A pilot study examining the relationship among Crohn disease activity, glucagon-like peptide-2 signalling and intestinal function in pediatric patients

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BACKGROUND/OBJECTIVES: The relationship between the enteroendocrine hormone glucagon-like peptide-2 (GLP-2) and intestinal inflammation is unclear. GLP-2 promotes mucosal growth, decreases permeability and reduces inflammation in the intestine; physiological stimulation of GLP-2 release is triggered by nutrient contact. The authors hypothesized that ileal Crohn disease (CD) affects GLP-2 release.

METHODS: With ethics board approval, pediatric patients hospitalized with CD were studied; controls were recruited from local schools. Inclusion criteria were endoscopy-confirmed CD (primarily of the small intestine) with a disease activity index >150. Fasting and postprandial GLP-2 levels and quantitative urinary recovery of orally administered 3-O-methyl-glucose (active transport) and lactulose/mannitol (passive) were quantified during the acute and remission phases.

RESULTS: Seven patients (mean ± SD age 15.3±1.3 years) and 10 controls (10.3±1.6 years) were studied. In patients with active disease, fasting levels of GLP-2 remained stable but postprandial levels were reduced. Patients with active disease exhibited reduced lactulose/mannitol recovery; all normalized with disease remission. The change in the lactulose/mannitol ratio was due to both reduced lactulose and increased mannitol absorption.

CONCLUSIONS: These findings suggest that pediatric patients with acute ileal CD have decreased postprandial GLP-2 release, reduced glucose absorption and increased intestinal permeability. Healing of CD resulted in normalization of postprandial GLP-2 release and mucosal functioning (nutrient absorption and permeability), the latter due to an increase in mucosal surface area. These findings have implications for the use of GLP-2 and feeding strategies as a therapy in CD patients; further studies of the effects of inflammation and the GLP-2 axis are recommended.

Key Words: 3-O-methyl glucose; Intestinal permeability; Lactulose; Mannitol; Nutrient absorption

The enteroendocrine trophic hormone glucagon-like peptide-2 (GLP-2) has been shown to promote mucosal growth in the small intestine, which, over time, increases fluid, electrolyte and nutrient transport (1-4). GLP-2 is a component of the physiological regulation of intestinal nutrient transport capacity. Under normal conditions, GLP-2 release is triggered by direct nutrient contact with L-cells, which are primarily located in the distal ileum and colon. L-cells extend from the cell body resting on the basal side of the mucosal basement membrane to the mucosal surface so that the cell can ‘taste’ the content of the intestinal chyle (5,6). This phenomenon is further modified by neuronal input (5). In the paradigm of patients with short bowel syndrome, the increased delivery of intraluminal nutrients (especially fats) to L-cells results in an increased postprandial GLP-2 response compared with controls (7,8); it is the postprandial levels that appear to be relevant because basal levels remain stable. In turn, GLP-2 acts to slow proximal motility and, in doing so, likely acutely improves nutrient contact with the absorptive mucosa and, over time, increases mucosal mass and nutrient absorptive capacity (8,9).

The interplay between GLP-2 in the physiological regulation of nutrient absorption and the pathophysiological state of intestinal
inflammation is less clear. GLP-2 has significant anti-inflammatory actions in the intestinal mucosa, acting via a novel neuronally mediated pathway (10-13). It has been shown that the administration of exogenous GLP-2 significantly decreases permeability characteristics in the small intestine in normal animals and in animals adapting following a massive small bowel resection (8,14). This may be significant because very few known drugs or therapies can reduce gut leakiness (15,16). Conversely, a variety of systemic disease states and postinflammatory states have been related to increases in intestinal permeability. Given the theory that Crohn disease may, in part, be caused by an initial abnormality in intestinal permeability, it is possible that this initial permeability defect may be related to abnormalities in GLP-2 release or in the response to the hormone (17-20).

