Increased Nitric Oxide in Infective Gastroenteritis

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Nitric oxide (NO) production is increased in several inflammatory disorders, although the role of this gas is not clear. The purpose of this study was to determine whether luminal NO in the intestine is increased in infective gastroenteritis. Rectal gas was sampled in 17 patients with gastroenteritis and 10 healthy volunteers, with balloon catheters made of 100% silicone and analyzed for NO by chemiluminescence. Plasma nitrate and nitrite levels were determined by capillary electrophoresis. Rectal NO was (mean ± SEM) 9441 ± 3126 parts per billion (ppb) in the patients and 74 ± 13 ppb in controls (P < .0001). There was no individual overlap. Plasma nitrite but not nitrate was significantly increased in patients compared with controls. These data indicate that luminal NO is greatly increased in gastroenteritis. The high levels of NO are easily measurable by rectal sampling, and measurement of luminal NO seems to be useful for evaluating local NO production in the gut in health and disease.

Two centuries ago, Prout [1] found abundant nitrate in the urine of a febrile patient. Little did he know that nitrate is the stable end product of nitric oxide (NO) when this diatomic gas is produced in the human body. In the last 15 years, we have become aware that NO is an important molecule in mammals, with effects that include vasodilatation, neural transmission, and immunologic activity. The nitrate that Prout discovered was probably derived from NO produced during the inflammatory process of infectious disease. This gas is produced from L-arginine by a family of enzymes called NO synthases (NOS). Two isoforms are calcium-dependent and named constitutive NOS (cNOS). The third isoform is known as inflammatory or inducible NOS (iNOS) and is activated by certain proinflammatory cytokines and bacterial toxins. This isoform is regulated at the gene level by transcription factors such as NF-κB. Indirect evidence for activation of the L-arginine–NO pathway in inflammation in the gut include determination of the coproduct citrulline [2], immunohistochemical staining [3], and quantification of nitrate [4]. Direct analysis of NO in most biologic systems is difficult because of the short-lived nature of this gas:

NO reacts rapidly with hemoglobin and other iron-containing compounds. However, NO in the gaseous phase is much more stable, enabling direct measurements in hollow organs such as the airways [5] and intestine [6]. Determination of gaseous NO has revealed increased levels of the gas in inflammatory disorders that include asthma [5], cystitis [7], and colitis [8–10].

The first measurements of luminal NO were made in connection with colonoscopy in patients with inflammatory bowel disease, where gas was sampled directly from the colon through the instrument [8]. This concept has been further developed, and we recently showed that rectal measurement of luminal NO is a possible way of detecting inflammation [9]. As for infective gastroenteritis, Dykhuizen et al. [4], following Prout, showed increased plasma levels of nitrate in a group of patients. In this study, we measured luminal concentrations of NO and plasma nitrate/nitrite in patients with gastroenteritis and in controls.

Patients and Methods

We randomly selected 17 adults with no previous intestinal disorders who visited the clinic for infectious diseases at Huddinge Hospital, Stockholm, for treatment of diarrhea. All were examined by a specialist, and diagnoses were based on clinical history, symptoms, and laboratory findings. Control subjects were 10 nonsmoking, healthy, volunteer laboratory and clerical staff with no symptoms of gastrointestinal disease (ages, 25–45 years; 9 men, 1 woman). Fecal samples were analyzed by established methods for enteropathogenic bacteria, including Salmonella, Shigella, Campylobacter, and Aeromonas species and Yersinia enterocolitica, Plesiomonas shigelloides, and Clostridium difficile. Slide preparations were used...
Figure 1. A, Rectal concentrations of NO in patients with gastroenteritis and controls. y axis, logarithmic scale (***(p < .001). B and C, Plasma concentrations of nitrite and nitrate in patients with gastroenteritis compared with controls (***p < .001; ns = not significant).

for ova and parasite examinations. Stool specimens were also examined for viruses by negative contrast electron microscopy, as described elsewhere [11].

C-reactive protein (CRP), hemoglobin, white blood cell count, platelet count, and serum electrolyte concentrations were analyzed at the hospital laboratory. Blood samples for measurement of nitrate and nitrite were spun, and plasma was stored at −70°C until analysis. Concentrations of nitrate and nitrite were determined by capillary electrophoresis, as described elsewhere [12]. This method is very specific, and there is no interference from factors such as hemoglobin.

For NO measurements, we used a chemiluminescence analyzer (CLD 700; Eco Physics, Dürnten, Switzerland). The detection limit for NO was 1 part per billion (ppb) of NO in nitrogen administered via an electromagnetic flow controller (Environics, Middletown, CT). The chemiluminescence assay is highly specific for NO, and there is no interference from other nitrogen oxides.

For sampling, we used 100% silicone Foley urinary catheters (Argyle; Sherwood Medical, Tullamore, Ireland). The catheters were inserted in the rectum by use of lubrication gel free of local anesthetics. The cuff was inflated with 10 cc of ambient air containing <5 ppb NO and incubated for 10 min. In all experiments, the studied gas was injected directly into the analyzer and the peak levels of NO were monitored.

To determine the rate of recovery for the catheters, we did experiments with incubation in known concentrations of NO for different periods. Gas was flushed through a canister in which the catheters were inserted. Concentrations were continuously monitored, and samples from the catheters were compared with known concentrations inside the canister. The catheters were found to have a maximum recovery rate of 91% at 20 min of incubation time. The recovery at 10 min was 84%, and this incubation time was used for the sake of clinical feasibility.

For statistical analysis, we used the Mann-Whitney U test. P < .05 was considered significant. Data are expressed as mean ± SEM.

