Dietary Vitamin E Supplementation Protects the Rat Large Intestine from Experimental Inflammation

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Abstract: Vitamin E, the most potent antioxidant in the lipid phase, was tested for antiinflammatory activity in trinitrobenzenesulfonic acid-induced rat colitis. Rats were fed a nonpurified diet (saline and control groups) or a vitamin E supplemented diet (treated group, 300 mg/kg nonpurified diet). Vitamin E supplementation, which resulted in increased colonic vitamin E levels, reduced colonic weight and damage score, prevented lipid peroxidation and diarrhea, reduced interleukin-1β levels and preserved glutathione reductase activity and total glutathione levels. However, it did not modify myeloperoxidase levels, which are indicative of neutrophil infiltration in the inflamed colon. Vitamin E protects the rat colon from oxidative stress associated with inflammation.

Key words: Vitamin E, trinitrobenzenesulfonic acid, rat colitis, colonic oxidative stress

Introduction

Inflammatory bowel disease (IBD) refers to two major clinical conditions: Crohn’s disease and ulcerative colitis. The inflammatory response in the intestine is associated with significant oxidative stress both in IBD [1] and in experimental models of colitis [2–5], due to the extensive release of highly reactive oxygen and nitrogen species. Oxidative stress may be one of the most important components in the pathophysiology of IBD [1, 6]. In fact, these reactive substances may play a role in the upregulation of the inflammatory sequence of events that take place in the inflamed intestine via activation of NF-kB, an expression factor involved in the regulation of several cytokines, and stimulation of the expression of intercellular adhesion molecule-1 (ICAM-1) and P-selectin [7, 8].

Considering this, the use of antioxidant compounds may be useful in limiting the damage in IBD. In fact, antioxidant therapy has shown beneficial effects in several experimental models of rat colitis [3, 4, 9, 10]. Furthermore, it has been proposed that antioxidant activity may be responsible for the beneficial effects showed by sulfasalazine and other salicylates in human IBD [11]. Vitamin E has two qualities that make it a good candidate for IBD therapy: first, it is a powerful antioxidant and certainly the most important one in the lipid phase, i.e. in biological membranes [12], and second, it has a generally safe side effect profile [13]. In the present study we establish the therapeutic effect of vitamin E dietary supplementation on experimental colitis in rats.

Materials and Methods

This study was carried out in accordance with the European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes, as adopted by the Council of Europe. Female Wistar rats (180–220 g) obtained from the Laboratory Animal Service of the University of Granada were used, housed in makrolon cages and maintained in air-conditioned animal quarters with a 12 h light-dark cycle. Animals had free access to tap water and food.

Materials and Reagents. All reagents, including trinitrobenzenesulfonic acid (TNBS), were obtained from Sigma (Madrid, Spain), except glutathione reductase (Boehringer Mannheim, Barcelona, Spain). All diets were provided by Panlab (Barcelona, Spain). The vitamin E-supplemented diet was prepared by the manufacturer by adding 300 mg of dl-α-tocopherol per kg of standard diet Panlab A04. The composition of the control diet was: 17.2% protein, 2.7% fat, 59.7% carbohydrates, 3.95% fiber, 4.4% minerals, and 12% humidity. Total energy was 3100 kcal/kg. The vitamin E content of the control diet was 30 mg/kg.

Induction of colitis. Colitis was induced by the method originally described by Morris et al with minor modifications [14]. Animals were fasted overnight and anesthetized with diethyl ether. Under these conditions, a single intracolonic dose of 10 mg TNBS dissolved in 0.25 mL of 50% ethanol (v/v) was administered. TNBS-induced colonic damage is dose-dependent and the dose used was originally described to elicit a response qualitatively identical to the maximal dose of 30 mg, albeit quantitatively lower [14]. Rats in the saline control group received 0.25 mL of phosphate buffered saline.

Experimental design. Rats were randomly assigned to 3 groups, which received nonpurified diet (control and TNBS groups) or vitamin E supplemented diet (treated group) for 4 weeks. At the end of this period, 7 rats from the control and treated groups were killed to measure vitamin E levels in colon. All the remaining animals from the treated group and all of the TNBS group were rendered colitic by TNBS administration. Animals from all the groups were killed by a diethyl ether overdose at 1, 2, 3 and 4 weeks after colitis induction. The body weight and total food intake for each group were recorded daily throughout the experiment.

