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Evidence against a systemic arterial defect in patients with inflammatory bowel disease

Luke A. Neill, BA¹, Christine M. Saundry, BS², Neil H. Hyman, MD³, and George C. Wellman, PhD^{2,3}

¹University of Vermont College of Medicine, Burlington, VT 05405

²Dept of Pharmacology, University of Vermont, College of Medicine, Burlington, VT 05405

³Dept of Surgery, University of Vermont, College of Medicine, Burlington, VT 05405

Abstract

Background—Despite increasing interest in local microvascular alterations associated with inflammatory bowel disease (IBD), the potential contribution of a primary systemic vascular defect in the etiology of IBD is unknown. We compared reactivity of large diameter mesenteric arteries from segments affected by Crohn's Disease (CD) or ulcerative colitis (UC) to an uninvolved vascular bed in both IBD and control patients.

Methods—Mesenteric and omental arteries were obtained from UC, CD and non-IBD patients. Isometric arterial contractions were recorded in response to extracellular potassium (K⁺) and cumulative additions of norepinephrine (NE). In addition, relaxation in response to pinacidil, an activator of ATP-sensitive K⁺ channels was examined.

Results—Contraction to K⁺ and sensitivity to NE were not significantly different in arteries from CD, UC and controls. Relaxation to pinacidil, was also similar between groups.

Conclusions—Potassium-induced contractions and sensitivity to NE and pinacidil were not significantly different in large diameter mesenteric and omental arteries obtained from IBD patients. Further, there was no difference in the sensitivity to K⁺, NE and pinacidil between mesenteric and omental arteries of CD and UC patients and those from non-IBD patients. Our results suggest an underlying vascular defect systemic to CD or UC patients is unlikely to contribute to the etiology of IBD.

Keywords

Crohn's Disease; Ulcerative Colitis; Inflammatory Bowel Disease; Mesenteric Artery; Potassium; Norepinephrine; Pinacidil

Corresponding Author: Neil H. Hyman, MD, Department of Surgery, Fletcher 465, Fletcher Allen Health Care, 111 Colchester Ave., Burlington, Vermont 05401, Telephone: (802) 847-0629; Fax: (802) 847-5579; neil.hyman@vtmednet.org.

Author contributions:

Luke Neill: Analysis and interpretation of data, writing and approving final version of the manuscript. Christine Saundry: Collection, analysis and interpretation of the data and approving final version of the manuscript. Neil Hyman: Conceiving and designing the study, data interpretation, writing and providing critical revisions, approving the final version of the manuscript. George Wellman: Designing the study, collection, analysis and interpretation of the data, writing the manuscript and providing critical revisions, approving the final version of the manuscript.

Introduction

Inflammatory bowel disease (IBD) is characterized by a chronic inflammatory state often leading to significant derangements in gastrointestinal structure and function.¹ As many as 2.2 million people in Europe and 1.4 million people in the United States suffer from IBD.² Crohn's disease may be characterized by abrupt transitions between unaffected tissue and ulcerated segments as well as transmural inflammation which can be complicated by perforation, fistula, stricture, and abscess.^{3,4} Ulcerative colitis is characterized by continuous inflammation involving only the colorectal mucosa.⁵ Although the clinical manifestations, complications, treatment and outcomes of both of these diseases have been well studied and described, the fundamental cause of IBD remains largely unknown.

The majority of research into the pathogenesis of IBD has been centered on the interaction between microbial, environmental, and genetic factors leading to immune system dysregulation. However, both diseases typically follow predictable regional patterns of inflammation that cannot be readily explained by primary immunological phenomena alone and may be indicative of a distinct vascular pathogenesis. Further, the concomitant occurrence of IBD with vasculitis and hypercoagulability has suggested a potential vascular origin of disease.⁶ Although CD can affect any region of the gastrointestinal tract from the mouth to the anus, it most commonly affects the terminal ileum with up to 75% having ileal disease both with and without colonic involvement.^{7,8} In UC, inflammation always involves the rectum and extends proximally through the colon in a continuous fashion to a limited or universal extent.⁹ It has been suggested that the extent of colitis is determined by the limit of the marginal artery and that characteristics of the mucosal microvasculature in the territory of the inferior mesenteric artery could predispose the colon to ulcerative colitis.¹⁰

