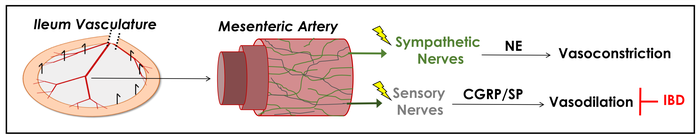
Decreased vascular expression of endothelin converting enzyme-1 and neprilysin in Inflammatory Bowel Disease

Benjamin W. Jones, Elizabeth A. Grunz-Borgmann and Erika M. Boerman

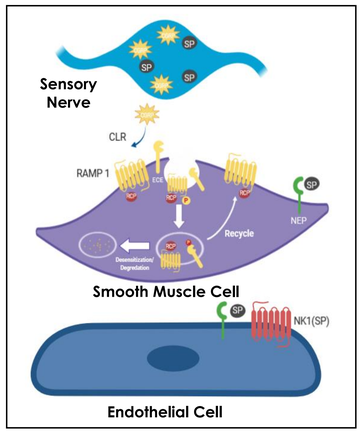
Medical Pharmacology and Physiology, School of Medicine, University of Missouri, Columbia, MO

Medical Pharmacology and Physiology
School of Medicine

Background



- Inflammatory Bowel Diseases (IBD) are chronic diseases that are diagnosed in around 70,000 Americans each year, and 1.6 million Americans in total.
- Inflammatory Bowel Diseases (IBD) are linked to impaired intestinal blood flow and comorbid with cardiovascular diseases, despite the absence of traditional risk factors
- Perivascular sensory nerves that increase blood flow are impaired with IBD
- Sensory nerves release calcitonin gene-related peptide (**CGRP**) and substance P (**SP**) which bind downstream to CGRP (**RAMP1, CLR, RCP**) and SP (**NK1**) receptors
- Endothelin-converting enzyme-1 (**ECE-1**) and neutral endopeptidase (**NEP**) regulate CGRP and SP signaling pathways through peptide degradation and receptor recycling



Previous ELISA results show decreased NEP and ECE-1 concentration in mesenteric arteries (NEP & ECE), colon (NEP), Aorta (ECE) and perivascular adipose (ECE).

Hypotheses

IBD alters the expression and/or localization of ECE and NEP in mesenteric arteries

Methods

- C57BL/6, IL10$^{-/-}$ mice are inoculated with Helicobacter hepaticus by gastric gavage after weaning and develop IBD over 90 days. Non-gavaged C57BL/6 mice serve as controls.
- **Immunofluorescence:** Vessels were incubated in CGRP/SP for an hour before being fixed with 1% paraformaldehyde, blocked (1% BSA + 0.2% Triton X-100) and incubated in primary antibody (ECE-1, NEP, RAMP1, NK1; [1:250]) overnight. Vessels were washed and incubated in secondary antibody [1:500] Mounting media containing DAPI was used. Images were taken on a Leica SP8 confocal microscope at 25x, with a 2x optical zoom.
- **Confocal imaging:** (Leica TCS SP8) of cannulated, immunolabeled mesenteric arteries will be used to determine how IBD affects CGRP/SP receptor recycling and NEP/ECE expression and localization.
- **Human Samples:** Samples were obtained from the University of Missouri Biorepository. IBD samples originated from the colon of IBD human patients, control samples from the colon of non-IBD patients.
- **Data Analysis:** Resulting images were analyzed for vascular area fraction of the staining for ECE, NEP, RAMP1 and NKI using ImageJ, with statistical analysis by nested t-tests

Human Samples

Representative Images

Control

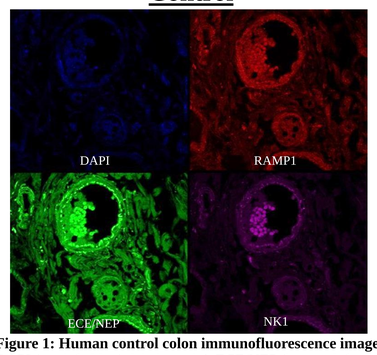


Figure 1: Human control colon immunofluorescence images: DAPI (top left, blue): labels nuclei, ECE/NEP (bottom left, green): labels ECE or NEP, RAMP1 (top right, red): labels CGRP receptors, NK1 (bottom right, magenta): labels SP receptors

IBD

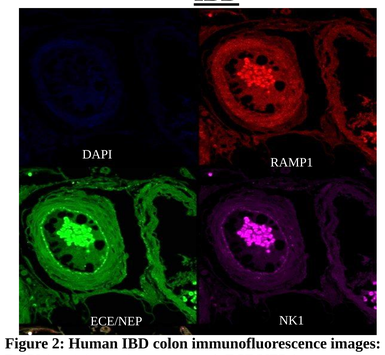


Figure 2: Human IBD colon immunofluorescence images: DAPI (top left, blue): labels nuclei, ECE/NEP (bottom left, green): labels ECE or NEP, RAMP1 (top right, red): labels CGRP receptors, NK1 (bottom right, magenta): labels SP receptors

