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Original Article

The combination of fructooligosaccharides and resistant starch shows prebiotic additive effects in rats

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SUMMARY

Different types of dietary fiber can be distinguished considering their rate of fermentability, thus determining the location of the large intestine where they exert their beneficial effect. Their combination could be interesting to obtain health-promoting effects throughout the entire colon. The aim of the present study was to evaluate the synergistic effect of two dietary fibers with different fermentation patterns, fructooligosaccharides (FOS) (Beneo®-95) and resistant starch (Fibersol®-2), after their administration to healthy rats or in trinitrobenzenesulphonic acid-(TNBS) colitic rats, with an altered colonic immune response. In healthy rats, the administration of the combination of FOS and resistant starch induced changes in the intestinal microbiota, by increasing lactobacilli and bifidobacteria in caecum and colonic contents. Furthermore, its administration up-regulated the expression of the trefoil factor-3 and MUC-2 in comparison with untreated rats, thus improving the intestinal barrier function. The beneficial effects observed with this combination were confirmed in the TBNS model of rat colitis, since it was able to exert intestinal anti-inflammatory effect, associated with an increase of protective bacteria and up-regulation of epithelial defense mechanisms. In conclusion, the combination of two different dietary fibers may result in a synergistic prebiotic effect, and may confer greater health benefits to the host.

1. Introduction

A prebiotic is defined as a selectively fermented ingredient, mainly carbohydrates, that allows specific changes, both in the composition and/or activity in the gastrointestinal microflora that confers benefits upon host well being and health.[1] The prebiotic health effects must be demonstrated for each carbohydrate, and they will mostly depend on their chemical properties and concentration. Prebiotic carbohydrates are dietary fibers, as they are not digested by human enzymes but fermented by the flora of the large intestine. Thus, they increase biomass, feces weight, and feces frequency, having a positive effect on constipation and on the health of the mucosa of the large intestine.[2,3] In addition, the fermentation of these products produces short chain fatty acids (SCFA), mainly acetate, propionate and butyrate, which clearly contribute to their beneficial properties. These seem to be multifactorial and include the role of butyrate in modulating the colonic immune system, through interactions with the mediators and cells involved.[4]

Several types of prebiotic fibers can be distinguished considering their rate of fermentability, which depends on the carbohydrate chain length as it has been demonstrated in an *in vitro* system of fermentation, showing that short chain fructooligosaccharides (FOS) are rapidly fermented whereas long chain prebiotic, like inulin, are steadily fermented.[5] These observations have been

Abbreviations: CINC-1, cytokine-induced neutrophil chemoattractant-1; FOS, fructooligosaccharides; IBD, inflammatory bowel disease; iNOS, inducible nitric oxide synthase; ICAM-1, intercellular adhesion molecule-1; IL-1β, interleukin 1β; IL-17, interleukin 17; MCP-1, monocyte chemotactic protein-1; MPO, myeloperoxidase; SCFA, short chain fatty acids; TNBS, trinitrobenzenesulphonic acid; TFF-3, trefoil factor-3; TNFα, tumor necrosis factor α.

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confirmed *in vivo* once the different prebiotics reach the large intestine: FOS are rapidly fermented,[6] whereas resistant starch is more slowly degraded.[7] In consequence, the particular kinetics would determine the region of the intestine where the effects will be clearer. Thus, FOS would be more active in the first parts of the large bowel whereas RS would reach the distal part of the colon. In fact, Le Blay et al. have reported that administration of FOS or raw potato starch induces different changes in wet-content weights, bacterial populations and metabolites in the caecum, proximal and distal colon, as well as in feces: as compared with resistant starch, FOS doubled the pool of caecal fermentation products, like lactate, while the situation was just the opposite distally.[8] These observations confirm that each prebiotic shows particular properties, which should be considered before their application for intestinal diseases; thus, rapidly fermentable prebiotics are particularly useful in those affecting the proximal part of the large intestine, while slowly fermentable prebiotics should be chosen for more distal intestinal conditions.[9] Moreover, an association with different prebiotics with complementary kinetics should be considered when a health-promoting effect throughout the entire colon is required.

The aim of the present study was to study the potential synergistic effect in healthy rats of the combination of two prebiotics with different fermentation patterns, FOS (Beneo®-95) and resistant starch (Fibersol®-2). For this purpose, the effects on microbiota composition and activity in the different caeco-colonic compartments were characterized and compared. In order to evaluate the impact of this prebiotic combination in an intestinal condition with an altered immune response, the different prebiotics were assayed in the trinitrobenzenesulphonic acid (TNBS) model of rat colitis. This experimental model is characterized by an intestinal inflammation in the colonic segment associated with an exacerbated immune response, in which different prebiotics, like dietary fiber and lactulose, have shown beneficial effects.[10,11]

2. Material and methods

2.1. Reagents and animals

FOS (Beneo®-95) was provided by Orafti (Tienen, Belgium) and resistant starch (Fibersol®-2) was purchased from ADM, Matsutani Chemical Industry Co., Ltd (Decatur, Illinois, USA). All other chemicals were obtained from Sigma Chemical (Madrid, Spain), unless otherwise stated.

