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Research Article

Effects of Sulfasalazine, Lactobacillus Plantarum (Lp-115) and Fish Oil in Experimental Colitis

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Abstract

Ulcerative Colitis (UC) is a chronic inflammatory disease that affects the gastrointestinal tract, and studies have shown the association of inflammation and oxidative stress on its occurrence. The use of immunomodulatory nutrients and probiotics has resulted in remission phase prolongation and are considered a promising strategy for prevention and treatment of UC. The long chain n-3 fatty acids from fish oil have also shown favorable results in UC decreasing symptoms. This study aimed to evaluate n-3 fatty acids from fish oil and/or the probiotic Lactobacillus plantarum (Lp-115), combined with sulfasalazine, at inflammatory markers in Trinitro-Benzene Sulfonic Acid (TNBS)-induced colitis in male Wistar rats. The study lasted 21 days. Nutritional intervention was performed during all experiment and colitis induction was performed in the 14th day. The rats were divided into five groups: (A), control group; (B), sulfasalazine; (C), sulfasalazine with Lactobacillus plantarum fermented milk; (D), sulfasalazine with n-3 fatty acids and (E) sulfasalazine, n-3 fatty acids and Lactobacillus plantarum. All groups received water and food ad libitum and environmental conditions were controlled. Tissue samples were collected for Myeloperoxidase (MPO) determination. A Kruskal-Wallis test with Dunn's post-test was applied to determine differences at level of 5 % significance ($p<0.05$) between groups. The results were demonstrated by reduction of tissue levels of myeloperoxidase. Beneficial effects were seen with the use of sulfasalazine with probiotic, but sulfasalazine association with n-3 fatty acids obtained results that were more favorable.

Introduction

Inflammatory bowel disease (IBD) is characterized by periods of remission and reactivation and Crohn's Disease (CD) and Ulcerative Colitis (UC) are the principal forms of presentation. Its etiology is multi factorial and complex: the immune system is involved, as well as genetic, environmental, and intestinal micro biota factors [1].

Treatment consists in drugs to heal the acute phase and then maintain remission of the disease; it comprises aminosalicylates, corticosteroids and immunosuppressant's [2]. Sulfasalazine is effective in treating mild to moderate ulcerative colitis and Meta-analyzes has demonstrated their effectiveness [3].

Therapeutic options, especially in UC, have the objective of increasing the effectiveness of selective blockade of inflammatory mediators and to increase innate immunity [4]. Nutritional therapy has shown to be an auxiliary therapeutic measure in cases of poor clinical progress at different stages of disease activity [5].

UC treatment with immunomodulator nutrients is a therapeutic modality that has shown promising perspectives. Although acting through different pathophysiological mechanisms, probiotics and n-3 polyunsaturated fatty acids from fish oil has been highlighted for this proposal [6]. Once these nutrients work by modulating immuno-inflammatory response, there is maintenance of intestinal mucosa integrity, improving clinical and nutritional status of patients [6].

Among the markers of inflammation, a widely used to assess neutrophil infiltration of tissues is the activity of Myeloperoxidase (MPO) [7]. MPO is also an inflammatory marker frequently used in the experimental colitis model, because it is an important enzyme found in the neutrophils granules and its increase is involved in tissue damage [8]. Thus, the higher activity of this enzyme is related to higher inflammation [9,10].

Therefore, this study aimed to evaluate n-3 fatty acids from fish oil and/or the probiotic Lactobacillus plantarum (Lp-115) combined with sulfasalazine, at MPO on Trinitro-Benzene Sulfonic acid (TNBS)-induced colitis in male Wistar rats.

