Available online at www.sciencedirect.com
ScienceDirect
Magnetic Resonance Imaging 28 (2010) 1022 – 1029
MAGNETIC RESONANCE IMAGING

Similarity in the metabolic profile in macroscopically involved and un-involved colonic mucosa in patients with inflammatory bowel disease: an in vitro proton (^{1}H) MR spectroscopy study

Uma Sharma[a], Rajiv R. Singh[a], Vineet Ahuja[b], Govind K. Makharia[b], Naranamangalam R. Jagannathan[a,*]

[a]*Department of NMR and MRI Facility, All India Institute of Medical Sciences, New Delhi-110 029, India*
[b]*Department of Gastroenterology and Human Nutrition, All India Institute of Medical Sciences, New Delhi-110 029, India*

Received 11 January 2010; accepted 14 March 2010

Abstract

Background: The histological extent of inflammatory bowel disease (IBD) is greater than that evident by colonoscopic evaluation. We hypothesized that metabolic profile in macroscopically un-involved colonic mucosa in IBD is similar to that of controls with healthy colon. We thus assessed the differences in metabolic profile in macroscopically involved and un-involved colonic mucosa of IBD patients to further substantiate the extent of disease.

Patients and Methods: Colonic mucosal biopsies were obtained and snap frozen from both the macroscopically un-involved and involved colonic mucosa of IBD patients and macroscopically normal colonic mucosa of controls and were subjected to in-vitro high-resolution proton (^{1}H) magnetic resonance (MR) spectroscopy and the concentrations of metabolites were determined.

Results: Thirty-two metabolites were assigned in the proton MR spectrum of colonic mucosa of IBD patients. The concentrations of amino acids (isoleucine, leucine, valine, arginine, lysine, glutamine/glutamate, alanine), membrane metabolites (choline, glycerophosphorylcholine/phosphorylcholine), glycolytic product (lactate) and short chain fatty acid (formate) were significantly lower while significantly high level of glucose were observed in the macroscopically un-involved colonic mucosa of IBD patients compared to the macroscopically normal mucosa of controls. There was no significant difference in the concentrations of metabolites in macroscopically involved and un-involved colonic mucosa of IBD patients.

Conclusions: The metabolic profile in macroscopically un-involved colonic mucosa of IBD patients is similar to that of macroscopically involved mucosa but different from colonic mucosa of controls. This suggests that even macroscopically un-involved colonic mucosa is metabolically abnormal and may explain the increase in extent of disease with time.

Keywords: Inflammatory bowel disease; Ulcerative colitis; Crohn's disease; Involved and un-involved mucosa; In-vitro proton (^{1}H) magnetic resonance spectroscopy; Metabolite concentration

1. Introduction

Inflammatory bowel disease (IBD) is precipitated and perpetuated by complex interactions of environmental, genetic, microbial and immunoregulatory factors. Primarily, IBD includes ulcerative colitis (UC) and Crohn's disease (CD) [1]. The extent of disease in patients with IBD including both UC and CD varies and is evaluated by the colonoscopy and/or enteroscopy [2]. Microscopic extent of the disease is evaluated by the colonic biopsies obtained during colonoscopy and is generally more than the macroscopic extent of the disease [3]. The extent of colonic involvement in UC is positively associated with the risk of colonic dysplasia and colorectal cancer. Patients with UC up to or beyond the hepatic flexure have a cumulative incidence of colorectal cancer of 5–15% at 20 years; while patients with disease distal to the hepatic flexure have a reported cumulative incidence of 2–5% [4,5]. The implications of the histological extent of colitis for the risk of dysplasia and colorectal cancer are unknown. With time, there is an

* Corresponding author. Tel.: +91 11 2659 3253; fax: +91 11 2658 8663.
E-mail address: jagan1954@hotmail.com (N.R. Jagannathan).

0730-725X/$ – see front matter © 2010 Elsevier Inc. All rights reserved.
doi:10.1016/j.mri.2010.03.039

extension of the disease in both UC and CD [6,7]. The mechanism of the extension of the lesion is not known and most likely is related with the basic cause of the disease.

Magnetic resonance spectroscopy (MRS) detects early biochemical changes that occur prior to any morphological changes that could signal initiation of the disease processes [8–14]. In recent years, the applications of MRS in studying the tissue metabolism of living systems have grown immensely. These studies provide information on the biochemistry of the disease processes as alterations in the levels of metabolites that are manifestations of the regulatory changes that occur in response to physiological stress and disease progression [8–14]. Human samples such as breast tissue [9], lymph nodes [10], colon tissue and mucosa [11] and body fluids [12] have been studied by in-vitro proton (^{1}H) MRS providing insight into the alterations of metabolic pathways in the pathological state. Such information's are useful in choosing appropriate treatment or developing management strategy or serves as a basis for studying preclinical aspects of the disease.