Assessment of colonic damage. The colon was removed and placed on an ice-cold plate, cleaned of fat and mesentery, and blotted on filter paper. Each specimen was weighed and its length measured under a constant weight (2 g). The large intestine was longitudinally opened and scored by two blinded observers according to the following criterion: (0) no macroscopic change; (1) hyperemia and/or bowel wall thickening without fibrosis; (2) one or more ulcers with possible fibrosis; (3) a large ulcerated area in the process of healing (cobblestone appearance); (4) a large ulcerated area with extended necrosis of the mucosa. The colon was subsequently divided longitudinally in 6 pieces for biochemical determinations. The fragments were immediately frozen at –30°C, except for the sample used for glutathione content determination, which was immediately weighed and then frozen in 1 mL of 5% trichloroacetic acid.

Biochemical assays. All biochemical measurements were completed within 2 weeks of the time of sample collection and were performed in duplicate. Myeloperoxidase (MPO) activity was measured according to the technique described by Krawisz et al [15] and the results were expressed as MPO units per gram of wet tissue. One unit of MPO was defined as that degrading 1 µmol/minute of hydrogen peroxide at 25°C.

Total glutathione content was measured using the recycling assay described by Anderson [16] and results were expressed as nmol per gram wet tissue. Reduced glutathione levels were assayed by the glyoxalase I method [17] and the difference with total glutathione content considered as oxidized glutathione. For determination of glutathione-related enzymes, the colonic samples were thawed, diluted 1:6 (w/v) in ice-cold 0.1 M potassium phosphate buffer (pH = 7.0) containing 1 mM ethylenediamine tetraacetic acid (EDTA) and 0.1 g/L digitonin, minced, homogenized and centrifuged at 7000 g for 15 min at 4°C. A suitable sample (50 µg of protein) was immediately used for the enzyme assays. Glutathione peroxidase activity was determined according to the method of Fiohe and Gunzler [18] and expressed as nmol of oxidized glutathione reduced per minute per mg protein (i.e. mU/mg protein). Glutathione transferase activity was measured by the method described by Warholm et al [19] and expressed as nmol of reduced glutathione consumed per minute per mg protein (i.e. mU/mg protein). Glutathione reductase activity was determined as described by Acedo et al. [20] and the results are expressed as nmol NADPH oxidised per min per mg protein (i.e. mU/mg protein). The protein content was determined by the method of Bradford [21].

The determination of malondialdehyde (MDA) levels was performed in colonic membrane enriched fractions obtained according to the technique previously described by De la Cruz et al [22]: the samples were minced and diluted 1:10 (w/v) in buffer containing 50 mM TRIS, 0.32...
M sucrose, 0.1 mM NaCl, 0.5 mM KCl, 0.55 mM CaCl₂, 1 mM MgSO₄ and 0.55 mM KH₂PO₄ (pH = 7.4). The homogenate was cleared at 3000 g for 10 min at 4°C and the supernatant centrifuged at 35000 g for 30 min at 4°C. The resulting pellet was resuspended in 50 mM TRIS (pH = 7.4), and kept at −80°C for no more than 2 weeks until assay. MDA was determined as thiobarbituric acid-reacting substances (TBARS) and expressed as nmol of MDA per mg protein.

Levels of α-tocopherol in colonic tissue were measured by HPLC [23]. The samples (~300 mg) were diluted 1:3 (w/v) in distilled water at 4°C and homogenized. The homogenate was mixed with 1 mL ethanol and vortexed. The mixture was allowed to stand for 10 min and then 500 µl of hexane were added. The mix was vortexed for 1 min and the organic and aqueous phases separated by centrifugation at 3000 g for 5 min at 4°C. Finally, 450 µl of the organic phase were collected and analyzed by HPLC using a Merck RP-18 column and methanol:acetonitrile:chloroform (45:45:10) as the mobile phase, with a flux rate of 1.5 mL/min.

The levels of interleukin-1β in the colonic tissue 1 week after the TNBS challenge were determined by immunoblotting. Briefly, samples were homogenized (1:3 w/v) in phosphate buffered saline supplemented with 0.1% sodium dodecyl sulfate, 0.1% sodium deoxycholate, 1% Triton X-100 and protease inhibitors (aprotinin, 1,10-phenanthroline, phenylmethylsulfonylfluoride, iodoacetamide). After protein concentration was measured by the bicinchoninic acid method [24] and the homogenates were boiled for 4 min in Laemli buffer, 100 µg of protein were separated by 15% SDS-PAGE, transferred to nitrocellulose membranes, and immunoblotted with a rabbit monoclonal antibody (sc-7884, Santa Cruz Biotechnology, Heidelberg, Germany). Culture medium of the human monocytic cell line THP-1 stimulated with lipopolysaccharide (NEN Life Science Products, Zaventem, Belgium) was used as a positive control. The bands were detected by enhanced chemiluminescence (NEN Life Science Products, Zaventem, Belgium).

