

Dietary Polydextrose Prevents Inflammatory Bowel Disease in Trinitrobenzenesulfonic Acid Model of Rat Colitis

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ABSTRACT Inflammatory bowel disease (IBD) is a multifactorial intestinal disorder that involves interactions among the immune system, genetic susceptibility, and environmental factors, especially the bacterial flora. Polydextrose, a polysaccharide constituted by 90% nondigestible and nonabsorbable soluble fibers, has several physiological effects consistent with those of dietary fibers, including proliferation of colon microflora. Because sulfasalazine presents serious side effects through long-term use at high doses, the aim of the present study was to evaluate the preventative effect of polydextrose on trinitrobenzenesulfonic acid-induced intestinal inflammation and its effects on the intestinal anti-inflammatory activity of sulfasalazine. Results indicated that polydextrose and its association with sulfasalazine present an anti-inflammatory effect that reduces myeloperoxidase activity, counteracts glutathione content, and promotes reductions in lesion extension and colonic weight/length ratio.

KEY WORDS: • *intestinal inflammation* • *irritable bowel disease* • *prebiotics* • *sulfasalazine*

INTRODUCTION

INFLAMMATORY BOWEL DISEASE (IBD) is a chronic inflammatory process that is presented in two main distinct types: ulcerative colitis and Crohn's disease. The etiology has not yet been defined, but it is known that interactions between immune and genetic factors could be responsible for the development of chronic intestinal inflammation characterized by alternating periods of remission and active inflammation.¹ Furthermore, the intestinal microbiota is linked to IBD pathogenesis because of its role in modulating intestinal physiology and intestine immunological functions. Recently, complementary and alternative therapies based on the use of probiotics, prebiotics, and synbiotics have been reported as new therapeutic options for the treatment of human IBD.² Dietary fiber has been proven to be beneficial in maintaining remission in human ulcerative colitis because of an increase in the luminal production of short-chain fatty acids.^{3,4} Several studies have reported that some prebiotics, including dietary fiber, germinated barley foodstuff, inulin, and lactulose, exert beneficial effects in both human and experimental colitis models.^{2,5}

Polydextrose is a polysaccharide widely used as a bulking agent to replace sugar or fat in food industry. This carbohydrate is constituted by 90% nondigestible and nonab-

sorbable soluble fibers, a large amount of which is excreted in the feces. Fermentation of polydextrose by microbiota selectively allows the growth of beneficial bacteria, reducing the putrefactive ones, enhancing short-chain fatty acid production, especially that of butyric acid, and suppressing the production of carcinogenic metabolites.³ Given that polydextrose has several physiological effects consistent with those of dietary fibers³ and that sulfasalazine, a drug of choice for IBD treatment, presents serious side effects when used long-term at high doses,⁶ our objective was to evaluate the preventative effect of polydextrose in trinitrobenzenesulfonic acid (TNBS)-induced intestinal inflammation and its effects on the anti-inflammatory activity of sulfasalazine.

MATERIALS AND METHODS

Drugs

All chemicals were supplied by Sigma (St. Louis, MO, USA). The dietary fiber polydextrose (Litesse®) was manufactured and provided by Danisco Sweeteners Ltd. of Brazil (Cotia, SP, Brazil).

Animals

Male Wistar rats (weighing 180–200 g) from the Central Animal House, São Paulo State University-UNESP, Botucatu, SP, Brazil were housed in standard environmental conditions (21°C, 60–70% humidity) under a 12-hour light/dark cycle and air filtration. Animals had free access to

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water and food (BioBase AA Ltd., Águas Frias, SC, Brazil). Experimental protocols met the Guidelines of Animal Experimentation approved by the Commission of Ethics in Animal Experimentation (protocol number 042/04-CEEA), the Institute of Biosciences, São Paulo State University-UNESP.

Experimental design and assessment of colonic damage

The rats were randomly assigned to six groups. Two of them, a noncolitic group and a colitic group, received no treatment. Three groups received 5% polydextrose dissolved in drinking water for 21 days prior to colitis induction and 4 days thereafter: the first group received only 5% polydextrose, whereas the second and third groups received, in addition, sulfasalazine (50 mg/kg and 5 mg/kg, respectively) at 72, 48, 24, and 2 hours before the colitis induction as well as 24 hours after. For reference comparison the remaining group received only sulfasalazine (50 mg/kg) at 72, 48, 24, and 2 hours before colitis induction as well as 24 hours thereafter. Sulfasalazine was administered by means of an esophageal catheter (volume, 5 mL/kg). Rats from the noncolitic and nontreated colitic groups were orally administered water.

