Therapeutic Effect of Sodium Alginate in Experimental Chronic Ulcerative Colitis

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ABSTRACT

The aim of this study was to test the therapeutic efficacy of sodium alginate in a rat model of trinitrobenzene sulfonic acid (TNBS)-induced inflammatory bowel disease. This experiment was carried out using 77 Sprague-Dawley rats which were divided into six groups; normal, control, prophylactic, therapeutic and two experimental groups. Rats were sacrificed 1, 2, 3 and 6 weeks after colitis induction. Severity of colitis was graded macroscopically and assessed using serum and colonic mucosal cytokines and eicosanoids. Intrarectal TNBS (30 mg) produced a significant chronic ulcerative colitis. The lesions were most severe on day seven after TNBS instillation, and then declined, but lesions were still observed after six weeks. TNBS administration also significantly enhanced the serum and colonic mucosal cytokines (TNF-alpha and IL-6) and eicosanoids (LTB4 and PGE2) levels, which paralleled with the severity of colitis. Low viscosity sodium alginate (LVA) solution as therapeutic agent was administered orally as drinking water at concentration of 0.5% (W/V) for six weeks. Results showed that pre-treatment (in prophylactic group) and treatment with LVA were significantly able to reduce colonic damage score, serum level and colonic mucosal production of TNF-alpha, IL-6, LTB4 and PGE2 in pre-treated and treated animals compared with non-treated controls.

LVA therapy is able to suppress chronic ulcerative colitis in experimental model.

Key words: Colitis; Inflammatory bowel disease treatment; Sodium alginate

INTRODUCTION

Inflammatory bowel disease (IBD), typified by Crohn's disease and ulcerative colitis, is a common disorder characterized by recurrent and serious inflammation of the gastrointestinal tract.1 The IBD is associated with the rise of modern, westernized industrial society. Although the causes of these diseases remain incompletely understood, the prevailing model is that the intestinal flora drives an unmitigated intestinal immune response and inflammation in the genetically susceptible host.2 The genetic predisposition, nutritional and environmental influences, intestinal pathogens, psychological stress, and disturbed intestinal barrier function have been
accepted as important factors in the pathogenesis of chronic inflammatory bowel diseases. Uncontrolled reactions of T-cells and proinflammatory cytokines are the common denominators of all factors contributing to pathogenesis. Treatment of IBD, based on the anti-inflammatory agents sulphasalazine and steroids is still non-specific and the results of the treatment in some patients remain unsatisfactory. Therefore, the researchers are seeking new therapeutic targets for treatment of IBD.

Alginites are natural copolymers comprised of β-D-mannuronate (M-block) and α-L-guluronate (G-block) linked by 1→4 glycosidic linkage. They are synthesized by bacteria belonging to the genera Pseudomonas and Azotobacter and brown sea-weeds. The M-blocks of the bacterial, but not sea-weed polymers, are to a variable extent acetylated at positions O-2 and/or O-3. The variability in monomer block structures and acetylation which are associated with the source of alginate, strongly affect the physicochemical and rheological properties of the polymer and the biological basis for the variability are therefore of both scientific and applied importance.

The alginate gels are well known as biocompatible, degradable and nontoxic. Thus, they are widely used as carriers for drug delivery; haemostatic wound dressing and immunoisolation systems for transplantation using uncoated alginate microspheres and devices anastomosed to the vascular system as arteriovenous shunts such as alginate-impregnated polyester vascular graft. Moreover inhibitory effects of various types of alginic acid on hyaluronidase and mast cell degranulation have been examined, in which alginic acid with an M/G ratio of 1.0 exhibited the strongest inhibition of both activities. The protective and reparative effects of sodium alginate on radiation stomatitis and suppression of radioactive absorption by this compound in animals and human subjects have also been investigated.

In addition, we showed the therapeutic effects of sodium alginate in experimental models of acute colitis and immune complex glomerulonephritis in our previous works. In this study, sodium salt low viscosity alginate (LVA) purified from Macrocystis pyrifera (Kelp) was used. The aim of present research was to assess the therapeutic effect of LVA in experimental model of chronic ulcerative colitis.

