Metabolism of abnormal and normal colonic mucosa of patients with ulcerative colitis (UC) and Crohn's disease (CD): An in-vitro proton MRS study

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Introduction: Inflammatory bowel disorders (IBD) are chronic disorders which encompass a group of diseases precipitated and perpetuated by a complex interaction of environmental, genetic, microbial and immuno-regulatory factors. Primarily, the IBD includes ulcerative colitis (UC) and Crohn's disease (CD). UC typically presents with shallow, continuous inflammation and usually affects only the mucosa while CD can often spread deep into the layers of mucosa. The extent of colonic involvement varies both in patients with UC and CD and is associated with the risk of colonic dysplasia and colorectal cancer in UC. The disease show progression to normal area with time and the mechanism is not clear. In addition, the macroscopic appearance of uninvolved (normal) colonic mucosa of IBD patients is similar to that of controls, however, histology shows greater spread of disease compared to the macroscopic evaluation by colonoscopy. We recently reported significant differences in the metabolism of abnormal mucosa of UC and CD patients compared to controls contributing to low energy status and damage of mucosal membrane integrity using in-vitro high-resolution proton (^{1}H) MRS (1). 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 disease. The objectives of the present study are: (a) comparison of the metabolic profile of macroscopically normal colonic mucosa of UC and CD patients with that of controls, and (b) and intra-individual comparison between macroscopically abnormal and normal looking area of colonic mucosa obtained from UC and CD patients using in-vitro ^{1}H MR spectroscopy.

Patients and Methods: Thirty five patients with IBD were recruited and the diagnosis of UC and CD were made using clinical, endoscopic and histological examination. Twenty one subjects undergoing colonoscopic examination for obscure gastrointestinal bleeding and colonic polyps where the colon was observed to be normal served as controls. Approximately, 10 mucosal biopsies (wet weight 58±15mg) were endoscopically collected from the macroscopically abnormal mucosa [(UC, n=17, mean age 36±12.5 yrs), (CD, n=18, mean age 34.6±11.4 yrs)] and normal appearing mucosa [UC, n=10, CD, n=9] and were immediately frozen in liquid nitrogen and stored at -35°C until PCA extraction. The Institute Ethics Committee approved the study. ^{1}H MRS was carried out at 400 MHz (DRX-400, Bruker). The 1D spectrum with water suppression, 2D double quantum filtered correlation spectroscopy (DQF COSY), and total correlation spectroscopy (TOCSY) spectra were acquired for assignment of various metabolites. Concentration of metabolites was determined using tri-methyl-silyl propionate (TSP) as concentration standard (1). Concentration of metabolites among UC, CD and controls were compared using one way ANOVA with Bonferroni correction. The concentration values of normal and abnormal area in UC and CD patients were compared using paired t-test for each group.

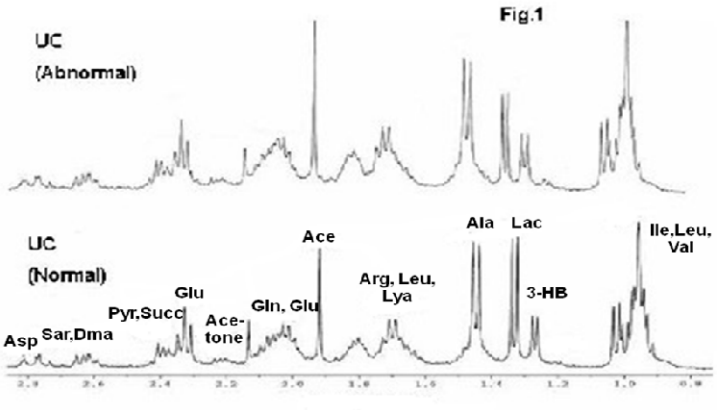

Table 1: Concentration (μM/g wet wt) of metabolites in colonic mucosa of UC and CD patients (normal and abnormal) and controls.