Previous studies looking at the regional intestinal blood flow in patients with CD or UC during surgery indicate that chronically inflamed tissues have reduced gut perfusion compared with controls.¹¹ This correlates directly with the histologic findings of fibrosis and ulceration seen histopathologically.¹² Furthermore, endoscopic Doppler laser flowmetry done on chronically inflamed CD bowel tissue has revealed significantly decreased perfusion.^{11,13} These studies demonstrate reduced blood flow to regions of the gut with morphologic features usually associated with chronic ischemia (e.g. ulceration and fibrosis). Although it is unclear whether these are primary or secondary phenomena, these features could potentially be explained by hyperreactivity (specifically vasoconstriction) of the arterial segments supplying the affected bowel.

Despite increased interest in the vascular pathology of IBD, few studies have examined whether there is an underlying systemic vascular defect occurring in CD and UC patients. In this study, we explored whether a local or systemic defect in blood vessel reactivity may be an important primary contributor to the pathogenesis of IBD. We compared the response of mesenteric and systemic (omental) arteries from surgical patients with CD or UC to comparable vessels from non-IBD control patients. We hypothesized that if there was systemic vascular dysregulation in IBD, both omental and mesenteric arteries would respond in an exaggerated manner to vasoactive stimuli; whereas an abnormal response limited to the mesentery would indicate a local vascular defect. However, our finding that arteries of IBD

patients tend to react in a similar fashion to controls suggests the “primary vascular hyperreactivity” hypothesis of IBD is unlikely.

Methods

Patients undergoing elective ileocolic resection for Crohn’s disease or total proctocolectomy for ulcerative colitis, and patients undergoing partial colectomy for either colon cancer or recurrent diverticulitis were offered participation in the study. For the IBD patients, mesenteric arteries supplying a grossly involved section of intestine was harvested. In the case of Crohn’s disease, the mesenteric artery feeding the worst segment of diseased bowel was selected. In ulcerative colitis, a sigmoidal artery was chosen. Both Crohn’s disease and UC patients also had segments of an omental artery procured. In the case of the non-IBD control patients, a mesenteric and omental arteries of similar size to those obtained from Crohn’s disease and UC patients were used. The sample size ranged from $n = 3$ to $n = 7$ arteries; only one artery per patient was used to examine a given ex vivo treatment and n values for each study parameter are provided in the figure legends. This protocol was reviewed and approved by the University of Vermont Institutional Review Board (IRB). Signed consent forms were obtained from patients prior to tissue removal. The University of Vermont has an approved assurance of compliance on file with the Department of Health and Human Services covering this protocol.

Vascular Isometric Force Measurements

Mesenteric and omental arteries were placed in cold (4°C), oxygenated physiological saline solution (PSS) containing (in mM): 118.5 NaCl, 4.7 KCl, 24 NaHCO_3 , 1.18 KH_2PO_4 , 2.5 CaCl_2 , 1.2 MgCl_2 , 0.023 EDTA, 11 glucose and immediately transported to the laboratory for ex vivo studies. Mesenteric and omental arteries were first dissected free of adipose tissue and cut into 3 mm length segments while submerged in cold (4°C), oxygenated PSS. Two stainless steel wires (35 gauge) were then placed through the lumen of the artery segments. One wire was attached to a fixed support, and the other was attached to a Grass (model FT 03, Quincy, MA) isometric force transducer mounted in a 50 ml water-jacketed tissue bath containing oxygenated PSS heated to 37°C . Isometric force measurements were acquired onto a PC using WinDaq software (Dataq Instruments Inc., Akron, OH).¹⁴ A resting tension was applied to each artery by moving the fixed support, placing them at an optimal position on their length-tension curve (determined in preliminary studies). Following a one hour period of equilibration, tissue viability was checked by exposing arteries to PSS containing 60 mM K^+ (iso-osmotic replacement of NaCl with KCl). Arteries that did not contract or contracted minimally to 60 mM K^+ ($< 50\%$ of anticipated maximum contraction) were discarded and replaced. Otherwise, arteries were washed back into 6 mM K^+ PSS and allowed to equilibrate for an additional 30 minutes. Subsequently, cumulative concentration response curves (10^{-8} M to 10^{-5} M) to norepinephrine (NE) were obtained using mesenteric and omental arteries from control, ulcerative colitis and Crohn’s disease patients. Peak contraction values are expressed as a percent of tissue maximum, which was determined using a combination of 120 mM K^+ and 10 μM NE at the end of the experiment. In other arteries, concentration-response curves to the vasodilator pinacidil were obtained from mesenteric and omental arteries. Pinacidil is an activator of ATP-sensitive K^+ channels