**MATERIALS AND METHODS**

**Animals**

Adult’s female Sprague-Dawley rats weighing 180±20 g were obtained from the Razi Institute (Karaj, Iran) and were housed in standard wire mesh cages. They were maintained in a room with controlled temperature (21±1°C) and light dark cycle (12h: 12h) with unlimited access to food and water. The animals were acclimatized for at least one week before the experiment. Rats were divided randomly into six groups. N: Normal group (n=7); C: Colitis induced group (n=20); P: Prophylactic group (n=20); T: Therapeutic group (n=20); E1 and E2: Experimental groups, «healthy controls receiving drugs for one and six weeks», respectively (n=5&5).

**Induction of Colitis and Treatment**

Colitis was induced according to Menozzi A. et.al. method. Briefly, rats were fasted for 24hr before induction of colitis. After an anesthesia with ether, a thin catheter was inserted 8cm into the rectum, proximal to the anus and 30 mg of TNBS (2, 4, 6-trinitrobenzene sulfonic acid) dissolved in 50% (v/v) ethanol was instilled slowly into the rectal lumen. Following the enema, they were kept in cages, with free access to water and food. For treatment, low viscosity sodium alginate (LVA) (Sigma-Aldrich, Lot. No. 89H9178) was dissolved in water and adjusted to a concentration of 0.5% (W/V). The prophylactic and therapeutic groups received 0.5% LVA solution as drinking water under a time-course table. To determine the therapeutic efficacy of LVA, rats (at least 5 per group) were killed 1 wk, 2 wk, 3 wk, and 6 wk after administration of TNBS. The onset of oral LVA administration for C and T groups was 24hr before and after the induction of colitis, respectively.

**Assessment of Colonic Damage and Inflammation**

At the end of these periods (1, 2, 3 and 6 weeks) the rats were anesthetized. The abdomen was opened along the median line, blood was withdrawn for cytokine and eicosanoid assay and an 8cm segment of distal colon including the major gross pathologic changes was removed. The colon was opened by a longitudinal incision and rinsed with isotonic saline, and was pinned down on a wax platform, and macroscopically visible damage was assessed according to the method of Morris et al, which was scored on a 0-5 scale based on...
on the extent and severity of inflammation and ulceration by two observers unaware of the treatments. Immediately after scoring, a 4-cm segment with the major gross pathologic changes was removed from the 8-cm segment and stripped longitudinally into two strips (40×5mm) for measurement of cytokine and eicosanoid.

Measurement of Cytokine and Eicosanoid

I. In serum: assessments of cytokines (IL-6 and TNF-α) and eicosanoids (LTB4 and PGE2) were carried out using enzyme immunoassay kits; ER2-IL-6 (Lot No: DA 53152 CA, Endogen Co, USA) for IL-6, ER-TNF-α (Lot No: DD 55747CA, Endogen Co, USA) for TNF-α, Correlate-EIATM (Lot No:901-068, Assay Designs, USA) for LTB4, and Correlate-EIA (Lot No: 901-001, Assay Designs, USA) for evaluating PGE2 levels in rats sera.

II. In tissue: Colonic tissue strips (40 × 5mm) were minced with scissors for 15s, suspended in 2 ml of 10mM phosphate buffer (pH 7.4), and incubated in a shaking water bath (37°C) for 20 min. The samples were then centrifuged (9000 g, 30s) and the supernatants were kept at -70°C until IL-6, TNF-α, LTB4, and PGE2 were assayed using above mentioned enzyme immunoassay kits.26

Statistical Analysis

All data were expressed as the mean ±SD. Comparison between groups of nonparametric data was made with the Mann Whitney U-test. Comparison between groups of parametric data was made with the Student’s t-test or ANOVA. Differences were considered significant at P<0.05.

RESULTS

Effect of LVA on Colonic Damage

I. Macroscopic findings: Intracolonic administration of TNBS resulted in acute (week 1) and chronic (week 6) IBD in control non-treated rats. The control animals developed colonic macroscopic damage such as diffuse hyperemia and ulcerations. Daily oral treatments with LVA (drinking 35.5 ±2.8 ml of 0.5% W/V LVA solution per rat which was equivalent to 0.65-0.77 mg/kg/day LVA) significantly reduced colonic damage scores in prophylactic and therapeutic groups at 1, 2, 3 and 6 weeks post induction colitis.

