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Inulin supplementation in rat model of pouchitis

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Available data indicates potential effectiveness of prebiotic therapy in alleviating inflammation and prolonging the remission in inflammatory bowel disease. Documented successes of such therapies were the basis for this study. So far, there is no data related to the effectiveness of inulin application in symptomatic or severe pouchitis in humans or in animal model. The aim of the study was to determine the effect of inulin supplementation on the expression of intestinal inflammation and feeding efficiency in rats with induced pouchitis. Twenty-four Wistar rats were operated. After induction of pouchitis animals were randomly divided into control and supplementation groups receiving, respectively, semi-synthetic diet with or without inulin (in a lower (LD) or higher (HD) dose: 2.5% or 5% of total dietary content of mass) for a period of 6 weeks. Selected nutritional parameters were assessed throughout the study. Histopathological and immunohistochemical analysis of pouch mucosa specimens was also performed. The energy intake, weight gain, feeding efficiency, quality of stools were comparable in all studied groups. The intensity of inflammation (Moskovitz scale) and adaptive changes (Laumonier scale) did not differ between compared groups. The tissue expression of pro- and anti-inflammatory interleukins (IL-1α, IL-6, IL-10 and IL-12) was not different either. Inulin supplementation does not improve the quality of stools or the expression of intestinal inflammation in rats with induced pouchitis. It has no impact on the intensity of pouch adaptation or on feeding efficiency.

Keywords: restorative proctocolectomy, pouchitis, inulin, rats

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INTRODUCTION

Restorative proctocolectomy carries the risk of postoperative complications. The most common complication following construction of the intestinal reservoir is pouchitis, which occurs in 5–60% of operated patients (Hoda *et al*., 2008). Typical symptoms of pouchitis comprise abdominal pain, loose stool mixed with mucus and blood, fecal incontinence and an elevated body temperature. However, diarrhea is the most difficult problem, since it can quickly lead to severe dehydration (Ferrante *et al*., 2008). The etiology of the inflammation in pouchitis is still unknown. Among the factors likely to influence its course are stasis of intestinal contents with subsequent proliferation of bacteria, mucosal edema and its loss of proteins. Additionally, a role of bile acids and short-chain fatty acids is considered. More commonly, the profile of intestinal flora and the role of both anaerobic and aerobic bacteria are emphasized. In support of this concept, the use of metronidazole in post-operative reservoir rinsing and probiotic application have been documented to significantly reduce both the incidence and severity of inflammation (Pari & Sndborn, 2006).

Prebiotics are non-digestible food ingredients that can selectively stimulate the growth and activity of advantageous bacterial strains, and improve the health of the host by a favorable change in intestinal flora composition. One of the most popular prebiotics is inulin, a soluble polysaccharide and a part of dietary fiber. It stimulates growth and activity of bifidobacteria and lactobacteria (Novak& Katz, 2006). Use of animal models dominates studies evaluating the effect of prebiotics in non-specific inflammation of the bowel (Hoentjen *et al*., 2005; Lara-Villoslada *et al*., 2006). The impact of different prebiotics (inulin, fructooligosaccharide, lactulose) on colitis induced by chemical substances or expressed under the influence of certain genetic modification has been evaluated. Enrichment of the diet with prebiotiocs alleviated symptoms and improved biochemical and histopathological parameters (Hoentjen *et al*., 2005). The supply of prebiotics also leads to a significant reduction in myeloperoxidase activity and a decline in tissue concentrations of inflammatory mediators TNF-α and leukotriene (LTB4) (Lara-Villoslada *et al*., 2006).

