Effects of dietary gingerol-enriched ginger supplementation on distribution of colon function markers in rats with diabetic neuropathic pain

Emily R. Stephens[1,2], Julianna M. Santos[2], Rui Wang[2], Chwan-Li Shen[2,3,4]

[1]School of Medicine, [2]Department of Pathology, [3]Center of Excellence for Translational Neuroscience and Therapeutics, [4]Center of Excellence for Integrative Health, Texas Tech University Health Sciences Center, Lubbock, TX

Background

- **Inflammation and physiologic stress** present in diabetes are **associated with increased intestinal permeability** known as "leaky gut."[1]
- Loss of epithelial integrity may contribute to the pathogenesis of diabetic neuropathic pain (DNP) by **perpetuating systemic inflammation.** [1,2,3]
- **Ginger** has been used in the treatment of a variety of pain disorders for its **anti-inflammatory, antioxidant, and analgesic** effects.[4,5]
- We previously reported that **gingerol-enriched ginger (GEG) supplementation suppressed the DNP-induced mRNA expression levels of the following proteins** in the colons of rat models for DNP
  - **Claudin-3** = tight junction protein
  - **PINK1** & **DRP1** = mitophagy markers
  - **GFAP** = enteric nervous system (ENS) marker
- Alterations in the expression of these proteins suggests an inflammatory response. [6,7,8]

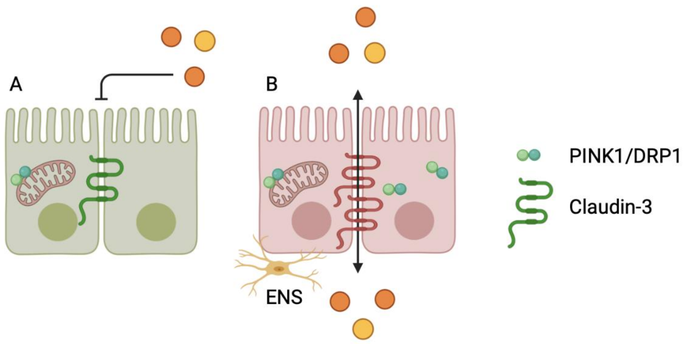

Figure 1. (A) Normal intestinal epithelium and (B) Intestinal epithelium ("leaky gut") with inflammation and compensatory upregulation of mitophagy markers PINK1/DRP1, tight junction protein claudin-3, and ENS.

Specific Aims

We aim to quantify the changes in expression of Claudin3, PINK1, DRP1, and GFAP following GEG supplementation in the colon of DNP rats using immunohistochemistry and confocal fluorescence microscopy.

Methods

- Male Sprague-Dawley rats were assigned to three groups:
  - Low-fat diet (Control)
  - High-fat diet (HFD)+ streptozotocin (DNP)
  - HFD+ streptozotocin+ 0.75% GEG w/w in diet (DNP+GEG)
- Colons were collected and preserved at -80 degrees, sectioned at 10-microns, and stained with fluorescent markers for confocal microscopy
- Protein levels were quantified using average fluorescence intensity in cross-sections of intestinal crypts within pre-determined regions of interest (ROIs), normalized by the background ROI, as described previously. [9]
- The comparisons between groups were made using an unpaired t-test.