Material and Methods

The study was approved by the Ethics Committee in Animal Experimentation of the Londrina University Hospital. It was used 50 Wistar rats, males, weaned, from Central Animal Facility of

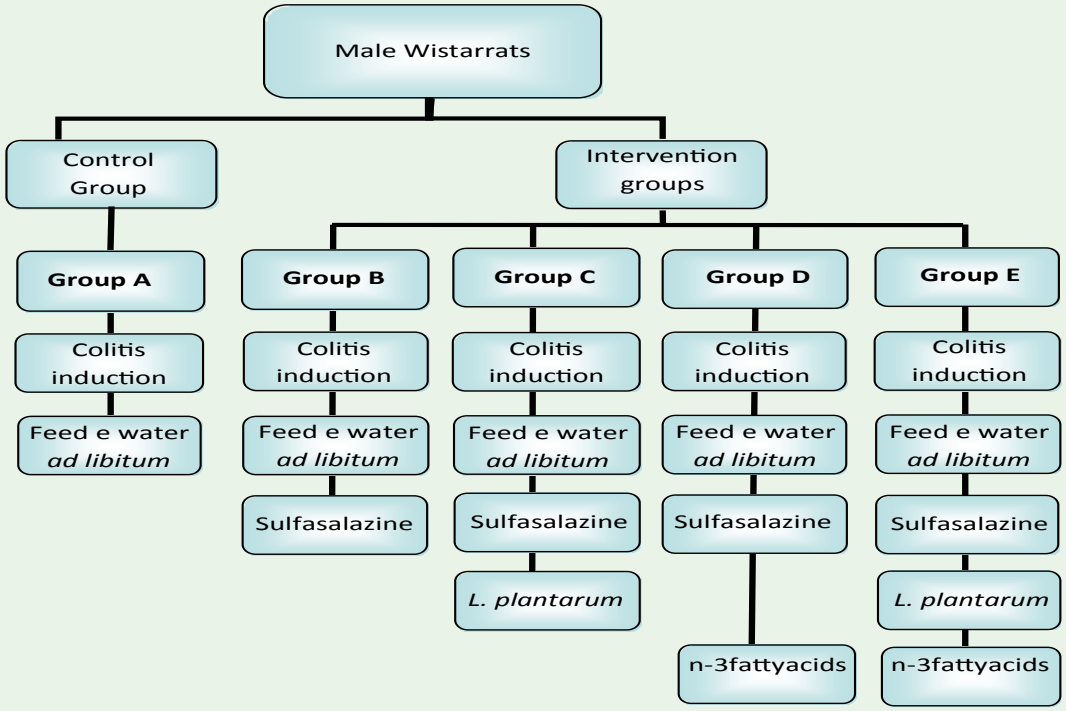

Figure 1: Changes in Mean Facial (± S.E.) Width-to-Height Ratios of Major League Baseball Players Active 1870-1989.

the Paraná Institute of Technology (Tecpar) Curitiba / PR / BR. The animals were maintained twenty days with water and normoprotein commercial feed (brand Nuvilab CRL®) and libitum in order to adapt themselves with the coop, respecting the light-dark cycle of 12/12 hours, with temperature of 22 ± 2 °C and humidity of 55% ± 10.

The study lasted 21 days. Nutritional intervention was performed during all experiment and colitis induction was performed in the 14th day.

The rats, weighing 180 to 230 grams, were submitted to colitis induction and the procedure was performed using 0.5 ml of TNBS at a concentration of 30 mg / ml in 50% ethanol by intra colonic instillation of a probe with 2 mm diameter, kept for 60 seconds in the Trendelenburg position.

For the procedure, the rats were anesthetized with 0.1 ml xylazine Anased an® (2% xylazine) and 0.1 ml of ketamine Dopalen® (10% ketamine) applied intramuscularly [11-14].

All animals were fed and received water ad libitum, and were randomly distributed in five groups (A, B, C, D and E). The group A was considered as positive control group (PC) and did not receive therapeutic intervention. The B, C, D and E groups were submitted to therapy with sulfasalazine, while group C, received also L. plantarum(LP-115) fermented milk, group D received n-3 fatty acids from fish oil and group E received L. plantarum(LP-115) and n-3 fatty acids (Figure 1).