So far, there have been only few clinical trials evaluating the effect of prebiotic application in patients with inflammatory bowel disease (IBD). All of them covered the combination of conventional therapies with the supply of prebiotics showing potential interesting results (Lindsay *et al*., 2006; Leenen & Dielman, 2007). According to the PubMed database, only one randomized controlled trial evaluating the effect of prebiotics on the inflamed intestinal mucosa of the J-pouch has been published (Welters *et al*., 2002). Welters and coworkers studied a group of 20 patients with asymptomatic pouchitis. Patients were treated with inulin for 3 weeks with a dose of 24 g/day. Significant reduction in the severity of the disease when compared with placebo, both in the endoscopic and histological assessment using pouchitis disease activity index (PDAI), was stated. So far, there is no data related to the effectiveness of inulin applica-

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Abbreviations: ABC, avidin-biotin complex; HD, high dose group; IBD, inflammatory bowel disease; LD, low dose group; PDAI, pouchitis disease activity index; UC, ulcerative colitis.

tion in symptomatic or severe pouchitis in humans or in animal model.

The experimental model of pouchitis is applicable to investigating several key etiologic mechanisms purportedly related to pouchitis and to the future development of new therapeutic modalities. Ileal pouch-rectal anastomosis in rats is a confirmed and accepted model for the study of pouchitis (Lichtman *et al*., 1998; Shebani *et al*., 2002).

The aim of the present study was to assess the effect of inulin supplementation on the expression of intestinal inflammation of the J-pouch and feeding efficiency in rats with induced pouchitis.

METHODS

After a seven-day period of adaptation and 24-hour fasting, twenty-four animals underwent restorative proctocolectomy with the creation of J-pouch. In all animals, feeding was stopped 24 hours before surgery. After an intraperitoneal injection of pentobarbital anesthesia (pentobarbital sodium, 50 mg/kg body mass) a 3–4 cm midline laparotomy was performed. Total proctocolectomy was performed by resecting the colon and ligating the mesentery with 4–0 silk. The intestinal segment was excised from 0.1 cm proximal to the ileocecal junction. The rectum was resected at the level of the pelvic floor, with a 0.5 cm rectal stump. Small-intestine contents was washed with 0.9% warm saline and a 2-cm ileal J-pouch was created by duplication of the distal end of the small intestine by a single-layer interrupted 6–0 prolene suture. The pouch anal anastomosis was performed with a single layer interrupted 6–0 prolene suture. The sufficiency of the anastomosis was controlled by injection of 3–5 ml of 0.9% warm saline through the anus.

After one day of fasting (exclusive supply of 8% glucose solution), the animals received semi-synthetic diet without fiber (AIN-93) in increasing doses for 10 days (respectively 5, 8, 10 and 12 g/day in the following days, up to 25 g/day). The semi-synthetic diet AIN-93 was composed of casein (20%), sunflower oil (7%), wheat starch and sucrose (53.2% and 10%), potato starch (5%), L-cystine (0.3%), vitamin mixture (1%) and mineral mix. For the following 11 days rats received the same diet (up to 25 g/day). Subsequently, the inflammation of J-pouch was induced. For that purpose for the next 7 days all animals were administered feed AIN-93 supplemented with fiber (in growing quantities, 1% on the first day, 2% in the second, up to a maximum of 4%).

Subsequently, rats were allocated by body mass and randomly divided into three equal subgroups (the control group and two supplementation groups). Animals from the control group were fed *ad libitum* the AIN-93 diet for a period of 6 weeks. The high dose (HD) group of rats received semi-synthetic AIN-93 diet enriched in inulin (Hortimex, Konin, Poland) at a dose of 5% of total dietary content by mass. The low dose (LD) group received semi-synthetic AIN-93 diet enriched in inulin at a dose of 2.5% of total dietary content. The quality of stools, body mass gain and energy intake were assessed throughout the period of the study. The feeding efficiency (ratio of daily energy intake to the average daily mass gain) was also calculated. At the end of the study animals were sacrificed and specimens of J-pouch mucosa were obtained for histopathological and immunohistochemical analysis.

All surgical procedures were conducted by one qualified surgeon in accordance with the guidelines of the European Community Council directives (86/609/EEC) and with approval of the Local Ethic Committee (42/2006).