Results

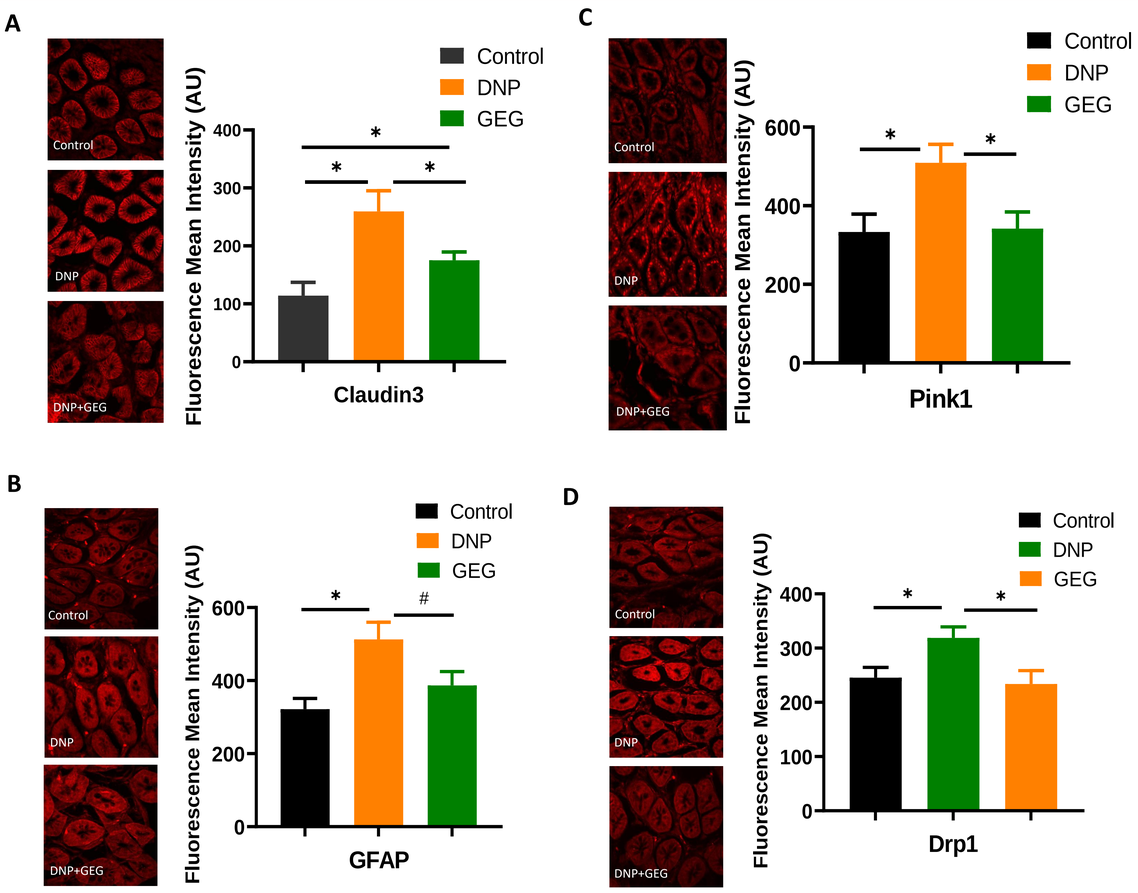

Figure 2. Fluorescence mean intensity for (A) claudin-3, (B) GFAP, (C) PINK1, and (D) DRP1. Compared to controls, the DNP group had higher expression levels of **claudin-3** (Control, n=6; DNP, n=5; p<0.005), **PINK1** (Control, n=7; DNP, n=7; p<0.05), **DRP1** (Control, n=6; DNP, n=7; p<0.01), and **GFAP** (Control, n=8; DNP, n=6; p<0.005).GEG supplementation into diets significantly suppressed DNP-induced expression levels of **claudin-3** (DNP, n=7, DNP+GEG, N=6; p<0.05), **PINK1** (DNP, n=7, DNP+GEG, n=5; p<0.05), and **DRP1** (DNP, n=7, DNP+GEG, n=6; p<0.05). There were no significant differences in expression between the control group and the DNP+GEG group (p>0.05) for PINK1 and DRP1, and GFAP.

Conclusions

- Compared to the Control group, the DNP group had higher expression levels of claudin-3, PINK1, and DRP1, and GFAP in colons of animals.
- GEG supplementation into diets significantly suppressed the DNP-induced expression levels of claudin-3, PINK1, and DRP1.
- There were no significant differences in expression between the Control group and the DNP+GEG group.
- GEG supplementation shows promise to reverse the inflammatory changes the gut-associated with DNP.

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