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Original Article

Effect of kale and papaya supplementation in colitis induced by trinitrobenzenesulfonic acid in the rat

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SUMMARY

Background & aims: Papaya and kale are usual vegetables in the Brazilian diet that have antioxidant activity. This study proposed to evaluate the effect of dried vegetables as a prebiotic and as an intestinal anti-inflammatory in the rat colitis model.
Methods: Rats received, orally, 500 mg/kg of rat weight of three treatments of dried vegetables: papaya, kale and the mixture of both vegetables (60% of kale plus 40% of papaya). In the prebiotic study, after two weeks of treatment, bacteria counts were determined. In the anti-inflammatory study, after the two weeks of treatment, colitis was induced by intracolonic administration of trinitrobenzenesulfonic acid (TNBS), and one week after, damage score and biochemical parameters were evaluated.
Results: Only the administration of the mixture was able to modulate the bacterial flora in healthy rats, as well as in rats with colitis induced by TNBS. In addition, the mixture showed intestinal antiinflammatory effect in the colitic rats. This effect was evidenced by a reduction in damage score, by the colonic iNOS expression downregulated, by the decrease in the production of the TNFα and IL-1β and by the decrease in the MPO activity.
Conclusion: The combination of both vegetables showed prebiotic and anti-inflammatory effects in the TNBS model of rat colitis, when compared to each single vegetable alone.

1. Introduction

Inflammatory bowel disease (IBD) is a chronic disease of the digestive tract, and the name usually refers to two related conditions, namely, ulcerative colitis and Crohn' s disease, which are characterized by chronic and spontaneously relapsing inflammation, with the participation of different inflammatory mediators, including cytokines, eicosanoids and nitrogen and oxygen free radicals.[1] Although the etiology of IBD remains unknown, there is increasing experimental evidence to support a role for luminal bacteria in the initiation and progression of these intestinal conditions; this is probably related to an imbalance in the intestinal microflora, the relative predominance of aggressive bacteria, and an insufficient amount of protective species.[1] This could explain the remission achieved in intestinal inflammation after treatment with antibiotics such as metronidazole or ciprofloxacin[2] or the fact that germfree animals may fail to develop experimental intestinal inflammation.[3] In consequence, a possible approach to IBD therapy is to modify the intestinal microflora in these patients by the administration of probiotics. In fact, it has been reported that the administration of a mixture of Bifidobacterium and Lactobacillus[4] species or of nonpathogenic, viable *Escherichia coli*,[5] which have been proposed to act by preventing the colonization of the intestine by microbial pathogens, prolongs remission in ulcerative colitis.[5] Another possible way to modify the intestinal microflora in these intestinal conditions may be through the administration of prebiotics, defined as nondigestible food ingredients that beneficially affect the host by selectively stimulating the growth and/or the activity of limited bacteria in the colon, especially bifidobacteria.[6] In fact, different prebiotics, including dietary fiber, germinated

Abbreviations: IBD, inflammatory bowel disease; TNBS, trinitrobenzenesulphonic acid.

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barley foodstuff, and inulin, have been reported to exert beneficial effects in both human and experimental colitis.[7]

Kale (*Brassica oleracea*) is a vegetable that has been long considered to promote and maintain human health, due to its components: minerals, carotenoids, like lutein and beta-carotene, as well as vitamins B and C.[8] Papaya fruit (*Carica papaya*) is an excellent source of carotenoids, like beta-carotene, beta-zeacarotene, zea-carotene, among others[9] and ascorbic acid.

Given the described chemical composition of kale and papaya, they may constitute attractive sources of dietary fiber and other components to be used as a complementary treatment in IBD. The aim of the present study was to test the preventative effects of these two vegetables usually ingested in Brazilian diet in the trinitrobenzenesulphonic acid (TNBS) model of rat colitis, a well-established model of intestinal inflammation with some resemblance to human IBD.[10] Special attention was paid to its effects on the production of some of the mediators involved in the inflammatory response, such as tumor necrosis alpha (TNFα), interleukin-1β (IL-1β), glutathione content, myeloperoxidase (MPO) activity, nitric oxide synthase (iNOS) and cyclooxygenase-2 expression (COX-2).

2. Materials and methods

This study was carried out in accordance with the 'Guide for the Care and Use of Laboratory Animals', as promulgated by the National Institute of Health, and was approved by the Animal Research and Ethic Committee of the University of Granada (Spain).