Female Wistar rats (180–200 g) were obtained from the Laboratory Animal Service of the University of Granada (Granada, Spain) and maintained in specific pathogen free conditions. They had free access of water and standard rodent food (Panlab A04 diet) provided by Panlab S.A. (Barcelona Spain). This study was carried out in accordance with the Guide for the Care and Use of Laboratory Animals, as promulgated by the National Institutes of Health, and the care and treatment protocols were approved by the Institutional Animal Care and Use Committee at the University of Granada (Granada, Spain).

2.2. Experimental design

The rats were randomly assigned to four groups: control group did not receive treatment ($n = 30$), and the treated groups ($n = 20$) were given FOS (2 g/rat/day), resistant starch (2 g/rat/day) or the mixture of both fibers (37.5% FOS and 62.5% resistant starch) (2 g/rat/day) incorporated in the drinking water. The body weight, water and food intake were recorded daily throughout the experiment, and every 4–6 days, the production of feces and their water content and consistence was evaluated, in order to assess the potential laxative effect of the prebiotics.

Two weeks after starting the experiment, ten rats of each group were sacrificed, with an overdose of halothane, to characterize the prebiotic effect exerted by the different dietary modifications in the fiber intake. The remaining animals were fasted overnight and those from the untreated control ($n = 10$) and treated groups ($n = 10$) were rendered colitic by the method described previously.[10] Briefly, they were anaesthetized with halothane and given 10 mg of TNBS dissolved in 0.25 ml of 50% ethanol (v/v) by means of Teflon cannula inserted 8 cm through the anus. Rats from the non-colitic group were administered intracolonically 0.25 ml of phosphate buffered saline instead of TNBS. After colitis induction, rats were maintained on the same dietary fiber intake for one additional week and then killed with an overdose of halothane.

2.3. Evaluation of prebiotic effect

The caecum and colonic contents were collected after sacrificing the animals and divided into aliquots to study fecal moisture, pH value and bacterial concentrations. The water content of the luminal stools was calculated by weight differences between fresh (immediately after collection) and dried (kept during 24 h at 65 °C) samples. For pH measurement, the caecum and colonic content samples were suspended in water and their pH values measured using a GLP21-21 pH-meter (Crison, Barcelona, Spain). The microbiological studies were performed on the luminal content samples, which were weighed, homogenized and serially diluted in sterile peptone water. Serial 10-fold dilutions of homogenates were plated on specific media for *Lactobacillus* (MRS media, Oxoid) or *Bifidobacterium* (MRS media supplemented with 0.5 mg/L dicloxacilin, 1 g/L LiCl and 0.5 g/L L-Cysteine hydrochloride) and incubated under anaerobic conditions in an anaerobic chamber for 24–48 h at 37 °C. After incubation, the final count of colonies was reported as $\log_{10}$ colony-forming units (CFU) per gram of material. Finally, a colonic sample was used for RNA isolation, as described below.

2.4. Evaluation of intestinal anti-inflammatory effect in the TNBS model of rat colitis

Once the rats were sacrificed, the colon was removed aseptically and placed on an ice-cold plate, longitudinally opened and the luminal contents were collected for the measurements of microbiological concentrations and short chain fatty acid (SCFA) production. Afterwards, the colonic segment was cleaned of fat and mesentery, blotted on filter paper; each specimen was weighed and its length measured under a constant load (2 g). The colon was scored for macroscopically visible damage on a 0–10 scale by two observers unaware of the treatment, according to the criteria previously reported,[11] which takes into account the extent as well as the severity of colonic damage. The colon was subsequently divided into different segments for biochemical determinations: myeloperoxidase (MPO) activity, tumor necrosis factor α (TNFα) and interleukin 1β (IL-1β) protein concentrations, as well as the expression of inducible nitric oxide synthase (iNOS). Another sample was used for RNA isolation. All biochemical measurements were completed within one week from the time of sample collection and were performed in duplicate.

MPO activity was measured according to the technique described by Krawisz et al.;[12] the results were expressed as MPO units per gram of wet tissue; one unit of MPO activity was defined as that degrading 1 μmol hydrogen peroxide/min at 25 °C. Colonic samples for TNFα and IL-1β determinations were immediately weighed, minced on an ice-cold plate and suspended (1:5 w/v) in a lysis buffer containing 20 mM HEPES (pH 7.5), 10 mM ethylene