The quality of stool. For the assessment of stool quality a 5-point scale was used (1 — lack of stool, 2 — diarrhea, 3 — blob of stool, 4 — textured stool, 5 — normal stool). Since such an assessment was expected potentially to be subjective, the observer was "blinded" (MT).

Histopathological examination. Microscopic assessment was performed according to standard histological techniques (hematoxylin and eosin staining). In addition to routine histopathological examination, the collected specimens were evaluated for the intensity of inflammation (Moskowitz scale) and the adaptation changes with intestinal villous shortening (Laumonier scale) (Sandborn *et al*., 1994).

Immunohistochemical examination. Tissue expression of selected pro- (IL-1α, -6, -12) and anti-inflammatory (IL-10) interleukins was evaluated with the use of ABC technique (Avidin-Biotin Complex) using LSAB PLUS kit Kit/HRP (DakoCytomation, Glostrup, Denmark) (Nagle *et al*., 1983). Preparations were contrasted with Mayer's hematoxylin. The presence or absence of cells with immunohistochemical reaction was assessed (assuming that honey-brown cytoplasm staining is equivalent to positive reaction). Colonic histopathological preparations of patients with active ulcerative colitis (UC) served as positive control. Preparations in which the addition of first antibody was omitted, during the immunohistochemical staining, served as a negative control.

Both histological and immunohistochemical assessment was performed by a blinded histopathologist (PM).

Statistical analysis. For the parametric values means and standard deviations (S.D.) were calculated, while for the nonparametric ones ranges (and for the quality of stool — 1–3 quartile) were given. For both, medians were provided. Statistical analysis of data was performed using the Kruskal-Wallis test.

RESULTS

The changes in energy intake, body mass and feeding efficiency were comparable in all studied groups (Table 1).

Table 1. Average body mass gain (BWG), energy intake (EI), feeding efficiency (FE) and quality of stools (QS) during the supplementation period of the study

Parameter	Group		
	HDG	LDG	Control
BWG (g/day) mean ± S.D. [median]	1.9 ± 0.6 [1.7]	1.9 ± 0.5 [1.9]	1.9 ± 0.5 [1.8]
EI (kcal) mean ± S.D. [median]	101.4 ± 7.1 [101.9]	102.7 ± 8.4 [99.9]	98.5 ± 8.0 [99.1]
FE (kcal/g) mean ± S.D. [median]	58.7 ± 20.6 [54.4]	56.5 ± 11.1 [54.7]	54.8 ± 14.4 [53.9]
QS range [median]	2–4 [4]	2–4 [4]	2–5 [4]

Table 2. Changes of stool quality during supplementation

The quality of stool	Week of supplementation	Group		
		HD	LD	Control
Median [range] (1–3 quartile)	1	3 [2–3] (3–3)	3 [2–3] (3–3)	3 [3–3] (3–3)
	2	3 [2–3] (2–3)	2.5 [2–3] (2–3)	3 [2–3] (3–3)
	3	2.5 [2–3] (2–3)	3 [2–3] (2.75–3)	3 [2–3] (3–3)
	4	2 [2–3]* (2–2.25)	3 [2–3] (2–3)	3 [3–3]* (3–3)
	5	3 [2–3] (2–3)	3 [3–3] (3–3)	3 [3–3] (3–3)
	6	3 [2–3] (2.75–3)	3 [3–4] (3–3)	3 [3–4] (3–3.25)

*$p<0.01$

The quality of stool during the whole study did not differ between studied groups (Table 1). However, the quality of stool was worse in the HD group than in the control group in the 4th week of dietary modification (Table 2).

The severity of inflammation expressed in the Moskowitz scale was greater in HD than in the control group. The intensity of the intestinal adaptive changes in the reservoir as assessed by the Laumonier scale did not differ between the groups. The tissue expression of pro-inflammatory interleukins (IL-1α, IL-6 and IL-12) and anti-inflammatory interleukin IL-10 assayed in pouch samples was not different either (Table 3).