Results

Rectal NO concentrations were 9441 ± 3126 ppb (range,
increased (vs. 13.3 ± 10,000) in patients with infectious gastroenteritis and 73.5 ± 13.4 ppb (range, 10–150) in the control group (figure 1A). Plasma levels of nitrite were also significantly increased in patients compared with controls (9.62 ± 1.59 vs. 1.49 ± 0.36 μM, P < .001; figure 1B); however, nitrate was not significantly increased (126.1 ± 17.6 vs. 97.7 ± 13.3 μM, P > .05; figure 1C). There was no significant correlation between nitrate or nitrite and rectal NO in the patient group (not shown).

In 11/17 patients, an enteropathogen was detected in fecal specimens. All but 2 patients had an elevated CRP value (mean, 101 mg/L). Patient characteristics are summarized in table 1.

### Discussion

We found that rectal NO levels in patients with infective gastroenteritis were >100× higher than in healthy controls. The measurements were made with a simple and minimally invasive method. Plasma nitrite was also increased in the patient group, indicating augmented production of NO. The exact source of the luminal NO is not known but is likely to be epithelial cells lining the intestinal mucosa. If NO was produced in the deeper layers of the gut, this reactive gas ought to be scavenged by hemoglobin and other proteins. Moreover, colon epithelial cells contain large quantities of activated iNOS when the mucosa is inflamed [3]. The expression of iNOS and the subsequent large production of NO locally in the mucosa are likely triggered by the invading pathogens. Indeed, bacterial products such as lipopolysaccharide are powerful inducers of iNOS, possibly through activation of NF-κB. One might argue that the high levels of NO found in disease derive from feces. However, this seems unlikely since, in an earlier study, we found no difference in rectal NO between subjects prepared for colonoscopy (no feces present) and those not prepared [9].

In this study it was not possible to determine the localization and extension of the inflammatory process in the intestine. Nevertheless, all patients had increased levels of NO in the rectum. This may be explained by NO being evenly distributed throughout the colon and rectum from a more proximal production site. Alternatively, NO production might be enhanced in the rectal mucosa during gastroenteritis regardless of primary location. We studied too few patients to make any conclusions on the correlation between rectal NO and etiologic agent.

The production of NO is increased in various inflammatory responses, although the role of this gas is not clear. There is no consensus as to whether NO should be considered beneficial or harmful. Some studies show harmful effects [13], whereas others indicate a more protective role of this gas [14]. In infection, the obvious benefit would be that NO has antimicrobial properties. Inflammatory cells can, when activated, produce substantial amounts of NO and use it to kill invading microorganisms (viruses, bacteria, fungi, and parasites) [15]. The epithelium-derived NO should work in the same manner. If this is so, the epithelial cells (the first cells to come into contact with invading pathogens) should not be regarded purely as a passive barrier but as an active part of the nonspecific host defense system.

The mechanism by which NO kills or inhibits microorganisms seems to be related to its binding to iron-containing enzymes, resulting in activation or inactivation of key enzymes in the respiratory cycle and in synthesis of DNA [16]. Obviously these mechanisms are also potential threats to host cells if there is excessive NO production. The harmful and beneficial effects of NO in inflammation need to be further investigated and future possible pharmacologic interventions clarified.

In 6/17 patients with gastroenteritis, no bacterial or viral agent was found in stool samples. However, the diagnosis of infective gastroenteritis was also based on other parameters such as symptoms, laboratory data, and history. The number of patients with gastroenteritis of unknown etiology in this

<table>
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<th>No.</th>
<th>Etiology</th>
<th>Age (years), sex</th>
<th>CRP (mg/L)</th>
<th>Hb (g/L)</th>
<th>Na⁺ (mM)</th>
<th>K⁺ (mM)</th>
<th>Nitrite (μM)</th>
<th>Nitrate (μM)</th>
<th>Rectal NO (ppb)</th>
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<td>22.7</td>
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<td>145</td>
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<td>2.2</td>
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NOTE. x, missing data; CRP, C-reactive protein; Hb, hemoglobin; ppb, part per billion.
study is in agreement with previous findings at our hospital in a large group of gastroenteritis patients (unpublished data).

Plasma measurements of nitrate and nitrite might be regarded as a simple way to monitor the production of NO in the body. However, this method has numerous disadvantages. First, dietary intake of nitrate influences plasma concentrations. Second, there is a temporal difficulty since the accumulated nitrate and nitrite may reflect NO produced earlier; it does not necessarily reflect current NO production. Third, circulating NO metabolites may theoretically originate from anywhere in the body and thus not be as specific as local measurements of NO.

In this study we found that plasma nitrite but not nitrate was markedly increased in persons with infectious gastroenteritis compared with controls. This could be because the control group was not on a restricted diet. Healthy people obviously eat more than persons with gastroenteritis, thus contaminating plasma levels with more dietary nitrate. Hence nitrite probably is a better marker of systemic NO production than nitrate.

Could measurement of rectal NO in patients with diarrhea have any applicability in clinical practice? In some clinical situations it is difficult to confirm the correlation between enteropathogens in stool and patient symptoms. A positive culture might in many cases just reflect a state of colonization, (i.e., detection of *C. difficile* in elderly and chronically ill patients) and not be the main cause of the symptoms. Simple measurement of rectal NO may in these cases differentiate active infection from carrier state and in selected cases be a marker for need for antibiotic treatment. Larger studies are needed to confirm this hypothesis.

We conclude that luminal levels of NO are greatly increased in the rectum of persons with gastroenteritis compared with levels in healthy controls, indicating enhanced production of this gas in the infected mucosa. The role of NO in gastroenteritis is not clear but may include involvement in primary host defense. Measurement of rectal NO is simple, rapid, and safe and can be used to estimate local mucosal NO production in the gut in health and in disease.

References