Colitis was induced using the method originally described by Morris *et al.*⁷ After having fasted overnight, animals were anesthetized with halothane. Under anesthesia, they were given 10 mg of TNBS dissolved in 0.25 mL of 50% (vol/vol) ethanol by means of a Teflon[®] (Dupont, Wilmington, DE, USA) cannula inserted 8 cm into the anus. During and after TNBS administration, the rats were kept in a head-down position until they recovered from the anesthesia. Rats from the noncolitic (normal) group received 0.25 mL of saline. Animal body weights, occurrence of diarrhea (as detected by perianal fur soiling), and total food intake for each group were recorded daily. Animals from all groups ($n=8$) were euthanized, 48 hours after colitis induction, by an overdose of halothane. The colonic segments were obtained after laparotomy, and the eventual occurrence of adhesions between the colon and adjacent organs was noted. The segments were placed on an ice-cold plate, cleaned of fat and mesentery, and blotted on filter paper. The entire colon was weighed, and its length was measured under a constant load (2 g). The colon was longitudinally opened and scored for macroscopically visible damage on a 0–10 scale by two observers unaware of the treatment, according to the criteria described by Bell *et al.*⁸ (Table 1).

The colon was subsequently divided into different longitudinal pieces to be used for the following biochemical determinations: myeloperoxidase (MPO) activity, alkaline phosphatase activity, and total glutathione (GSH) content.

MPO activity was determined according to the technique described by Krawisz *et al.*⁹ The results are expressed as MPO units/g of tissue. One unit of MPO activity was defined as the amount required to degrade 1 μ mol of hydrogen peroxide/minute at 25°C.

Alkaline phosphatase activity was determined spectrophotometrically, using disodium nitrophenylphosphate (5.5 mM) as substrate in 50 mM glycine buffer with 0.5 mM MgCl₂ at pH 10.5.¹⁰ The enzymatic activity is expressed as mU/mg of protein.¹¹

GSH content was quantified with the recycling assay described by Anderson,¹² and the results were expressed as nmol/g of wet tissue.

Statistics

All results are expressed as mean \pm SEM values, and differences between means were tested for statistical significance using one-way analysis of variance and *post hoc* least significance tests. Nonparametric data (scores) are expressed as the median (range) and were analyzed with the Kruskal-Wallis test. Differences between proportions were analyzed with the χ^2 test. Statistical significance was set at $P < .05$.

RESULTS

TNBS administration resulted in colonic inflammation, which was evidenced after 48 hours by severe necrosis of the mucosa, typically extending 3.8–5.8 cm along the colon, bowel wall thickening, and hyperemia (Table 2). This inflammatory process was associated with an increase in the colonic weight/length ratio and with a reduction in food intake compared to noncolitic rats (data not shown), in addition to signs of diarrhea in 100% of the colitic animals (Table 2). Biochemically, the colonic damage was characterized by a reduction in colonic GSH levels (Fig. 1) and increases in MPO (Fig. 2) and alkaline phosphatase (data not shown) activities.

Our results demonstrate that polydextrose incorporation in drinking water (5% wt/vol) facilitated recovery from TNBS-induced colonic damage in rats, evidenced by a reduction in colonic damage and weight/length ratio (Table 2),

TABLE 1. CRITERIA FOR ASSESSMENT OF MACROSCOPIC COLONIC DAMAGE

Score	Criteria
0	No damage
1	Hyperemia, no ulcers
2	Linear ulcer with no significant inflammation
3	Linear ulcer with inflammation at one site
4	≥ 2 sites of ulceration/inflammation
5	≥ 2 major sites of ulceration and inflammation or one site of ulceration/inflammation extending >1 cm along the length of the colon
6	If damage covers >2 cm along the length of the colon, the score is increased by 1 for each additional centimeter of involvement

TABLE 2. EFFECTS OF EXPERIMENTAL GROUPS ON DAMAGE SCORE, EXTENSION OF LESION, CHANGES IN COLONIC WEIGHT, AND INCIDENCE OF DIARRHEA IN ACUTE TRINITROBENZENESULFONIC ACID COLITIS