Table 3. Severity of inflammation (Moskowitz scale — MS), degree of pouch adaptation (Laumonier scale — LS) and tissue expression of pro-inflammatory and anti-inflammatory interleukins in pouch samples

Parameter		Groups		
		HD	LD	Control
Histopathological assessment	MS median [range]	3.5* [3–5]	4 [2–5]	2* [2–3]
	LS median [range]	2 [1–4]	1 [1–2]	1 [1–2]
Intrerleukin expression	IL-1α median [range]	1.5 [1–2]	1 [1–2]	1 [1–1]
	IL-6 median [range]	1 [1–2]	1 [0–1]	1 [1–1]
	IL-12 median [range]	1.5 [1–3]	2 [1–2]	1 [1–2]
	IL-10 median [range]	3 [1–3]	2 [1–3]	2 [1–2]

*$p<0.01$

DISCUSSION

The impact of inulin supplementation on the effectiveness of feeding and induction of inflammation of the pouch mucosa of rats subjected to restorative proctocolectomy was assessed in the study. The use of inulin as a factor potentially modulating inflammation was suggested by the promising results obtained by Welters *et al.* (2002), who showed that the supply of inulin in asymptomatic patients with pouchitis reduces the severity of disease as compared with placebo. However, we failed to document a positive impact of inulin supplementation on the induced pouchitis in the rat model.

Restorative proctocolectomy demands the removal of the entire large intestine. For a period of about 3 months, patients are given temporary decompressive ileostomy. In rats, this phase of the surgical treatment is omitted and immediately after the operation the pouch is exposed to feces, which results in severe diarrhea. Feces of rats in the present experiment underwent gradual normalization. However, the quality of the stool was worse in the HD group than in the control group in the 4th week of dietary modification. Our results differ from the data obtained in the human study (Welters *et al.*, 2002). In 20 patients who underwent restorative proctocolectomy, a 3-week dietary inulin supplementation did not change the frequency of bowel movements or the frequency of the presence of blood in the stool. Patients received 12 g of inulin twice daily, irrespective of sex or body mass. However, it should be noted that none of the patients had any symptoms of pouchitis. Therefore, this clinical situation was different from that analyzed in the present animal model. The dose of inulin in our study, although somewhat lower than typical for healthy rats, seems to be slightly higher than that applied in the above human study. It might have affected the quality of feces in the test groups. As an osmotically active substance, inulin leads to softening of fecal mass and in consequence changes the consistency of stool. Additionally, inulin has the ability to increase fecal mass by stimulating the growth of intestinal microflora. Hydrogen, methane and carbon dioxide appearing as a result of bacterial fermentation soften the stool as well. In the case of excessive fermentation, there may be a deterioration in the stool quality (Badiali *et al.*, 1995; Macfarlane *et al.*, 2006).

The quantification of cytokines in patients with pouchitis has become more common recently. Laboratory evaluation provides information on the etiology and pathogenesis of pouchitis, and it also helps practicing clinicians with accurate diagnosis, differential diagnosis, disease stratification, and management of ileal pouch disorders (Leal *et al.*, 2008; Navaneethan & Shen, 2009). For example, quantification of mucosal proinflammatory cytokines, such as IL-1β and IL-8, differentiates in a simple and objective way between irritable pouch syndrome and pouchitis (Schmidt *et al.*, 2007).

Some of the best known pro-inflammatory cytokines are interleukins, whose levels were determined in the rat intestinal biopsies of the reservoirs for the purpose of the present study. An increased production of pro-inflammatory IL-1α and IL-6 in patients with pouchitis has been documented, as compared to that in subjects with non-inflammed ileoanal pouch (Gionchetti *et al.*, 1994; Patel *et al.*, 1995). Similarly, a relationship between IL-10 levels and intensity of pouchitis has been shown (Bulois *et al.*, 2000). Therefore, we assessed in the present study the expression of four selected interleukins assuming that their levels reflected the intensity of inflammation. Tissue levels of pro- (IL-1α, IL-6 and IL-12) and anti-inflammatory (IL-10) cytokines were comparable in

all analyzed rat groups in the present study. The available evidence on the influence of prebiotic supplementation on interleukin expression is sparse. Four weeks of fructooligosaccharide supplementation (at doses of 2.5% and 7.5%) in a mice model did not significantly affect the function of the immune system (Hosono *et al*., 2003). However, *ex vivo* culture of immune cells isolated from Peyer's patches in mice fed with diet supplemented fructooligosaccharide and *Bifidobacterium pseudocatenulatum* showed an increase in total IgA, IFN-γ, IL-5, IL-6 and IL-10 levels as compared to control mice without the supplementation.