The effects of acute inflammation on GLP-2 release and activity are not well understood. In patients with Crohn disease, GLP-2 levels may be predicted to vary depending on disease activity and the segment of intestine affected. From the results obtained in normal patients, patients with acute Crohn disease with associated malabsorption and diarrhea would be expected to have an increase in meal-stimulated GLP-2 levels (21,22) unless the inflammation itself affects L-cell sensitivity. Furthermore, an increase in GLP-2 production may be a feature of the natural healing or anti-inflammatory response of the intestine, which in turn may also influence intestinal permeability (12,13). In previous studies involving adults with both Crohn disease and ulcerative colitis, fasting levels of GLP-2 were found to be elevated compared with controls; however, disease activity was not quantified (23). A study involving patients with mixed inflammatory bowel disease (IBD) with mild disease showed a normal GLP-2 response to a meal (24). In a preliminary study involving children hospitalized with acute Crohn disease (25), we demonstrated reduced postprandial GLP-2 levels; the effect of active ileal Crohn disease on stimulated (postprandial) release of GLP-2 levels is unclear.

In the present study, we investigated meal-stimulated GLP-2 production and, at the same time, intestinal function in pediatric patients with an acute flare of Crohn disease, comparing them with controls and with themselves once the disease was in remission. We hypothesized that acute inflammation would reduce postprandial GLP-2 release, despite ongoing nutrient malabsorption, and that this would normalize once the inflammation subsided.

METHODS

The present study was designed as both a comparative study, with affected patients compared with normal controls, and a longitudinal study, comparing patients with themselves after resolution of the acute flare of IBD. Patients were evaluated at intake and Crohn disease activity was quantified using a clinical Crohn index tool. At study commencement, there was uncertainty regarding the responsiveness of the pediatric index due to the inclusion of linear growth as an input; therefore, the adult index was used (26,27). GLP-2 levels were quantified at baseline (fasting) and after a mixed meal of 6 kcal/kg to 8 kcal/kg (postprandial). Intestinal function was quantified using the urinary recovery of carbohydrate probes. Patients were subsequently treated by their primary physician and, after symptom resolution (six to eight weeks), patients were re-evaluated and comparisons made both with their initial values and with contemporaneous healthy controls.

Subjects

Ethical considerations: Patients were recruited after evaluation at a tertiary pediatric gastroenterology clinic for IBD symptoms and the decision for admission for acute treatment had been made by the treating team. The study inclusion criteria were: a diagnosis of Crohn disease (primarily confined to the small intestine) confirmed by endoscopy; and a Crohn disease activity index (CDAI) >150 (27). Patients were enrolled at the initial diagnosis or during a flare; typically they were on oral medication (5-aminosalicylic acid or low-dose steroids) only. On admission, the treating physicians would notify the study team, who would approach the patient and their family regarding enrollment; informed consent and assent were obtained. Controls were recruited by advertisement from local schools.

Patients were excluded if they had a current need for or had previously undergone intestinal surgery (regardless of whether related to IBD or other causes), or had significant obstructive symptoms, evidence of fixed intestinal stricture, renal impairment, current or recent documented small bowel infection, diabetes mellitus or major endocrine abnormalities requiring treatment.

Interventions: After informed consent, all patients completed a baseline standard case report form with a study investigator, including a full gastroenterological functional survey, with questions specifically on the use of acetylsalicylic acid and other anti-inflammatory medications, alcohol and smoking habits. The patients completed a CDAI questionnaire (26). Typically, on the night of admission, intestinal permeability studies were performed using previously described methods (28). Briefly, patients consumed a regular evening meal; however, 3 h before bedtime they drank a solution containing lactulose 5 g, mannitol 2 g, and 3-O-methyl-glucose (3-OMG) 5 g in 200 mL of water (all sugars USP; from Biosource, Canada). All urine for the subsequent 12 h was collected, with 5 mL of thymol (5%) added to the storage container for preservation and stored frozen. Urine samples were subsequently analysed using high-performance liquid chromatography (28,29).