Experimental group	Score (0–10) ^a	Extension of lesion (cm) ^b	Colon weight (mg/cm) ^b	Diarrhea (%)	Adherence (%)
Noncolitic	0**	0**	115.75 ± 6.06**	0**	0
TNBS control	7 (6–8)	3.4 ± 0.45	155.70 ± 7.47	100	0
Sulfasalazine (50 mg/kg)	6 (5–7)	3.45 ± 0.28	160.65 ± 7.08	100	0
Polydextrose 5%					
Alone	4 (0–7)	1.45 ± 0.55*	125.34 ± 8.27*	100	12.5
+ Sulfasalazine (50 mg/kg)	5 (0–6)	1.18 ± 0.49**	146.07 ± 2.77	100	0
+ Sulfasalazine (5 mg/kg)	6 (3–8)	2.31 ± 0.53	153.40 ± 7.86	100	40

^aScore data are expressed as median (range).

^bExtension of lesion and colonic weight data are mean ± SEM values.

* $P < .05$, ** $P < .01$ versus trinitrobenzenesulfonic acid (TNBS) control group.

a maintenance of the colonic GSH level (Fig. 1), and a reduction in MPO activity, in comparison to the colitic control (Fig. 2). The treatment with polydextrose and sulfasalazine (50 mg/kg) showed results similar to those produced by polydextrose dietary treatment (Table 2). Curiously, the only reduction promoted by the association of polydextrose with sulfasalazine (5 mg/kg) was in MPO activity (Fig. 2).

Sulfasalazine treatment of colitic rats, at the dose of 50 mg/kg, showed a preventative effect in acute colitis evidenced by a diminished colon weight/length ratio (Table 2) and a counteraction of the GSH level depletion

(Fig. 1). For all treated groups, no significant difference was found in alkaline phosphatase activity (data not shown).

DISCUSSION

The intestinal microbiota has been found to be an implicating factor in the etiology of IBD² and to play a crucial role in perpetuating the inflammation in experimental assays and patients with Crohn's disease.¹³ Therapeutic changes of the luminal microenvironment produced by administration of several probiotics and prebiotics offer great promise for nontoxic treatment of IBD.¹⁴ Prebiotics are nondigestible