The determination of the appropriate inulin dose was based on the results of Józefiak *et al.* (2005). They documented that dietary content of 10% of inulin caused loosening of stool in healthy rats through an increase in water content. Such an effect was not recorded with a diet containing of 5% of inulin. Considering the differences between the Józefiak and coworkers and our study model, including the presence of the intestinal reservoir, diets with lower inulin content by mass (5% and 2.5% of the total dietary content) were adopted. The prebiotic dosage in different human studies assessing the impact of inulin supplementation on the health status of adult patients ranged from 7 to 40 g per day (Lindsay *et al*., 2006; Leenen *et al*., 2007). Relating it to dry mass (as in the present model), the higher dose of inulin in the present study seems to be larger than those applied in patients with IBD.

The analysis of available published data and our results points to a need of further research on the prebiotic therapy in the treatment of pouchitis, potentially with lower doses of oligosaccharides. It should be underlined that although the quality of the stool was worse in the HD group, the increases in body mass, energy intake and feeding efficiency in the present study were similar in all studied rat groups.

Inulin supplementation does not improve the quality of stools and the expression of intestinal inflammation in rats with induced pouchitis, neither has it an impact on the intensity of pouch adaptation or feeding efficiency.

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REFERENCES

Badiali D, Corazziari E, Habib FI, Tomei E, Bausano G, Magrini P, Anzini F, Torsowi A (1995) Effect of wheat bran in treatment of chronic nonorganic constipation. A double-blind controlled trial. *Dig Dis Sci* **40:** 349–356.

Bulois P, Tremaine WJ, Maunoury V, Gambiez L, Hafraoui S, Leteurtre L, Cortot A, Sandborn WJ, Colombel JF, Desreumaux P (2000) Pouchitis is associated with mucosal imbalance between interleukin-8 and interleukin-10. *Inflamm Bowel Dis* **6:** 157–164.

Ferrante M, Declerck S, De Hertogh G, Van Assche G, Geboes K, Rutgeerts P, Penninckx F, Vermeire S, D'Hoore A (2008) Outcome after proctocolectomy with ileal pouch-anal anastomosis for ulcerative colitis. *Inflamm Bowel Dis* **1:** 20–28.

Gionchetti P, Campieri M, Belluzzi A, Bertinelli E, Ferretti M, Brignola C, Poggioli G, Miglioli M, Barbara L (1994) Mucosal concentrations of interleukin-1 beta, interleukin-6, interleukin-8, and tumor necrosis factor-alpha in pelvic ileal pouches. *Dig Dis Sci* **39:** 1525–1531.

Hoda KM, Collins JF, Knigge KL, Deveney KE (2008) Predictors of pouchitis after ileal pouch-anal anastomosis: a retrospective review. *Dis Colon Rectum* **5:** 554–560.

Hoentjen F, Welling GW, Harmsen HJ, Zhang X, Snart J, Tannock GW, Lien K, Churchill TA, Lupicki M, Dieleman LA (2005) Reduction of colitis by prebiotics in HLA-B27transgenic rats is associated with microflora changes and immunomodulation. *Inflamm Bowel Dis* **11:** 977–985.

Hosono A, Ozawa A, Kato R, Ohnishi Y, Nakanishi Y, Kimura T, Nakamura R (2003) Dietary fructooligosaccharides induce immunoregulation of intestinal IgA secretion by murine Peyer's patch cells. *Biosci Biotechnol Biochem* **67:** 758–764.

