Role of In-Vitro Proton NMR Spectroscopy in Inflammatory Bowel Disorders

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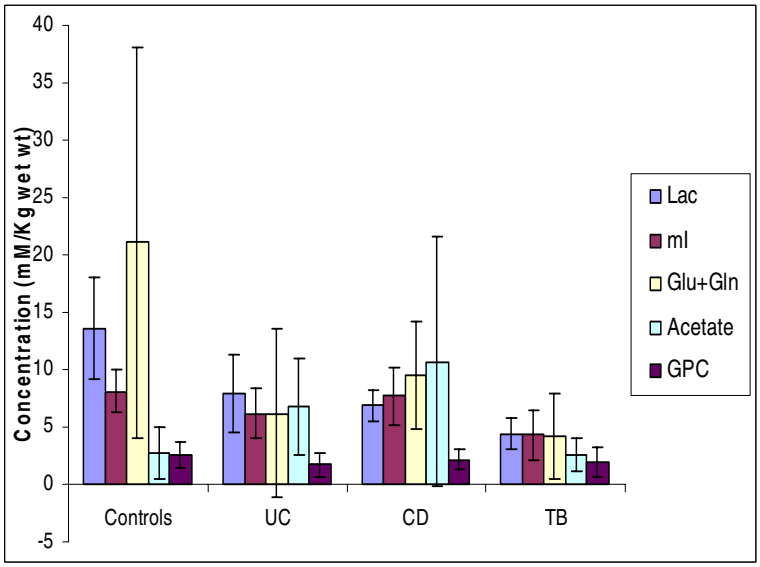
Introduction: Inflammatory bowel disorder (IBD) is a disease that primarily affects the small intestine and the colon. It encompasses a group of diseases triggered and perpetuated by diverse genetic, environmental, and immunologic factors that share similar clinical manifestations. The two most common types of IBD are ulcerative colitis (UC) and Crohn's disease (CD). Magnetic Resonance Spectroscopy (MRS) has the potential to be used for the diagnosis of the two types of IBD. MRS detects early, pre-morphological biochemical changes that signal the initiation of the disease and the extent of inflammation. It can provide information of diagnostic and prognostic importance that can help in choosing the appropriate treatment and management strategy or help to study the preclinical aspect of this disease. The objectives of this study were (i) to evaluate the biochemical profile of the colon tissues from IBD patients and normal subjects using NMR spectroscopy, and (ii) to quantify the various metabolites observed in the NMR spectra and identify the possible metabolic/biochemical markers exclusive for UC, CD and TB that would aid in their exact distinction.

Methodology: The mucosal biopsies were collected from control (n = 27) and IBD patients (UC = 15, CD = 10, TB = 6) recruited for colonoscopy. The frozen tissues were weighed, crushed and then thoroughly homogenized in 6% perchloric acid (PCA). The homogenate was then centrifuged at 10,000 rpm for 10 minutes following which the supernatant was collected. The supernatant was then neutralized using 3M potassium hydroxide (KOH) and the precipitated perchlorate salts ($KClO_4$) were removed by centrifugation. The supernatant obtained was lyophilized and the resulting sample was dissolved in deuterium oxide (D_20). Following PCA extraction of the water soluble metabolites, the samples were subjected to proton NMR spectroscopy on DRX-400 spectrometer at 400.13 MHz. Assignment of the various metabolites observed in the 1-D NMR spectra were carried out using 2-D COSY and TOCSY experiments. Concentrations of the metabolites were determined by comparing the integrated intensity of the isolated resonances of the compounds of interest with that of the TSP signal that served both as a chemical shift reference and concentration standard for the proton NMR studies.

Results: 21 metabolites were assigned unambiguously using 2-D NMR methods. The concentration of metabolites which gave well-resolved resonance peaks in the 1-D spectrum were determined. In all, 10 metabolites were quantified. Significant decrease in lactate (Lac), glutamate+glutamine (Glu+Gln) myo-inositol (mI) and glycerophosphorylcholine (GPC) (Fig. 1) were observed in patients with UC compared to controls. Patients with CD showed a significant decrease in Lac and Glu+Gln while those with tuberculosis (TB) showed a decrease in Lac, Glu+Gln and mI in comparison to controls. UC and CD patients showed increased acetate (Ace) concentration compared to controls. Patients with TB showed a significant decrease in Lac and Ace in comparison to those with UC and a decrease in Lac, Ace and mI compared to those with CD.



Discussion: Decrease in mI concentration was observed in IBD patients. *Myo*-inositol, the major nutritionally active form of inositol, is vital to many biological processes of the body, participating in a diverse range of activities. *Myo*-inositol is metabolized to phosphatidylinositol which makes up a small but very significant, component of cell membranes. Decrease in this metabolite in patients with IBD indicates a decreased membrane synthesis in regions of inflammation. Patients with IBD showed a decrease in glutamate+glutamine concentration which is a primary source of energy for the intestines. Hence, a decrease in the concentration of this metabolite in IBD indicates a damage of the mucosal lining of the intestines that occurs due to inflammation and ulcerations. Decrease in lactate observed in IBD patients could be attributed to the increased blood flow in the regions of inflammation due to which there is an increased oxygen supply in these regions. Increased acetate concentration could be due to increased production of ketone bodies that occurs during starvation (which the patient had to undergo prior to colonoscopy).

Conclusion: Our study demonstrated that there is a general decrease in the metabolic activity in patients with IBD. It also shows that NMR spectroscopy can be useful for the differentiating between UC, CD and ITB. This study thus paves a way for yet another application of MRS in the early and accurate diagnosis of IBD.

REFERENCES

1. Bezabeh T, Ray L. Somorjai, Smith ICP et al. Am J Gastroenterol.2001; 96:442-8.