Therapeutic interventions

For gavage therapeutic interventions, a seven centimeters long syringe was used for the administration of exact doses for all animals, and daily, respecting the same time, the animals were previously anesthetized with sulfuric ether inhalation [13,14].

Except for the control group, all animals daily-received sulfasalazine by gavage, purchased from Sigma®, in powder form. Therefore, each animal took a dose of 100mg / kg [15], adopting the average of 25mg / animal, diluted in sterile saline (0.9%).

The animals in groups D and E received 1g / kg body weight n-3 fatty acids derived from fish oil13 averaging 250 mg. The fish oil (Sundown Naturals®) was purchased at local retail.

The rats from groups C and E, received Lactobacillus plantarum (Lp-115) (Danisco®) prepared according Costa et al., (2011) [16] and Barreto et al., (2014) [17], with viable bacteria in the range 108-109 CFU / g of the final product.

After three weeks treatment with sulfasalazine, L. plantarum (LP-115) and n-3 fatty acids of fish oil, the animals were anesthetized for obtaining the samples. The analysis was equally carried out in the five groups and all animals were sacrificed with cardiac puncture (exsanguination). This procedure was performed after intramuscular administration of ketamine and xylazine (0.15 ml each, corresponding to 10 mg / kg).

Obtaining the colonic mucosa and determination of myeloperoxidase activity

On the 7th day after the induction of colitis the rats were anesthetized and the colon was rapidly excised, approximately 10 cm proximal to the anus, slit longitudinally and gently rinsed with 10 mm, pH 7.4, sodium phosphate buffer (SPB). Then, the segment was placed on a flat glass plate with the mucosal surface up. Soon after, the mucosa was scraped off with a microscope slide onto a cold Petri dish and all mucosa specimens were homogenized in SPB (5 ml) using a mechanical PotterElvejhem. After that, they were quickly

immersed in dry ice and kept in freezer at -70º C. Myeloperoxidase (MPO) activity in colonic mucosa was determined using the method originally described by Bradley et al., (1982) [18] and modified by Nieto et al., (1998) [13]. One unit of activity was defined as the amount of enzyme present that produces a change in absorbance per minute of 1.0 at 37º C in the final reaction volume containing the sodium acetate. The results were expressed in units per 100 mg of protein (U/100 mg of protein).

Statistical analysis

The results were expressed in U/100 mg of protein. Data were treated as median and inter quartile (25% -75%) and analyzed by Kruskal-Wallis test with subsequent Dunn test.

Results

We began the study with 50 rats, 10 animals in each group; three animals died during the experiment; one in the second group (sulfasalazine), one in the fourth group (sulfasalazine and fish oil) and one in the fifth group (sulfasalazine, fish oil and probiotic). Thus, the final n in each group was as follows: Group 1 (controls - 10 rats); Group 2 (sulfasalazine - 9 rats); Group 3 (L plantarum - 10 rats); Group 4 (sulfasalazine and fish oil - 9 rats) and Group 5 (sulfasalazine, fish oil and L plantarum - 9 rats).

The results of the study are shown in (Table 1). There was a statistically significant decrease in MPO activity in groups C ($p<0.01$) and D ($p<0.0001$) in relation to group A and significant decrease in group D ($p<0.05$) compared to group B. Groups A and B did not show any significant difference as well as there were no differences between groups C, D and E.

Discussion

It was observed a significant reduction of myeloperoxidase levels in the adjunct treatments compared to the treatment with sulfasalazine only. Although probiotc has demonstrated a beneficial effect, treatment with sulfasalazine and fish oil showed even better results. Several studies have emphasized the benefits of immunonutrition with n-3 fatty acids in many clinical situations. Campos et al., (2002) [19] verified the results of parenteral administration of different lipid emulsions in acute colitis in male Wistar rats. The authors concluded that while the emulsions containing long chain triglycerides with low ratio of n-3 / n-6 did not alter inflammatory colitis, the association of medium chain triglycerides and long-chain fish oil established beneficial impact regarding inflammation and especially MPO levels.