of arterial myocytes leading to enhanced K^+ efflux, membrane potential hyperpolarization, a decrease in the open-state probability L-type voltage-dependent Ca^{2+} channels and relaxation of vascular smooth muscle. ** Luke please add Wellman J. Physiol. and Nelson AJP reference here***Prior to the addition of pinacidil, arteries were contracted to approximately 70 % of tissue maximum using a combination of 20 mM K^+ and 10 μ M NE. Pinacidil-induced relaxations are expressed as a percent of maximal relaxation.

Statistical Analysis—Data are presented as mean $\pm$ SEM. ANOVA followed by Tukey test was used to determine statistical significance at the level of $P < 0.05$.

Results

Vasoconstrictor responses are not altered in arteries from IBD patients

Influx of Ca^{2+} through voltage-dependent Ca^{2+} channels (VDCCs) is an important contributor to the contraction of vascular smooth muscle caused by a variety of physiological stimuli.¹⁵ To examine VDCC function, extracellular K^+ was increased from 6 mM to 60 mM by iso-osmotic replacement of NaCl with KCl in PSS bathing arteries obtained from CD, UC and control (non-IBD) patients. An elevation of extracellular K^+ to 60 mM causes a depolarizing shift in the K^+ equilibrium potential to approximately -22 mV leading to an increase in the open-state probability of VDCCs and arterial contraction. With mesenteric arteries obtained from CD and UC patients, 60 mM K^+ caused a contraction equivalent to approximately 80 % of tissue maximum. This response was not significantly different from 60 mM K^+ -induced contractions of omental arteries from UC and CD patients, or omental or mesenteric arteries from non-IBD controls (Figure 1). This data indicates that direct activation of VDCCs via membrane potential depolarization is not impaired in mesenteric or omental arteries from CD and UC patients.

To examine if receptor-mediated contractions are altered in arteries from IBD patients, concentration-response curves (10^{-8} M to 10^{-5} M) were generated using the alpha adrenergic receptor agonist, norepinephrine (Figure 2). As anticipated, norepinephrine potently constricted mesenteric and omental arteries obtained from control patients.¹⁶ Interestingly, the amplitude of norepinephrine-induced contractions observed in mesenteric and omental arteries from CD and UC patients were similar to that obtained in tissue from control patients (Figure 2 A, B). Further, norepinephrine efficacy, assessed by calculation of the concentration needed to evoke 50 % of the maximal response (EC_{50} value) was similar between groups (Figure 2 C).

Vasodilator responses are not altered in arteries from IBD patients

The above findings indicate that vasoconstrictor sensitivity is not enhanced in large diameter arteries obtained from IBD patients. However, a systemic vasodilatory impairment could also potentially contribute to the etiology of IBD. To assess vasodilator function, responses to pinacidil, an activator of smooth muscle ATP-sensitive K^+ channels,¹⁷ were examined in arteries from control, UC and CD patients. Mesenteric and omental arteries were contracted to approximately 70 % of tissue maximum using a combination of 20 mM K^+ and 10 μ M NE prior to the cumulative addition of pinacidil (10^{-8} M to 10^{-5} M).¹⁸ We found that the

Pinacidil responses were also similar in mesenteric and omental arteries obtained from Control, UC and CD Patients (Figure 3). This data indicates that an underlying defect in vascular smooth muscle relaxation is not present in the vasculature of IBD patients.