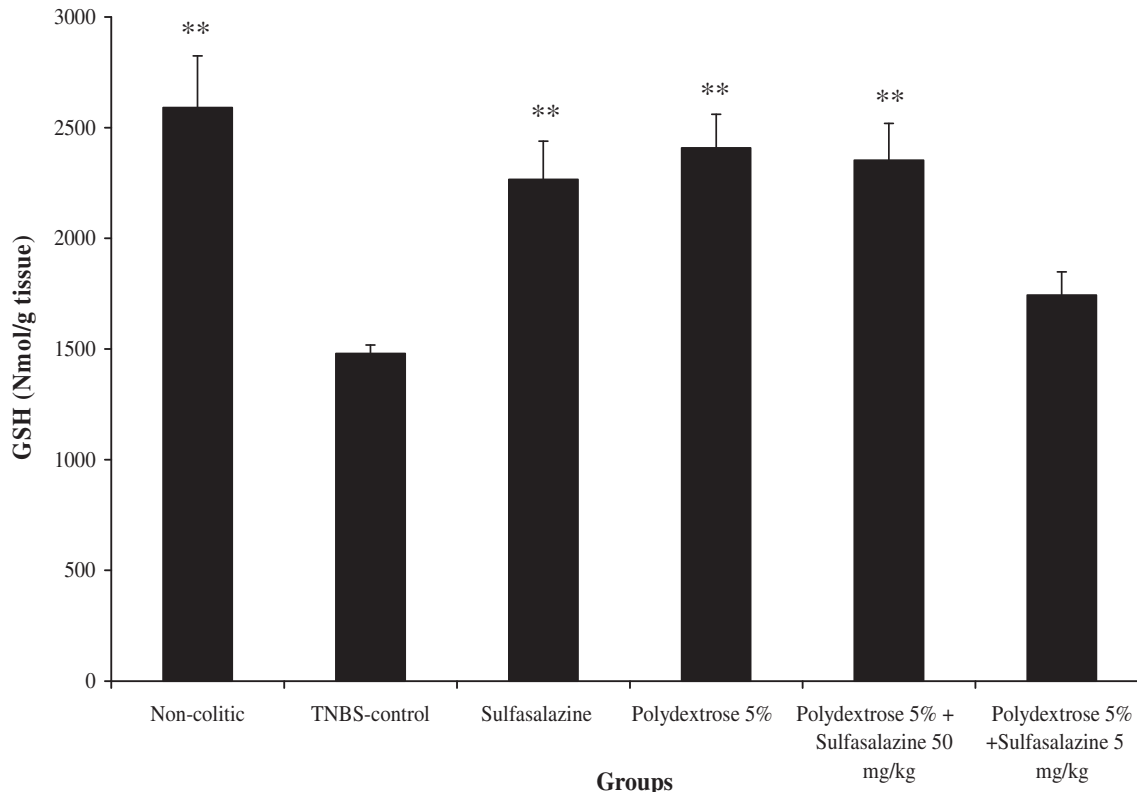


FIG. 1. Effects in experimental groups on total glutathione (GSH) level in acute TNBS colitis. Data are mean ± SEM values. ** $P < .01$ versus TNBS control group. All groups differ significantly from the noncolitic group ($P < .01$, data not shown).

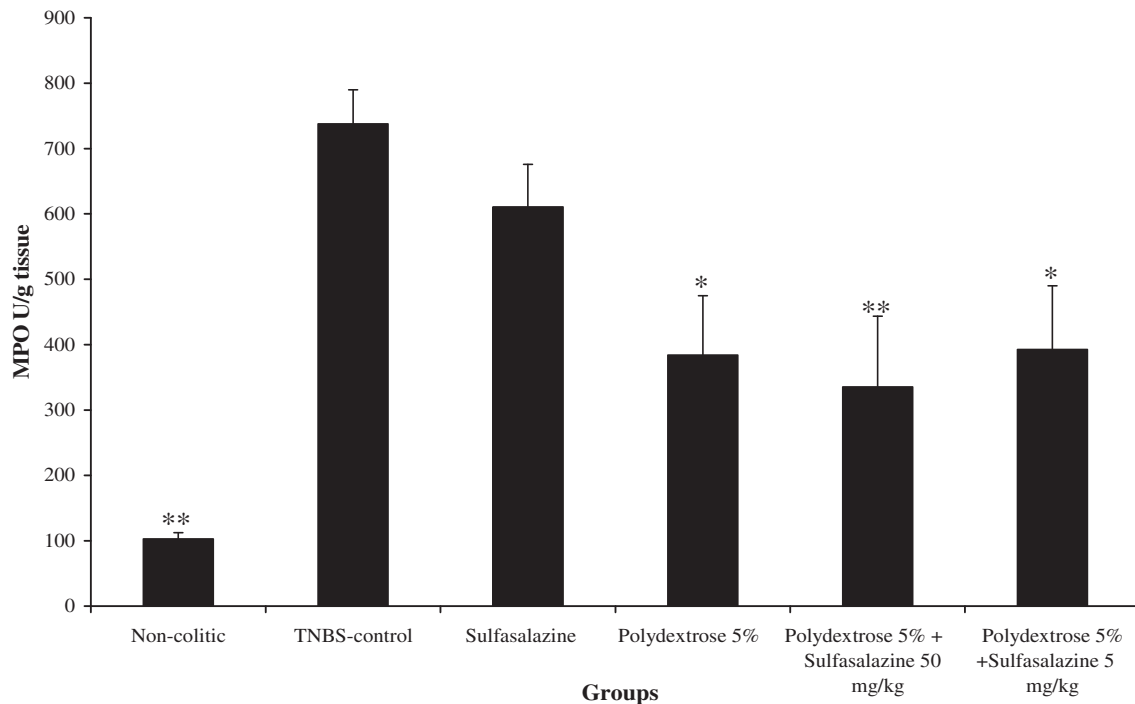


FIG. 2. Effects in experimental groups on myeloperoxidase (MPO) activity in acute TNBS colitis. Data are mean \pm SEM values. * $P < .05$, ** $P < .01$ versus TNBS control group. All groups differ significantly from the noncolitic group ($P < .01$, data not shown).