Similarly, Nieto et al., (1998) [13] performed a study with TNBS to induce experimental colitis in mice. After induction, the animals were submitted to different diets; the main component in each was olive oil, fish oil or purified brain phospholipids pork. The authors concluded that n-3 fatty acids can act beneficially when administered to prevent inflammation in UC.

In humans, our group verified that treatment with sulfasalazine (2g / day) was superior to the use of n-3 fatty acids of fish oil (5.4g / day - 18 capsules) in patients with mild to moderate UC [20]. Thereafter, it was found that association of n-3 fatty acids (4.5 g / day - 15 capsules) and sulfasalazine resulted in a decrease in oxidative stress and an increase plasma antioxidant capacity, which did not occur with the isolated use of sulfasalazine [21].

Regarding treatment with probiotics in the control of inflammation, several studies have shown satisfactory results. Gionchetti et al., (2000) [22] evaluated 40 patients with pouchitis, an inflammation that may occur after ileoanal anastomosis, which is performed in UC patients in order to cure the disease. To half of them were given a mixture of probiotics containing three strains of bifid bacterium, four strains of Lactobacillus and Streptococcus thermopiles, while the other half was treated with placebo. All patients in the latter group reactivated pouchitis, while in the group receiving the mixture of probiotics, only 15% reactivated, demonstrating the importance of nutrition therapy with probiotics, especially for patients with UC who underwent pouchitis.

Peran et al., (2007) [14] reported a decrease in the production of mediators involved in the intestinal inflammatory response, such as TNF-α (tumor necrosis factor), in TNBS induced colitis using L. salivariusssp; TNF-α acts as a potent inducer of chemo taxis, contributing to neutrophil recruitment in the colon and starting the inflammatory cascade and its inhibition by probiotic contributed to the improvement of colonic inflammation. The same authors found an improvement in the inflammatory response as compared to the group without treatment with probiotics, due to an anti-inflammatory effect demonstrated by the significant reduction in inflammation and colonic necrosis extension.

The combination of L. acidophilus ($3{,}33x10^6$UFC / g), B. bifidum ($3{,}33x10^6$UFC / g) E. faecium ($3{,}33x10^6$UFC / g) and Probiotic Vetnil® was rectally administered in rats with induced colitis in a study [23]. Probiotics combination enhanced the mucosal trophism, resulting in beneficial effects for treatment, as it has increased the metabolic change between the tissues, improving nutrition.

Lactobacillus plantarum increased IL-10 synthesis, secretion of macrophages and T-cells derived from inflamed colon through immunomodulation in vitro on extracted wounded cells of human colon [24]. In addition, the Lactobacillus plantarum species was

Table 1: Myeloperoxidase – MPO comparison of therapeutic intervention.

Group	Treatment	Median U/100 mg of protein	Interquartile U/100 mg of protein	Statistical Significance
A	Colitis control	73.85	(68.78 – 85.81)	
B	Colitis + Sulfasalazine	68.50	(58.99 – 108.3)	
C	Colitis +Sulfa+*L.plantarum*	60.16	(58.24 – 62.83)	Compared to Group A $p<0.01$
D	Colitis +Sulfa+Fish oil	55.72	(47.45 – 63.33)	Compared to Group A $p<0.0001$ Compared to Group B $p<0.05$
E	Colitis +Sulfa+*L. plantarum*+ Fishoil	65.10	(63.52 – 69.99)	

the first of its kind to have its genome sequenced and studies have attributed to L. plantarum ability to modulate the immune system [25], reduce the risk of cardiovascular disease [26], mitigate intestinal disorders [27] antimicrobial activity against pathogens (Helicobacter pylori, Listeria monocytogenes, Clostridium difficile) [28], among other health benefits as the production of Short Chain Fatty Acids (SCFA) that inhibit carcinogenic products by reducing the enzyme activity [29,30].

In conclusion, beneficial effects were seen with the use of sulfasalazine with probiotic, but sulfasalazine association with n-3 fatty acids obtained more favorable results.

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