JOURNAL OF SURGICAL RESEARCH 191 (2014) 318–322

Available online at www.sciencedirect.com

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journal homepage: www.JournalofSurgicalResearch.com

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

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ARTICLE INFO

Article history:
Received 18 October 2013
Received in revised form
6 January 2014
Accepted 9 April 2014
Available online 16 April 2014

Keywords:
Crohn disease
Ulcerative colitis
Inflammatory bowel disease
Mesenteric artery
Potassium
Norepinephrine
Pinacidil

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 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 adenosine triphosphate-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. Furthermore, there was no significant 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.

1. 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 disease (CD) 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 (UC) 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.

Most 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

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http://dx.doi.org/10.1016/j.jss.2014.04.021

regional patterns of inflammation that cannot be readily explained by primary immunologic phenomena alone and may be indicative of a distinct vascular pathogenesis [6–8]. Furthermore, the concomitant occurrence of IBD with vasculitis and hypercoagulability has suggested a potential vascular origin of disease [9]. 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 [10,11]. In UC, inflammation always involves the rectum and extends proximally through the colon in a continuous fashion to a limited or universal extent [12]. 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 UC [6].

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 [13]. This correlates directly with the histologic findings of fibrosis and ulceration seen histopathologically [14]. Furthermore, endoscopic Doppler laser flowmetry done on chronically inflamed CD bowel tissue has revealed significantly decreased perfusion [13,15]. These studies demonstrate that 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.

2. Methods

Patients undergoing elective ileocolic resection for CD or total proctocolectomy for UC, 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 were harvested. In the case of CD, the mesenteric artery feeding the worst segment of diseased bowel was selected. In UC, a sigmoidal artery was chosen. Both CD and UC patients also had segments of an omental artery procured. In the case of the non-IBD control patients, mesenteric and omental arteries of similar size to those obtained from CD and UC patients were used. The sample size ranged from $n = 3$ to $n = 7$ arteries for each group; 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. Signed consent forms were obtained from patients before 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.

2.1. *Vascular isometric force measurements*

Mesenteric and omental arteries were placed in cold (4°C), oxygenated physiological saline solution (PSS) containing (in millimolar): 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 personal computer using WinDaq software (Dataq Instruments Inc, Akron, OH) [16]. 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 1-h 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 [17,18]. Otherwise, arteries were washed back into 6 mM K^+ PSS and allowed to equilibrate for an additional 30 min. Subsequently, cumulative concentration–response curves (10^{-8} M to 10^{-5} M) to norepinephrine (NE) were obtained using mesenteric and omental arteries from control, UC, and CD 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 adenosine triphosphate-sensitive K^+ channels of arterial myocytes leading to enhanced K^+ efflux, membrane potential hyperpolarization, a decrease in the open-state probability of L-type voltage-dependent Ca^{2+} channels (VDCCs) and relaxation of vascular smooth muscle [19,20]. Before the addition of pinacidil, arteries were contracted to approximately 70% of tissue maximum using a combination of 20 mM K^+ and 10 μM NE. Pinacidil-induced relaxations are expressed as a percent of maximal relaxation.

2.1.1. *Statistical analysis*

Data are presented as mean ± standard error of mean. Analysis of variance followed by Tukey test was used to determine statistical significance at the level of $P < 0.05$.