Results

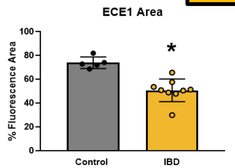


Figure 3: ECE1 Percent Area of Human Colon: Data are individual values and mean ± SE; (Control: n=5) (IBD: n=9) ($p \leq 0.05$)

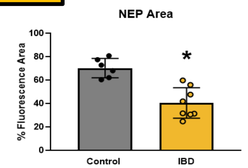


Figure 4: NEP Percent Area of Human Colon: Data are individual values and mean ± SE; (Control: n=6) (IBD: n=8) ($p \leq 0.05$)

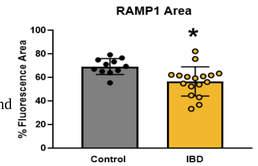


Figure 5: RAMP1 Percent Area of Human Colon: Data are individual values and mean ± SE; (Control: n=11) (IBD: n=18) ($p \leq 0.05$)

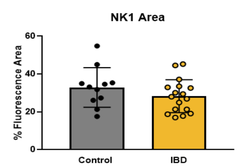


Figure 6: NK1 Percent Area of Human Colon: Data are individual values and mean ± SE; (Control: n=11) (IBD: n=17)

3D Confocal Microscopy

Representative Images

Control

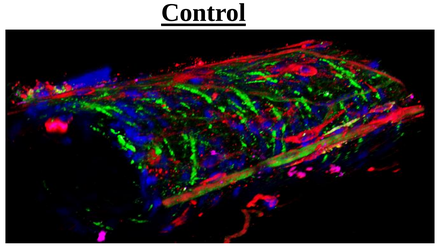


Figure 7: Confocal Imaging of a cannulated Control mesenteric artery. Green staining labels for NEP/ECE-1. Red staining labels for RAMP1 (CGRP receptors). Magenta labels for NKI (SP receptors). Blue stain labels for DAPI (nuclei). Figure 7 shows a representative image.

IBD

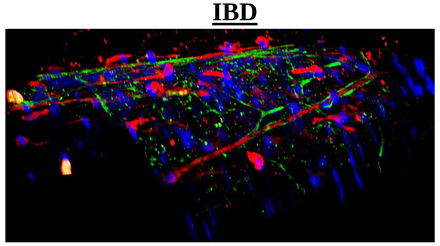


Figure 8: Confocal Imaging of a cannulated IBD mesenteric artery. Green staining labels for NEP/ECE-1. Red staining labels for RAMP1 (CGRP Receptors). Magenta labels for NKI (SP Receptors). Blue stain labels for DAPI (nuclei). Figure 8 shows a representative image.

Next Steps

3D Image Analysis

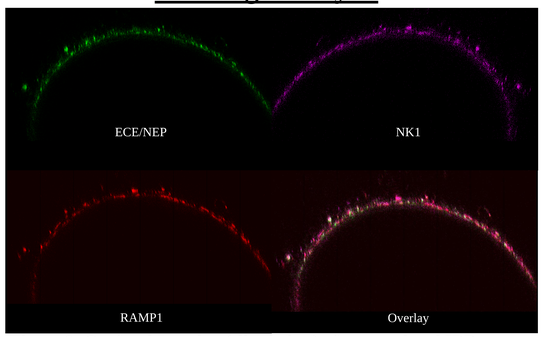


Figure 9: Confocal Imaging (sliced view) of a cannulated IBD mesenteric artery. Green staining labels for NEP/ECE-1. Red staining labels for RAMP1 (CGRP Receptors). Magenta labels for NKI (SP Receptors). Overlay shown. Figure 9 shows a representative image.

Current Work

- We are now beginning to take the head-on sliced images from the 3D confocal microscopy to be analyzed.
- Sliced are analyzed via Image J for percent area as well as the ratio of one staining to another; statistical tests will be run via GraphPad to find any significance.
- Analysis of the 3D images will provide further information, alongside the human sample analysis, to show what effect IBD has on ECE and NEP.

Results

- **ELISA results have shown decreased NEP and ECE expression in multiple tissues associated with vasculature (mesenteric arteries, aorta, perivascular adipose) and the colon.**
- **Confocal imaging of human samples of colon showed a decrease in percent area of ECE1, NEP and RAMP1 in IBD patients.**
- **Current work is being done to examine the vascular expression of ECE1, NEP, RAMP1 and NK1 in cannulated, incubated mouse arteries.**
- **Further analysis from mouse mesenteric arteries will provide more information about how IBD affects CGRP and SP receptor trafficking and degradation as they relate to blood flow through mesenteric arteries.**

Support

NIH grants K99HL129196, R00HL129196 (EMB) and R25 GM 056901(BWJ)