Discussion

Evidence of microvascular injury in areas of the gut affected by IBD has been used to suggest that an underlying systemic vascular defect may contribute to the etiology of CD and/or UC.^{10, 19–21} To explore this possibility, vasoconstrictor and vasodilator responses were examined in mesenteric and omental arteries obtained from patients with CD, UC and non-IBD pathologies. Artery segments, 0.8 to 1.5 mm in diameter and 3 mm in length, were connected to isometric force transducers mounted in tissue chambers containing warmed and oxygenated physiological saline solution (PSS). Vascular reactivity of mesenteric arteries from inflamed region of the gut of CD and UC patients was compared to their own systemic (omental) arteries, and mesenteric and omental arteries from non-IBD patients. We hypothesized that if a systemic vascular defect is present in CD or UC patients, then vascular beds from both affected and non-affected regions should exhibit enhanced contractile responses.

Improvements in the understanding of vascular biology have revealed an important role of the mesenteric microcirculation in the chronic inflammation and mucosal immunity shown in IBD.¹ Previous studies have shown that responses of mesenteric arteries and veins to ATP were reduced in IBD patients and responses to NPY were greater in mesenteric veins as well. Similar to our results, there were no significant differences in the response in both the larger omental/mesenteric arteries and the micro-vascular responses of mesenteric arteries and veins to norepinephrine.²² It has been shown that the vasodilator response of human intestinal arterioles (50–150µm diameter) with ACh was significantly reduced in CD patients suggesting a loss of NO dependent dilation contributing to hypoperfusion and poor wound healing in response to long term inflammation.²³ Decreased vasoconstriction in response to phenylephrine has also been shown in arterial segments (300–500µm diameter) in CD.²⁰ However, when examining the pathogenesis of IBD, it is important to consider the cause versus effect with respect to vascular function and whether the microvascular changes observed in IBD patients are primary or simply represent a secondary response to inflammation or other local phenomena.

The present study directly examined selected vasoconstrictor and vasodilator responses in human intestinal omental and mesenteric arteries in control subjects and in patients with UC and CD. The findings reported here indicate that sensitivity to K⁺, norepinephrine (NE) and pinacidil are not significantly different in large diameter mesenteric and omental arteries obtained from healthy, UC, and CD patients. Thus, our investigation shows no evidence of differences in vascular smooth muscle function between mesenteric arteries from inflamed regions of the gut and omental arteries from non-inflamed tissue in CD or UC patients when compared to omental and mesenteric vessels obtained from non-IBD patients. These findings provide important new information suggesting that there is not a vascular defect systemic to CD or UC patients.

A number of potential limitations should be considered with respect the present study. First, although ex vivo isometric force studies enable direct assessment of vascular smooth muscle function in response to vasoactive stimuli, these vascular preparations were removed from their native environment and were examined in the absence of neuronal input and exposure to paracrine and endocrine influences. In addition, the present study examined responses to a limited number of vasoactive stimuli (K^+ , norepinephrine and pinacidil); considering the vast number of vasoactive agents, it is possible that arteries from UC or CD patients may exhibit altered reactivity to other constrictor or dilator agents. Further, as colon resection is not typically done in truly healthy individuals, mesenteric and omental arteries were obtained from non-IBD patients undergoing partial colectomy for either colon cancer or elective recurrent diverticulitis. Theoretically these other disease states could also affect the local vasculature. Considering the nature of the surgery, only mesenteric and omental arteries were obtained; thus only one systemic vascular bed (omental) was examined in this study. Finally, this study examined reactivity of relatively large diameter arteries and did not investigate responses in veins or the microvasculature, including capillaries. In our study, we did not aim to describe the microvasculature reaction observed in the setting of inflammation, but rather sought to determine whether there is a primary dysregulation or hypersensitivity of mesenteric or system arteries to vasoactive stimuli. We found no evidence to support a primary arterial defect in the etiology of IBD.