carbohydrates that stimulate the growth of particular species of the microflora, mainly lactobacilli and bifidobacteria, resulting in improved enteric function. The most commonly used prebiotics in experimental intestinal inflammation models and clinical IBD trials were lactulose, lactosucrose, oligofructose, inulin, psyllium, germinated barley foodstuff, and fructo- and milk oligosaccharides.^{2,5} These products may act via modulating the endogenous flora or some of its fermentation products.¹⁵ A prebiotic mixture containing inulin and oligofructose administered for 2 weeks showed a 10-fold increase in the number of the bifidobacteria and lactobacilli in human intestine,¹⁶ in addition to their immunomodulatory properties in clinical IBD patient trials¹³ and in experimental colitis.^{17,18} Inulin alone or in combination with *Bifidobacterium infantis* strains significantly improved the disease activity indexes and decreased both colonic MPO activity and expression of inflammatory mediators.¹⁹ Lactulose, another synthetic prebiotic, also demonstrated a preventative anti-inflammatory effect in TNBS-induced colitis⁵ and IBD patients.²⁰ In light of these findings, therapeutic alteration of the luminal microenvironment by prebiotics may constitute a new therapeutic strategy to restore the balance of the gastrointestinal microbiota in order to reduce or prevent intestinal inflammation.

Polydextrose is a polysaccharide that is recognized and used as soluble fiber because of its fermentative properties in the lower gastrointestinal tract and production of short-chain fatty acids.²¹ It is partially fermented in the large intestine, leading to increased fecal bulk, reduced transit time, growth promotion of favorable microflora, diminished pu-

trifactive microflora, and suppressed production of carcinogenic metabolites.³ Among other important activities, polydextrose augments bifidobacteria levels,²² fecal pH, production of acetic, isobutyric, and 2-methylbutyric acids,²³ calcium absorption and bone mineralization,²⁴ and growth of normal cecal epithelial cells³ and slightly elevates fecal mass and stool consistency,²⁵ lipid and glucose regulation metabolism,²⁶ water and sodium reabsorption,²⁷ and blood flow in the colon²⁸ and intestinal immune system^{29,30} while reducing the formation of aberrant crypt foci in the rat rectum.³¹ Further studies assured the safety and efficacy of polydextrose as a bulk agent.³²

The results obtained in the present study showed a protective and preventative effect of polydextrose on the TNBS-induced inflammatory process in rats. Recovery from the TNBS-induced colonic damage in rats treated with polydextrose was evidenced by a reduction in colonic damage and weight/length ratio (Table 2), a counteraction of GSH depletion (Fig. 1), and a reduction in MPO activity (Fig. 2). Glutathione is an endogenous compound with important physiological functions, mainly in relation to intestinal integrity.³³ Compounds that prevent the colonic GSH reduction can be adjuvant options for IBD management. A reduction in MPO has been interpreted as a manifestation of the anti-inflammatory property of a given compound.³⁴ A similar effect was evidenced after treatment with polydextrose associated with sulfasalazine (50 mg/kg), whereas the sulfasalazine-treated group that did not receive simultaneous polydextrose treatment was only able to counteract the glutathione depletion that occurs subsequently to the

inflammation process (Fig. 1). One study attributed the beneficial protection exerted by the 5-aminosalicylic derivatives to their antioxidant and free radical scavenger properties.³⁵

These results suggest that anti-inflammatory activity after simultaneous administration of polydextrose and sulfasalazine is dependent on polydextrose action. Because sulfasalazine is a sulfonamide antibiotic that acts by blocking bacterial proliferation, it is possible that the observed protective effect is also related to effects of polydextrose on bacterial proliferation.³ The effect exerted by polydextrose in preserving the colonic mucosa from oxidative insult may be a factor in diminishing the neutrophil infiltration that occurs in response to TNBS. In fact, an inhibition of free radical generation could contribute to a lower level of leukocyte infiltration into the inflamed tissue, thus preventing inflammation of colonic tissue.

In conclusion, the present study showed that polydextrose prevents TNBS-induced colonic damage in rats while its observed anti-inflammatory effect was associated with improved intestinal oxidative stress, demonstrated by counteraction of GSH depletion and inhibition of the MPO activity. This finding is probably related to the prebiotic properties of this soluble fiber. However, the effects of polydextrose on the balance between beneficial and pathogenic bacteria populations in the colon must still be elucidated. Indeed, the effect of polydextrose on sulfasalazine anti-inflammatory activity is not synergistic because polydextrose improves only some effects of sulfasalazine. However, polydextrose may constitute an important dietary supplement in the prevention of human IBD.

AUTHOR DISCLOSURE STATEMENT

No competing financial interests exist.

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