Józefiak D, Krejpcio Z, Trojanowska K, Wójciak R, Tubacka M, Śmigiel-Papińska D, Rutkowski A (2005) Effect dietary inulin on microbial ecosystem and concentrations of volatile fatty acids in rat's caecum. *J Anim Feed Sci* **14:** 171–178.

Lara-Villoslada F, de Haro O, Camuesco D, Comalada M, Velasco J, Zarzuelo A, Xaus J, Galvez H (2006) Short-chain fructooligosaccharides, in spite of being fermented in theupper part of the large intestine, have anti-inflammatory activity in the TNBS model of colitis. *Eur J Nutr* **45:** 418–425.

Leal RF, Coy CS, Ayrizono ML, Fagundes JJ, Milanski M, Saad MJ, Velloso LA, Góes JR (2008) Differential expression of pro-inflammatory cytokines and a pro-apoptotic protein in pelvic ileal pouches for ulcerative colitis and familial adenomatous polyposis. *Tech Coloproctol* **12:** 33–38.

Leenen CH, Dieleman LA (2007) Inulin and oligofructose in chronic inflammatory bowel disease. *J Nutr* **37:** 2572–2575.

Lichtman SN, Wang J, Hummel B, Lacey S, Sartor RB (1998) A rat model of ileal pouch-rectal anastomosis. *Inflamm Bowel Dis* **4:** 187–195.

Lindsay JO, Whelan K, Stagg AJ, Gobin P, Al-Hassi HO, Rayment N, Kamm MA, Knight SC, Forbes A (2006) Clinical, microbiological, and immunological effects of fructooligosaccharide in patients with Crohn's disease. *Gut* **55:** 348–355.

Macfarlane S, Macfarlane GT, Cummings JH (2006) Review article: prebiotics in the gastrointestinal tract. *Aliment Pharmacol Ther* **24:** 701–714.

Nagle RB, Clark VA, McDaniel KM, Davis JR (1983) Immunohistochemical demonstration of keratins in human ovarian neoplasms. A comparison of methods. *J Histochem Cytochem* **3:** 1010–1014.

Navaneethan U, Shen B (2009) Laboratory tests for patients with ileal pouch-anal anastomosis: clinical utility in predicting, diagnosing, and monitoring pouch disorders. *Am J Gastroenterol* **104:** 2606–2615.

Novak J, Katz JA (2006) Probiotics and prebiotics for gastrointestinal infections. *Curr Infect Dis Rep* **8:** 103–109.

Patel RT, Bain I, Youngs D, Keighley MR (1995) Cytokine production in pouchitis is similar to that in ulcerative colitis. *Dis Colon Rectum* **38:** 831–837.

Pardi DS, Sandborn WJ (2006) Systematic review: the management of pouchitis. *Aliment Pharmacol Ther* **23:** 1087–1096.

Sandborn WJ, Tremaine WJ, Batts KP, Pemberton JH, Phillips SF (1994) Pouchitis after ileal pouch-anal anastomosis: a Pouchitis Disease Activity Index. *Mayo Clin Proc* **69:** 409–415.

Schmidt C, Häuser W, Giese T, Stallmach A (2007) Irritable pouch syndrome is associated with depressiveness and can be differentiated from pouchitis by quantification of mucosal levels of proinflammatory gene transcripts. *Inflamm Bowel Dis* **13:** 1502–1508.

Shebani KO, Stucchi AF, Fruin B, McClung JP, Gee D, Beer ER, LaMorte WW, Becker JM (2002) Pouchitis in a rat model of ileal J pouch-anal anastomosis. *Inflamm Bowel Dis* **8:** 23–34.

Welters CF, Heineman E, Thunnissen FB, van den Bogaard AE, Soeters PB, Baeten CG (2002) Effect of dietary inulin supplementation on inflammation of pouch mucosa in patients with an ileal pouch-anal anastomosis. *Dis Colon Rectum* **5:** 621–627.