Serum Cytokine and Eicosanoid Findings

Figure 1a shows that LVA therapy is significantly able to reduce the serum levels of TNF-α in P and T groups (during weeks 2, 3 and 6) compared with colitis induced (C) rats (p<0.05). Figure 1b represents a significant difference in serum levels of IL-6 in P and T groups (after 1, 2, 3 and 6 weeks) compared with C animals (p < 0.05). Moreover, as shown in Figure 2a, LVA administration could significantly diminish the serum concentration of LTB4 (after 1, 3 and 6 weeks) in P and (after 1 and 3 weeks) in T group compared with colitis induced group (p < 0.05). Figure 2b shows that LVA therapy is significantly able to reduce the serum levels of PGE2 in P (during 6 week) and (during 1 and 3 weeks) in T group compared with colitis induced rats (p < 0.05).
Tissue Cytokine and Eicosanoid Findings

Using the supernatant of homogenized colonic tissue samples, we found that there was a significant difference ($P < 0.05$), between P and T groups versus colitis induced group in connection with the amounts of IL-6, TNF-α, and LTB4 (during weeks 1 and 6), Figure 3a, Figure 3b and Figure 4a respectively. However, there was no significant difference between P and T groups versus C in colonic tissue PGE2 level following LVA therapy in experimental model of colitis (Figure 4b).

DISCUSSION

Our data in this experimental study revealed the potential therapeutic effect of LVA in rat model of inflammatory bowel disease. IBD, broadly classified as either Crohn's disease or ulcerative colitis is caused by a dysregulated mucosal immune response to a luminal antigen, possibly a bacterium, in a genetically predisposed host. A rapid expansion of knowledge in recent years has greatly increased our understanding of the pathophysiology of these disorders.27,28

Figure 2. Effects of LVA on serum levels of LTB4 (a) and PGE2 (b), one and six weeks after induction of colitis. C: colitis induced; P: Prophylactic; and T: Treated rats.

Figure 3. Amounts of IL-6 (a) and TNF-α (b), in colonic tissue homogenate following six weeks orally administration of LVA. C: colitis induced; P: Prophylactic; and T: Treated rats.

Figure 4. Amounts of LTB4 (a) and PGE2 (b), in colonic tissue homogenate following six weeks orally administration of LVA. C: colitis induced; P: Prophylactic; and T: Treated rats.
It has been reported that the production of some cytokines such as, IL-6\(^{29,30}\) and TNF-\(\alpha\)^{31,32} as well as some eicosanoids; LTB4\(^{26,33}\) and PGE2\(^{34}\) are increased significantly in patients with inflammatory bowel disease. In this study, induction of colitis enhanced the serum and colonic mucosal IL-6, TNF-\(\alpha\), LTB4 and PGE2 levels in colitis induced group compared with normal rats, which paralleled the severity of colitis and was in agreement with others findings. LVA therapy significantly decreased lesion formation in TNBS-induced colitis. This treatment also significantly reduced serum and colonic mucosal cytokines (IL-6 and TNF-\(\alpha\)) and eicosanoids (LTB4 and PGE2) levels in prophylactic and therapeutic groups.

On the other hand, the scientific evidence revealed a significant correlation between the secretion of some inflammatory mediators, such as histamine,\(^{35-37}\) hyaluronidase\(^{38,39}\) and development of colitis. In IBD, histamine content and secretion were found to be significantly increased particularly in affected mucosa of Crohn’s disease and ulcerative colitis.\(^{35}\) Furthermore, Heller \textit{et al.}\(^{38}\) and van der Wiel \textit{et al.}\(^{39}\) have reported increasing activity of hyaluronidase in IBD. Whereas, Asada \textit{et al.}\(^{16}\) showed the inhibitory effects of various types of alginic acid on hyaluronidase and on histamine release from mast cells. Alginates are known to be biocompatible, degradable and nontoxic. These are used extensively as carriers for drug delivery, cell immobilization, wound dressing and as a protective agent in radiotherapy.\(^{11,12,17,40}\)

It is of interest to note that the protective effect of LVA in IBD paralleled with the protective and reparative effects of sodium alginate on radiation stomatitis in animals and human subjects.\(^{18,19}\)

In conclusion, our findings in this study demonstrated that LVA therapy palliated the progression of colonic inflammatory lesions in experimental model of IBD. The beneficial effect of LVA is associated with a reduction of cytokines (IL-6 and TNF-\(\alpha\)) and eicosanoids (LTB4 and PGE2) synthesis. Thus, sodium alginate as a new therapeutic option can be recommended for IBD preclinical trials.

REFERENCES


