [16-21].

Glyphosate's Mechanism and Lignite's Protective Efficacy

Glyphosate, with its small size and ability to pass through the plasma membrane, likely affects tight junctions uniquely. It may disrupt intracellular F-actin fibers, leading to chaotic nuclei distribution. This study demonstrates this phenomenon and highlights lignite's protective efficacy against glyphosate-mediated tight junction dysregulation. While the experiment methodology provides simplicity, further research is needed to determine the specific mechanism by which glyphosate leads to tight junction dysregulation [22].

Substances Improving Tight Junction Integrity

Few studies have shown improvements in tight junction integrity and Transepithelial Electrical Resistance (TEER). Natural substances like quercetin, butyrate, L-glutamine, probiotic Bifidobacterium, and the lignite extract compound studied here have demonstrated such improvements. Larazotide, a synthetic pharmaceutical and zonulin inhibitor, has also shown positive effects on tight junction formation. Among these, lignite extract

stands out for its quick improvement in tight junction integrity within the first thirty minutes of introduction.

Conclusion

This study underscores the lignite extract's ability to mitigate glyphosate-mediated damage in small intestinal and colon epithelial membranes, even at levels 50 times higher than the allowable limit in certain foods like corn and soy. The direct impact of lignite extract on large and small bowel tissues reveals a swift enhancement of tight junction expression, indicating a reinforcement of intestinal barrier function. These findings carry significant public health implications, considering that tight junction dysregulation leading to intestinal permeability is recognized as a fundamental cause of chronic inflammatory conditions.

Tight junction injury, implicated in diverse conditions from allergies and asthma to Alzheimer's and Parkinson's diseases, raises ongoing debates about the factors contributing to the increased incidence of these ailments—whether it's heightened awareness or substantial changes in our food system.

This study illuminates how the consumption of glyphosate-containing food products may contribute to intestinal barrier permeability, potentially leading to unregulated antigen presentation and chronic inflammatory conditions. Future research is imperative to explore the clinical applications of lignite extract supplements.

Acknowledgments

The authors express gratitude to Barbara Brand for her valuable comments on the manuscript.

References

1. Van Itallie CM, Anderson JM (2014) Architecture of tight junctions and principles of molecular composition. Semin Cell DevBiol 36: 157-165.
2. Gunzel D, Yu AS (2013) Claudins and the modulation of tight junction permeability. Physiol Rev 93: 525-569.
3. Bauer H, Zweimueller-Mayer J, Steinbacher P, Lametschwandtner A, Bauer HC (2010) The dual role of zonulaoccludens (ZO) proteins. J Biomed Biotechnol 2010: 402593.
4. Anderson JM, Van Itallie CM (2006) Tight junction channels. In: Gonzalez-Mariscal L(ed.) Tight junctions. NY, USA: Springer.
5. Fromm M, Schulzke JD (2009) Molecular Structure and Function of the Tight Junction: From Basic Mechanisms to Clinical Manifestations. NY, USA: Wiley-Blackwell.
6. Wang W, Uzzau S, Goldblum SE, Fasano A (2000) Human zonulin, a potential modulator of intestinal tight junctions. J Cell Sci24: 4435-4440.
7. Fasano A (2012) Zonulin, regulation of tight junctions, and autoimmune diseases. Ann N Y AcadSci 1258: 25-33.
8. Fasano A (2012) Leaky gut and autoimmune diseases. Clin Rev Allergy Immunol42: 71-78.
9. Fasano A (2008) Physiological, Pathological, and Therapeutic implications of zonulin-mediated intestinal barrier modulation. Am J Pathol173: 1243-1252.
10. Fasano A (2011) Zonulin and its regulation of intestinal barrier function: the biological door to inflammation, autoimmunity and cancer. Physiol Rev 91: 151-175.
11. Samsel A, Seneff S (2013) Glyphosate's suppression of cytochrome P450 enzymes and amino acid biosynthesis by the gut microbiome: pathways to modern diseases. Entropy 15: 1416-1463.
12. Rudnev MI, Maliuk VI, Stechenko LA (1993) An electron microscopic analysis of the stimulating and toxic effects of mumie-containing preparations. LikSprava 12: 63-64.
13. Saper RB, Phillips RS, Sehgal A, Khouri N, Davis RB, et al. (2008) Lead, mercury, and arsenic in US- and Indian- manufactured Ayurvedic medicines sold via the Internet. JAMA 300: 915-923.
14. Quaroni A, Wands J, Trelstad RL, Isselbacher KJ (1979) Epithelioid cell cultures from rat small intestine. Characterization by morphologic and immunologic criteria. J Cell Biol 80: 248- 265.
15. Rousset M, Chevalier G, Rousset JP, Dussaulx E, Zweibaum A (1979) Presence and cell growth-related variations of glycogen in human colorectal adenocarcinoma cell lines in culture. Cancer Res 39: 531-534.
16. Sääf AM, Halbleib JM, Chen X, Yuen ST, Leung SY, et al. (2007) Parallels between global transcriptional programs of polarizing Caco-2 intestinal epithelial cells in vitro and gene expression programs in normal colon and colon cancer. Mol Biol Cell 18: 4245-4260.
17. Vasiluk L, Pinto, LJ, Moore MM (2005) Oral bioavailability of glyphosate: studies using two intestinal cell lines. Environ Toxicol Chem 24: 153-160.
18. Gildea JJ, Roberts DA, Bush Z (2016) Protection against gluten-mediated tight junction injury with a novel lignite extract supplement. J Nutr Food Sci 6: 547.
19. Ling X, Linglong P, Weixian D, Hong W (2016) Protective effects of Bifidobacterium on intestinal barrier function in LPS-induced enterocyte barrier injury of caco-2 monolayers and in rat NED model. Plos One 11: e0161635.
20. Peng S, Wang SB, Singh D, Zhao PY, Davis K, et al. (2016) Claudin-3 and claudin-19 partially restore native phenotype to ARPE-19 cells via effects on tight junctions and gene expression. Exp Eye Res 151: 179-189.
21. Gildea JJ, Seaton JE, Victor KG, Reyes CM, Bigler Wang D, et al. (2014) Exosomal transfer from human renal proximal tubule cells to distal tubule and collecting duct cells. ClinBiochem 47: 89-94.
22. Shen L, Weber CR, Raleigh DR, Yu D, Turner JT (2011) Tight Junction Pore and Leak Pathways: A Dynamic Duo. Annu Rev Physiol73: 283-309.