Metabolites	Control (a)	UC		CD		p- value
		Normal (b)	Abnormal (c)	Normal (d)	Abnormal (e)	
Lac	16.2±9.2	7.0±7.3	4.0±2.0	3.8±1.3	5.0±2.0	$P^{(a-b,c,d,e)}$
Pyr	1.4±0.7	1.1±0.9	0.9±0.5	1.0±0.4	1.0±0.3	$P^{(a-b,c,d,e)}$
ATP	3.1±1.9	1.5±0.9	1.9±1.3	1.4±1.2	1.5±0.7	$P^{(a-b,c,d,e)}$
Cr+PCr	2.2±1.3	1.0±0.7	0.9±0.5	1.0±0.3	0.9±0.3	$P^{(a-b,c,d,e)}$
For	3.2±1.9	1.6±1.0	1.7±1.6	1.6±0.5	1.5±0.6	$P^{(a-b,c,d,e)}$
GPC+PC	3.7±2.1	2.1±1.0	1.6±0.7	1.7±0.6	1.5±0.5	$P^{(a-b,c,d,e)}$
Arg+Leu +Lys	2.5±3.5	5.3±5.5	5.3±4.2	3.6±2.1	4.7±3.4	$P^{(a-b,c,d,e)}$

Results: 32 metabolites including 11 amino acids, 10 organic acids (glycolysis and citric acid cycle intermediates and products), short chain fatty acids, membrane and high energy containing metabolites were unambiguously assigned using 2D DQF COSY and TOCSY. Concentration of 25 metabolites including amino acids, organic acids, membrane components and sugars were compared between normal and abnormal area of colonic mucosa of UC and CD patients and controls (1). The concentrations of lactate (Lac), β- hydroxyl butyrate (β-OHB), n-butyrate, formate (For), glycerophoshoryl choline+phosphoryl choline (GPC+PC), myoinositol (mI), creatine+phosphocreatine (Cr+PCr) were significantly lower and the concentration of arginine (Arg), leucine (Leu) and lysine (Lys) were significantly higher in both normal and abnormal mucosa of UC and CD patients in comparison to controls (see Figure 1 and Table 1). However, the concentration of all 25 metabolites was similar in both the normal and abnormal area of the mucosa of patients with UC and CD.

Discussion: To our knowledge this is the first study that compared the metabolism of normal and abnormal area of colonic mucosa in patients with UC and CD using in-vitro ^{1}H MRS. Interestingly, the metabolic profile of the normal area of mucosa was similar to the abnormal mucosa in both UC and CD patients indicating progression of abnormal metabolic activity to the normal looking area of mucosa. The concentration of Lac, For Cr+PCr and ATP were significantly lower in both normal and abnormal mucosa in UC and CD patients compared to controls that suggest a low energy state. However, the concentration of Arg+Leu+Lys showed significant increase in patient groups (UC and CD) compared to controls. The decrease of the concentration of Cr+PCr might be due to reduced glycolytic and oxidative metabolism as well as reduced shuttle of Arg toward Cr+PCr. The concentration of membrane related metabolites like GPC, Cho and mI was also significantly lower in both normal and abnormal colonic mucosa of UC and CD patients compared to controls. Choline is an essential nutrient used in the synthesis of lipid components of cell membranes, phosphorylcholine (PC) and sphingomyelin (2). Deficiency of these metabolites may lead to anomalous membrane biosynthesis. In addition, the decrease in the concentration of energy containing metabolites may also be attributed to increase in the luminal permeability of the membrane. In IBD, the inflammatory reactions, characterized by increased cytokines lead to the mucosal damage and a further disturbance in epitheial barrier function thus contributing to the loss of mucosal membrane integrity [3]. Moreover, the colonocytes involve in various energy dependent process vital to health, including electrolyte exchange, mucin synthesis, lipid synthesis, structural protein synthesis and detoxification. Loss of cellular energy may affect these processes and impair the protective functions of colonic mucosa. Thus our results imply that these metabolic abnormalities observed in the normal looking area of mucosa in patients with UC and CD might probably one of the reasons for the progression of the disease.

References: (1) Balasubramanian K et al. Magn Reson Imaging. 2008 (In Press). (2) Michel V et. al. Exp Biol Med 2006; 231: 490-504. (3) Schmidt C, et al. Gastroenterol Dietol., 2005; 51: 127-145.