The morning following admission, before starting active therapy, patients underwent an assessment of GLP-2 levels. GLP-2 levels were analyzed from blood samples drawn after fasting and 45 min after beginning a standardized meal of 6 kcal/kg to 8 kcal/kg of Carnation Instant Breakfast (Nestle, Canada). Blood samples were drawn by venipuncture, specimens were immediately stored on ice in a 10% volume of Trasylol EDTA (Miles, Canada) and centrifuged within 30 min. The resulting plasma was stored at ~20°C for subsequent batch analysis as previously described. GLP-2 levels were measured using a radioimmunossay specific for the N-terminus of intact human GLP-2 (amino acid residues 1 to 33; antibody code 92160), with a sensitivity of 1 pmol/L and an interassay variation of 5% at a GLP-2 concentration of 40 pmol/L (21). Each sample was analyzed in duplicate.

Patients were subsequently treated by the admitting gastroenterology physician, and typically underwent endoscopy with biopsy to confirm the diagnosis, either at the initial hospitalization or as an outpatient. However, the biopsies were performed a variable time after beginning therapy and, therefore, were not well correlated with disease status but did confirm the disease and ileal involvement. Therapy was directed by the attending physician and typically consisted of a short course of high-dose steroids followed by an early introduction of an immunomodulator such as azathioprine and a rapid wean of steroids (30). Patients were followed as outpatients after discharge, and the follow-up sampling was completed from six to eight weeks following...
the initial hospital admission, after completion of the initial therapy and the weaning of ongoing steroids. All patients were off steroids at the time of the follow-up assessment. No other exclusions regarding other ongoing treatments were made. Control patients were similarly studied on an outpatient basis.

**Statistical methods**

GLP-2 levels, permeability and glucose absorption data and CDAI scores were compared within the same patients using the Student’s paired t test. Data from the different disease activity time points and the normal controls was compared using ANOVA, with post hoc testing using the Tukey method with significance set at P<0.05. Comparisons were performed using Prism 4.0 software (Prism Corporation, USA).

**RESULTS**

Over the years of the present study (2004 to 2007), 14 patients with appropriate clinical scenarios were approached for enrollment and 12 agreed. Three were started on treatment before the preliminary blood work or absorption studies could be initiated, and two underwent biopsies or endoscopic results that showed primarily a colitis picture and were therefore excluded from further analysis. This left seven patients who completed the studies; their baseline characteristics are shown in Table 1. All patients were treated with an acute dose of steroids and the majority were then treated with azathioprine; all were off steroids at the time of the remission studies. GLP-2 levels and sugar absorption results are presented in Tables 2 and 3, and the results of the control patients are shown in Table 4.

In patients with active Crohn disease, the CDAI showed significant disease as anticipated in a hospitalized population, ranging from 180 to 220 (Table 2). In these patients, the fasting GLP-2 levels were appropriate and not different from controls (Table 3). However, the postprandial levels of GLP-2 were lower in the patients with active Crohn disease and, in fact, showed no significant effect of the meal stimulation (meal-stimulated GLP-2 levels in patients with active disease 24.1±10.9 pmol/L versus controls 30.4±6.6 pmol/L; P<0.05). These normalized with resolution of the disease (patients in remission: 38.9±10.3 pmol/L; P<0.001). All patients were able to tolerate the meal stimulation of 6 kCal/kg.