Further, recent studies have combined multivariate analysis with MRS data and reported its diagnostic value in various diseases [12,13]. Bezabeh et al. reported the results of ex vivo ^{1}H MRS combined with multivariate spectral analysis of patients with IBD [13]. In addition, in vitro ^{1}H nuclear magnetic resonance (NMR) study of fecal extracts of patients with UC and CD showed high level of glycerol as a dominant feature in CD [14]. We recently reported using in-vitro ^{1}H NMR reduced level of several metabolites in IBD patients like lactate (Lac), glutamate (Glu)+glutamine (Gln), choline (Cho), glycerophosphoryl choline (GPC) and formate (For) [15]. These reduced levels of metabolites are suggestive of decreased carbohydrate, protein and short chain fatty acid metabolism thereby decreased energy status and deterioration of mucosa integrity during chronic inflammation. In the present study, we investigated the metabolic profile of macroscopically normal colonic mucosa of IBD patients to get an insight into mechanism of progression of the disease. The objectives of the present study are: (a) comparison of the metabolic profile of macroscopically normal colonic mucosa of UC and CD patients to that of controls and (b) intra-individual comparison between macroscopically abnormal (involved) and normal (un-involved) colonic mucosa obtained from UC and CD patients using ^{1}H MR spectroscopy.

2. Materials and methods

2.1. Patients

Twelve patients with UC (mean age 37.5±12.6 years) and nine with CD (mean age 43±12.4 years) were recruited in this study. Patients with less than 14 years of age and more than 65 years, unwilling patients and those with systemic disease like cardiac disease and renal failure excluded. All patients underwent full-length colonic examination using video-colonoscope (Olympus 160) to evaluate the extent of the disease. Diagnosis of CD was made on the basis of characteristic clinical manifestations (chronic diarrhea, hematochezia, abdominal pain and intestinal obstructive manifestations), endoscopic features (skip lesions, asymmetrical involvement, deep ulcers, aphthous ulcers, ileocecal valve involvement and terminal ileal involvement) and histological evidences (acute chronic colitis, presence of inflammation extending beyond muscularis mucosae, lymphoid follicles and granuloma). Barium meal followed through, small bowel enema and/or retrograde ileoscopy was used to evaluate the involvement of small intestine. The activity of CD was assessed using Crohn's Disease Activity Index [16] and the location and behavior of the disease was classified using modified Montreal classification [17]. The diagnosis of UC was based on the combination of clinical, endoscopic and histological characteristics. Truelove and Witts' criteria [18] was used to assess the severity of UC, and the extent of the disease was determined by using videocolonoscope. All the patients were treated according to standard guidelines.

2.2. Controls (normal mucosa)

Twenty-six subjects undergoing colonoscopic examination for obscure gastrointestinal bleeding and colonic polyps where the colon appeared normal and reported earlier by us [15] served as controls for this study. In subjects with colonic polyps, the biopsies taken from an area away from the polyps, while in those with occult gastrointestinal bleeding, biopsies were taken only if the colon appeared normal. All patients were treated according to standard treatment regimen. An informed consent taken from each patient and control and the Institute Ethics Committee approved the study.

2.3. Sample collection and processing

Approximately, 10 mucosal biopsies (weighing 58± 15 mg total) each were obtained from the macroscopically involved (abnormal) colonic mucosa and un-involved (normal appearing) mucosa from all patients. Following the collection, the tissue samples were snap-frozen in liquid nitrogen in order to freeze the enzymatic actions so that no metabolic changes occur in the tissue thereby keeping the tissues in a state close to its physiological state prior to excision. The tissues were then stored at −35°C until perchloric acid extraction (PCA) extraction was carried out.

2.4. PCA of the water-soluble metabolites

The procedure involved extraction of water-soluble metabolites from the tissues (i.e., biopsies) using perchloric acid [12–15]. The frozen tissues were weighed, crushed to powder and thoroughly homogenized in 6% PCA. The homogenate centrifuged at 10,000 rpm for 10 min following which the supernatant was collected. The supernatant obtained was neutralized with 3 M potassium hydroxide to pH 7. The precipitated perchlorate salts ($KClO_4$) was removed by centrifugation at 10,000 rpm for 10 min. The