2.1. Reagents and animals

All chemicals were purchased from Sigma (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 under standard conditions, with free access to standard food for rodents and tap water.

2.2. Preparation and administration of the plant material

Organic papaya and kale was purchased from Yamaguishi (Campinas, Brazil). Papaya was peeled and its seeds were withdrawn. The papaya pulp without seeds and kale leaves were dehydrated in an oven at 40 °C for 2 days, and then was powdered.

2.3. Evaluation of the prebiotic activity of kale and papaya in healthy rats

Female Wistar rats were randomly assigned to four groups ($n = 8$); one of them (non-treated group) received no treatment and the others received, orally, daily for 2 weeks, 500 mg/kg per rat weight (suspended in 2 ml of distilled water) of kale, papaya or a mix of 60% kale plus 40% papaya. During the treatments, parameters as body weight and food intake were periodically measured. After the treatment period, the rats were sacrificed with an overdose of halothane, the caecum and colon were removed aseptically and placed on an ice cold plate, and longitudinally opened; and then the luminal contents were collected for microbiological studies. The colon was subsequently divided into three segments for biochemical determinations.

2.4. Evaluation of the intestinal anti-inflammatory effect of kale and papaya in TNBS rat colitis

Female Wistar rats were randomly assigned to five groups ($n = 8$); two of them (non-colitic and control groups) received no treatment and the others received, orally, daily for 3 weeks, 500 mg/kg per rat weight (suspended in 2 ml of distilled water) of kale, papaya or a mix of 60% kale plus 40% papaya. Both non-colitic and TNBS control groups were given daily 2 ml of distilled water. Two weeks after the treatment was started, the rats were fasted overnight and those from the control and treated groups were rendered colitic by the method originally described by Morris et al.[11] 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 a Teflon cannula inserted 8 cm through the anus. Rats from the non-colitic group were administered intracolonically with 0.25 ml of phosphate-buffered saline instead of TNBS. Body weight and food intake were recorded daily throughout the experiment. All rats were killed with an overdose of halothane 1 week after induction of colitis, and the colon was removed aseptically and placed on an ice cold plate. It was longitudinally opened; then the luminal contents were collected for microbiological studies (see below). Afterwards, the colonic segment was cleaned of fat and mesentery, blotted on filter paper; each specimen was weighed and its length measured macroscopically visible damage on a 0–10 scale by two observers who were unaware of the treatment, according to the criteria described previously,[12] which take into account the extent as well as the severity of colonic damage. Representative whole gut specimens were taken from a region of the inflamed colon corresponding to the adjacent segment to the gross macroscopic damage and were fixed in 4% buffered formaldehyde. Cross-sections were selected and embedded in paraffin. Equivalent colonic segments were also obtained from the non-colitic group. Full-thickness sections of 5 μm were taken at different levels and stained with hematoxylin and eosin. The histological damage was evaluated on a 0–59 scale by a pathologist observer (Ana Nieto), who was blinded to the experimental groups, according to the criteria described previously by Stucchi et al. (Table 1) with modifications.[13] The colon was subsequently divided into segments for biochemical determinations. MPO, total glutathione content, iNOS and COX-2 expression, as well as TNFα and IL-1β levels were determined. All biochemical measurements were performed in duplicate and completed within 1 week of sample collection.

MPO activity was measured according to the technique described by Krawisz[14]; the results were expressed as MPO units per gram of wet tissue; 1 unit of MPO activity was defined as that degrading 1 μmol hydrogen peroxide/min at 25 °C. Total glutathione content

Table 1
Scoring criteria of full-thickness distal colon sections.

Mucosal epithelium and lamina propria
Ulceration: none (0); mild surface (0–25%) (1); moderate (25–50%) (2); severe (50–75%) (3); extensive-full thickness (more than 75%) (4).
Polymorphonuclear cell infiltrate
Mononuclear cell infiltrate and fibrosis
Edema and dilation of lacteals
Crypts
Mitotic activity: lower third (0); mild mid third (1); moderate mid third (2); upper third (3)
Dilations
Goblet cell depletion
Submucosa
Polymorphonuclear cell infiltrate
Mononuclear cell infiltrate
Edema
Vascularity
Muscular layer
Polymorphonuclear cell infiltrate
Mononuclear cell infiltrate
Edema
Infiltration in the serosa

Scoring scale: 0, none; 1 slight; 2, mild; 3, moderate; 4, severe. Maximum score: 59.