In examining nutrient absorption, patients with active disease demonstrated reduced glucose uptake (3-OMG: active disease 43.4±5.4 versus patients in remission 60.6±12.5 [P<0.01]; and controls 55.7±8.7 [P<0.05] [% uptake of orally administered 3-OMG]). Mucosal intestinal permeability as measured by the lactulose/mannitol absorption was increased in patients with active disease (Table 1: active disease 0.056±0.025 versus remission 0.032±0.010 [P<0.05]; and versus controls 0.029±.008 [P<0.01]). This was associated with a decrease in mannitol absorption in the patients with active Crohn disease (active disease 20.6±4.3 versus quiescent: 35.5±8.0 [P<0.01] and versus controls 32.8±6.9 [P<0.01] [% uptake of orally administered mannitol]. The normalization of the lactulose/mannitol ratio in the Crohn patients with quiescent disease was due to both a modest reduction in lactulose absorption and a significant increase in mannitol absorption.

The results of control patients are summarized in Table 4, and show typical profiles of GLP-2 production and permeability. It was difficult to recruit teenage patients; the tested subjects were largely recruited as siblings of participants in other studies (31).

**DISCUSSION**

The present study showed that in pediatric patients with active ileal Crohn disease, the expected postprandial production of GLP-2 was blunted despite evidence of reduced nutrient absorption. However, the...
TABLE 4
Control group

<table>
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<tr>
<th>Patient</th>
<th>Age, years</th>
<th>Sex</th>
<th>Tanner stage</th>
<th>GLP-2 levels, pmol/L</th>
<th>Sugar absorption, %</th>
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<td>Fasting</td>
<td>Postprandial</td>
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</table>

Mean ± SD 10.3±1.6 16.2±2.2 30.4±6.6 55.7±8.7 0.96±0.23 32.8±6.9 0.030±0.008

Glucagon-like peptide 2 (GLP-2) levels determined by radiomunnoassay using a specific antibody for intact GLP-2 (amino acid residues 1 to 33) (20). Sugar probe absorption is reported as the proportion of orally administered probe recovered in urine after an overnight collection, quantified using high-performance liquid chromatography (23,34). 3-OMG 3-O-methyl glucose.
limits of our methodology. This, coupled with the lack of change in permeability in this same group, suggests that the hypothesis relating the causality of Crohn disease to a fundamental lack of GLP-2 production and an associated increase in intestinal permeability that precedes or causes eventual disease is unlikely (19,20,36).

The findings of the present study suggest that patients with active ileal Crohn disease experience a reduced meal-stimulated release of GLP-2; this may impact nutrient absorptive capacity as well as trophic mucosal support. Enteral feeding is an effective therapy for pediatric Crohn disease but the specific mechanisms underlying the benefits of this treatment are not clear (41,42). GLP-2 and its analogues have significant anti-inflammatory effects in both animal models of IBD and in limited studies in humans (12,13,43). Meal-stimulated GLP-2 release may be a part of the therapeutic effects of enteral feeding; this relationship deserves further study. A further implication of this relationship would be that patients who have acute inflammation may benefit from nutrient regimens that optimize the release of native GLP-2 (ie, diets containing a high proportion of long-chain fats); this will require specific study (5).

Overall, the results of the present study suggest that acute ileal Crohn disease has an impact on GLP-2 release, which recovers with disease resolution. The findings regarding the relationship between active nutrient absorption and passive permeation by inert sugars confirm the degree of nutrient malabsorption apparent in this patient population but does not support an underlying permeability defect in these patients. The continued exploration of the relationship between nutrients and the response of the intestinal mucosa in IBD patients is encouraged, so that both the nutritional support of this vulnerable population and the opportunities to harness the beneficial anti-inflammatory actions of enteral nutrition are optimized.

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CONTRIBUTIONS: DL Sigalet led the study conception and design, interpretation of data, and drafting of manuscript; DK led data acquisition, and initial analysis, BH and JH contributed to sample analysis, all authors contributed to analysis of the data, critical review and editing of the manuscript. The technical assistance of Laurie Wallace and Kim Tran is gratefully acknowledged.

DISCLOSURES: DL Sigalet has acted as a paid consultant regarding the development of a glucagon-like peptide 2 ligand for Nycomed Corporation. The remaining authors declare that they have no competing interests.

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