Rectal Mucosal Nitric Oxide in Differentiation of Inflammatory Bowel Disease and Irritable Bowel Syndrome

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Background & Aims: **Differentiating patients with functional bowel disorders from those with inflammatory bowel disease (IBD) can be difficult. Rectal luminal levels of nitric oxide (NO) are greatly increased in IBD. To further evaluate this disease marker, we compared NO in patients with irritable bowel syndrome (IBS) with those found in patients with active IBD and in healthy control subjects.** ***Methods:*** **Rectal NO was measured with chemiluminescence technique by using a tonometric balloon method in 28 healthy volunteers, 39 patients with IBS, 86 with IBD (Crohn's disease and ulcerative colitis), and 12 patients with collagenous colitis. In addition, NO was measured before and after a 4-week treatment period in patients with active ulcerative colitis and repeatedly during 2 weeks in healthy volunteers.** ***Results:*** **NO was low in healthy control subjects (median, 45; 25th–75th percentile, 34–64 parts per billion [ppb]), and variations over time were small. In IBS patients NO was slightly increased (150, 53–200 ppb; $P < .001$), whereas patients with active IBD or collagenous colitis had greatly increased NO levels (3475, 575–8850 ppb, and 9950, 4475–19,750 ppb, respectively; $P < .001$). With a cutoff level of 250 ppb, NO had a sensitivity of 95% and a specificity of 91% in discriminating between active bowel inflammation and IBS. Rectal NO correlated with disease activity in IBD and collagenous colitis and decreased markedly in IBD patients responding to anti-inflammatory treatment.** ***Conclusions:*** **Rectal NO is a minimally invasive and rapid tool for discriminating between active bowel inflammation and IBS and a possibly useful add-on for monitoring patients with IBD.**

Inflammatory bowel disease (IBD) and irritable bowel syndrome (IBS) are chronic conditions that can present with similar symptoms, but they have different underlying pathophysiology. IBD is an idiopathic inflammatory condition affecting varying locations of the gastrointestinal (GI) tract. IBS is a chronic disorder of variable symptoms characterized by abdominal pain and changes in bowel habits in the absence of apparent organic disease.[1] It is one of the most frequent causes of chronic diarrhea in adult patients and the most common outpatient diagnosis among gastroenterologists, even though they only see a small fraction of the patients.[2] The Rome II criteria[3] are used in the diagnosis of IBS, but clinical differentiation between IBS and IBD remains problematic because many symptoms overlap. Therefore, many patients are extensively investigated with expensive invasive radiographic and endoscopic imaging to make a diagnosis of exclusion and to rule out malignant disease.[4] In the clinical setting it would be of value to achieve a feasible method to distinguish a disorder of inflammatory nature (eg, Crohn's disease or ulcerative colitis) from one presumed to be of noninflammatory origin, such as IBS.

The gas nitric oxide is a pluripotent biologic messenger involved in numerous physiologic and pathologic events in the GI tract.[5] During inflammation the mucosal production of NO is increased. An inducible NO synthase is up-regulated in response to proinflammatory cytokines released in the inflamed area, and this enzyme can generate large quantities of NO during an extended period of time. NO is very short-lived in most biologic systems,[6,7] and because of this, direct analysis is often difficult. However, in the gaseous phase NO is stable, which has allowed for direct measurements in hollow organs such as the airways[8,9] and the GI tract.[10] Patients with inflammatory intestinal disorders including IBD,[10,11] gastroenteritis,[12] and celiac disease[13,14] have greatly increased NO levels locally in the gut. Luminal NO can be measured in the rectum with a minimally invasive tonometric balloon technique.[11] It has been suggested that NO could serve as a sensitive marker of inflammation in the GI tract, which might be useful in screening of patients with suspected IBD and in monitoring patients with an established diagnosis of IBD.[15,16]

Abbreviations used in this paper: CI, confidence interval; GI, gastrointestinal; HBI, Harvey Bradshaw index; IBD, inflammatory bowel disease; IBS, irritable bowel syndrome; NO, nitric oxide; ppb, parts per billion.

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In further evaluating such a novel test, it will be essential to establish normal levels of NO in the gut and to study rectal NO levels in other intestinal disorders in which patients might present with symptoms resembling those seen in IBD.

The aim of this study was to determine rectal NO levels in patients with IBS and to compare them with those found in patients with active IBD and healthy control subjects. In addition, the variation in rectal NO over time was studied in healthy subjects and in symptomatic ulcerative colitis patients during conventional treatment.

Patients and Methods

This study was done in two parts, the first with a cross-sectional and the second with a longitudinal design in which NO measurements were performed repeatedly in control subjects and before and after medical treatment in patients with active disease. The studies were performed at the Karolinska University Hospital, Stockholm, Sweden between 1999 and 2004. Permission for the study was obtained from the Regional Ethics Committee, and all subjects gave informed consent before entering the study. The patients included were either hospitalized (IBD only) or were visiting an outpatient ward (IBS, IBD, and collagenous colitis patients). The same gastroenterologist (P.M.H.) examined all patients.

Cross-sectional Study

Table 1 shows the demographic details of control subjects and patients. The control group consisted of 28 healthy volunteers with no history of GI disease or symptoms.

The IBS group comprised 39 patients diagnosed according to the Rome II criteria,[3] and all had a typical history and symptoms. The patients were all of the alternating type, and no predominant diarrhea such as chronic diarrhea exceeding 14 days was studied. Patients with chronic constipation were not studied. Each patient had been investigated with a negative outcome of endomysium or transglutaminase antibodies for celiac disease and normal colonoscopy.

The IBD group consisted of 47 patients with ulcerative colitis, 39 with Crohn's disease, and 12 patients with collagenous colitis. All had an established diagnosis based on previous endoscopic and microscopic examinations. Duration of disease varied between 1 and 30 years. Patients with ulcerative colitis were classified according to the Truelove-Witts' scoring (0–3) of disease activity[17]: remission (0), mild (1) , moderate (2), and severe (3), whereas the Harvey Bradshaw index (HBI) was used for Crohn's disease.[18] For some calculations and presentations, disease activity in Crohn's disease was converted to the 4-graded scale used in ulcerative colitis patients: remission (HBI score $\leq$4), mild (HBI score 5–6), moderate (HBI score 7–9), and severe (HBI score $\geq$10). Patients in clinical remission had been free of symptoms for at least 6 months. Disease activity in collagenous colitits was also graded on a scale 0–3 on the basis of the number of bowel movements with diarrhea the patients had each day: remission (no diarrhea; score 0), mild ($<$4/day; score 1), moderate (4–5/day; score 2), and severe ($\geq$6/day; score 3).

Longitudinal Study

NO was measured repeatedly in 7 of the healthy volunteers from the cross-sectional study. In these subjects

Table 1. Demographic Characteristics of Patients and Control Subjects in the Cross-sectional Study

Characteristic	Control subjects	IBS	Ulcerative colitis	Crohn's disease	Collagenous colitis
N	28	39	47	39	12
Sex (M/F)	16/12	11/28	24/23	21/18	2/10
Age range (*y*)	20–47	18–81	23–82	20–84	37–87
Disease activity					
Remission			14	13	3
Mild			6	8	1
Moderate			12	7	2
Severe			15	11	6
Disease location					
Small bowel				4	
Ileum				3	
Ileocecal				8	
Colon			47	24	12
Extensive			14		
Left-side			33		
Treatment					
Corticosteroids			18	20	1
Sulfasalazine			3	2	
5-Aminosalicylic acid			20	10	
Azathioprine			2	2	