Conclusion

Our results suggest that sensitivity to K^+ , norepinephrine (NE) and pinacidil are not significantly different in large diameter mesenteric and omental arteries in patients with inflammatory bowel disease (IBD). Vascular reactivity, specifically sensitivity to K^+ , NE and pinacidil, of the large diameter mesenteric and omental arteries is not affected in patients with IBD.

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References

1. Hatoum OA, Miura H, Binion DG. The vascular contribution in the pathogenesis of inflammatory bowel disease. *Am J Physiol Heart Circ Physiol*. 2003; 285(5):H1791–H1796. [PubMed: 14561675]
2. Loftus EV Jr. Clinical epidemiology of inflammatory bowel disease: Incidence, prevalence, and environmental influences. *Gastroenterology*. 2004; 126(6):1504–1517. [PubMed: 15168363]
3. Ardizzone S, Porro GB. Perianal Crohn's disease: overview. *Dig Liver Dis*. 2007; 39(10):957–958. [PubMed: 17723323]
4. Loftus EV Jr, Sandborn WJ. Epidemiology of inflammatory bowel disease. *Gastroenterol Clin North Am*. 2002; 31(1):1–20. [PubMed: 12122726]
5. Danese S, Fiocchi C. Ulcerative colitis. *N Engl J Med*. 2011; 365(18):1713–1725. [PubMed: 22047562]
6. Ardizzone S, Bianchi Porro G. Biologic therapy for inflammatory bowel disease. *Drugs*. 2005; 65(16):2253–2286. [PubMed: 16266194]

7. Radford-Smith G, Pandeya N. Associations between NOD2/CARD15 genotype and phenotype in Crohn's disease--Are we there yet? *World J Gastroenterol.* 2006; 12(44):7097–7103. [PubMed: 17131470]
8. Baumgart DC, Sandborn WJ. Crohn's disease. *Lancet.* 2012; 380(9853):1590–1605. [PubMed: 22914295]
9. Seidelin JB, Coskun M, Nielsen OH. Mucosal Healing in Ulcerative Colitis: Pathophysiology and Pharmacology. *Advances in Clinical Chemistry.* 2013; 59:101–123. [PubMed: 23461134]
10. Hamilton MI, Dick R, Crawford L, Thompson NP, Pounder RE, Wakefield AJ. Is proximal demarcation of ulcerative colitis determined by the territory of the inferior mesenteric artery? *Lancet.* 1995; 345(8951):688–690. [PubMed: 7885124]
11. Angerson WJ, Allison MC, Baxter JN, Russell RI. Neoterminal ileal blood flow after ileocolonic resection for Crohn's disease. *Gut.* 1993; 34(11):1531–1534. [PubMed: 8244138]
12. Geboes K. Pathology of inflammatory bowel diseases (IBD): variability with time and treatment. *Colorectal Dis.* 2001; 3(1):2–12. [PubMed: 12791013]
13. Tateishi S, Arima S, Futami K. Assessment of blood flow in the small intestine by laser Doppler flowmetry: comparison of healthy small intestine and small intestine in Crohn's disease. *J Gastroenterol.* 1997; 32(4):457–463. [PubMed: 9250891]
14. Jewell RP, Saundry CM, Bonev AD, Tranmer BI, Wellman GC. Inhibition of Ca⁺⁺ sparks by oxyhemoglobin in rabbit cerebral arteries. *J Neurosurg.* 2004; 100(2):295–302. [PubMed: 15086238]
15. Nelson MT, Patlak JB, Worley JF, Standen NB. Calcium channels, potassium channels, and voltage dependence of arterial smooth muscle tone. *Am J Physiol.* 1990; 259(1 Pt 1):C3–C18. [PubMed: 2164782]
16. Toda N, Fujita Y. Responsiveness of isolated cerebral and peripheral arteries to serotonin, norepinephrine, and transmural electrical stimulation. *Circ Res.* 1973; 33(1):98–104. [PubMed: 4765703]
17. Wellman GC, Quayle JM, Standen NB. ATP-sensitive K⁺ channel activation by calcitonin gene-related peptide and protein kinase A in pig coronary arterial smooth muscle. *J Physiol.* 1998; 507(Pt 1):117–129. [PubMed: 9490826]
18. Dunn WR, Wellman GC, Bevan JA. Enhanced resistance artery sensitivity to agonists under isobaric compared with isometric conditions. *Am J Physiol.* 1994; 266(1 Pt 2):H147–H155. [PubMed: 8304495]
19. Wakefield AJ, Sankey EA, Dhillon AP, Sawyerr AM, More L, Sim R, Pittilo RM, Rowles PM, Hudson M, Lewis AA, et al. Granulomatous vasculitis in Crohn's disease. *Gastroenterology.* 1991; 100(5 Pt 1):1279–1287. [PubMed: 2013373]
20. Lebuffe G, Haddad E, Desreumaux P, Gambiez L, Colombel JF, Vallet B. Impaired contractile response of mesenteric arteries in Crohn's disease. *Aliment Pharmacol Ther.* 2000; 14(10):1279–1285. [PubMed: 11012472]
21. Knutson H, Lunderquist A, Lunderquist A. Vascular changes in Crohn's disease. *Am J Roentgenol Radium Ther Nucl Med.* 1968; 103(2):380–385.
22. Birch D, Knight GE, Boulos PB, Burnstock G. Analysis of innervation of human mesenteric vessels in non-inflamed and inflamed bowel--a confocal and functional study. *Neurogastroenterol Motil.* 2008; 20(6):660–670. [PubMed: 18298440]
23. Hatoum OA, Binion DG, Otterson MF, Gutterman DD. Acquired microvascular dysfunction in inflammatory bowel disease: Loss of nitric oxide-mediated vasodilation. *Gastroenterology.* 2003; 125(1):58–69. [PubMed: 12851871]

Figure 1. Responses to 60mM K⁺ are Similar in Mesenteric and Omental arteries from Control, Ulcerative Colitis and Crohn's Patients

Human mesentery (left) and omental (right) arterial contractions to 60 mM K⁺ expressed as a percent of tissue maximum. Bars represent control (con), ulcerative colitis (UC) and Crohn's (CD) patients. Values represent mean $\pm$ standard error of the mean. There were no significant differences between groups ($p > 0.05$). Mesenteric and omental arteries: Control: n = 6; UC: n = 4; CD: n = 5.

Figure 2. Norepinephrine Sensitivity is Similar in Mesenteric and Omental Arteries from Control, Ulcerative Colitis and Crohn's Patients

A) mesenteric and B) omental artery contractions to cumulative addition of increasing concentrations of norepinephrine. Arteries were obtained from non-IBD patients (squares, n = 5), patients with ulcerative colitis (circles, n = 5), and patients with Crohn's disease (triangles, n = 4) patients. C) Norepinephrine EC₅₀ values, expressed as a negative log molar concentration, in non-IBD control patients (Con), ulcerative colitis patients (UC) and Crohn's disease patients (CD). Values represent mean $\pm$ standard error of the mean. There were no significant differences between groups ($p > 0.05$).

Figure 3. Pinacidil-induced Relaxation is Similar in Mesenteric and Omental Arteries from Control, Ulcerative Colitis and Crohn's Patients

A) mesenteric and B) omental artery relaxation to cumulative addition of increasing concentrations of pinacidil. Arteries were obtained from non-IBD patients (squares, $n = 7$ mesenteric and $n = 6$ omental arteries), patients with ulcerative colitis (circles, $n = 5$ mesenteric and $n = 4$ omental arteries), and patients with Crohn's disease (triangles, $n = 5$ mesenteric and $n = 3$ omental arteries) patients. C) Pinacidil EC_{50} values, expressed as a negative log molar concentration, in non-IBD control patients (Con), ulcerative colitis

patients (UC) and Crohn's disease patients (CD). Values represent mean $\pm$ standard error of the mean. There were no significant differences between groups ($p>0.05$).