Statistical analysis. Results are expressed as mean ± SEM. One way analysis of variance, followed by least significance tests, on preselected pairs was used to compare means of multiple groups, while unpaired two-tailed Student’s t-tests were applied in the case of group pairs. Non-parametric data (score) are expressed as median (range) and were analyzed with the Mann-Whitney U test. Differences among proportions were analyzed with the χ² (chi-square) test. All statistical analyses were carried out with the Statgraphics 5.0 software package (STSC, Maryland), with statistical significance set at p < 0.05.

Results

Vitamin E supplementation. In the course of the 4-week period prior to TNBS administration, a higher food intake was observed in the rats receiving the vitamin E-supplemented diet than in the control group (20.3 ± 0.4 g vs. 17.7 ± 0.4 g/rat · day, p < 0.001). This resulted in greater body weight gain (37.9 ± 1.2% vs. 30.0 ± 0.9%, p < 0.001). No signs of diarrhea or other morbidity were noted in any of the rats. By the end of this period, i.e. at the time of colitis induction, levels of α-tocopherol were greater in the treated group in the colonic tissue (1.35 ± 0.18 vs. 0.44 ± 0.08 µg/g, p < 0.01).

TNBS-induced chronic colitis. The rats that received a TNBS/ethanol enema showed early signs of disease such as colonic hemorrhage (bloody feces), diarrhea, anorexia and loss of weight. Thus, rats of the TNBS group lost 7.4 ± 0.6% of their body weight in the first 3 days after TNBS administration, whereas those of the control group gained 0.2 ± 0.3% (p < 0.001). This was due to the difference in food intake, which was 7.7 ± 1.8 g/rat-day vs. 18.1 g/rat-day for the TNBS and control groups, respectively (p < 0.05). These differences were progressively reduced thereafter and vanished by 1 week post-TNBS. Most of the rats of the TNBS group showed signs of diarrhea at 1 week, but these were greatly diminished at 2 weeks and completely disappeared thereafter (Table I).

Table I: Effect of vitamin E dietary supplementation (300 mg/kg) on the incidence of diarrhea, damage score, colonic weight/length in TNBS chronic colitis.

<table>
<thead>
<tr>
<th>Group</th>
<th>Incidence of diarrhea (%)</th>
<th>Damage score (0–4)</th>
<th>Colonic weight/length (mg/cm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0</td>
<td>0</td>
<td>77.1 ± 0.7</td>
</tr>
<tr>
<td>One week</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TNBS</td>
<td>85.7</td>
<td>4 (3–4)</td>
<td>224.0 ± 22.5*</td>
</tr>
<tr>
<td>Vitamin E</td>
<td>50.0*</td>
<td>3 (1–4)*</td>
<td>148.8 ± 5.7***</td>
</tr>
<tr>
<td>Two weeks</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TNBS</td>
<td>42.9</td>
<td>2 (2–3)</td>
<td>119.4 ± 6.4*</td>
</tr>
<tr>
<td>Vitamin E</td>
<td>28.6</td>
<td>2 (1–2)</td>
<td>114.2 ± 30.3*</td>
</tr>
<tr>
<td>Three weeks</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TNBS</td>
<td>0</td>
<td>2 (1–3)</td>
<td>108.8 ± 2.4*</td>
</tr>
<tr>
<td>Vitamin E</td>
<td>0</td>
<td>2 (0–4)</td>
<td>110.1 ± 3.1*</td>
</tr>
<tr>
<td>Four weeks</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TNBS</td>
<td>0</td>
<td>2 (2–2)</td>
<td>94.6 ± 2.3</td>
</tr>
<tr>
<td>Vitamin E</td>
<td>0</td>
<td>2 (0–2)</td>
<td>87.9 ± 1.5</td>
</tr>
</tbody>
</table>

Incidence of diarrhea is expressed in percentage. Score data are expressed as median (range). Colonic weight/length ratio is expressed as mean ± SEM. * p < 0.05 vs. control group; ** p < 0.05, *** p < 0.01 vs. TNBS group.
ing 3–4 cm of the distal colon, with hyperemia, bowel wall thickening (Table I), fibrosis, and multiple adhesions to adjacent organs. The colon was regularly shortened and occasionally affected by stenosis. In addition, the colonic mucosa was necrotic, appearing typically as a firmly attached dark layer which tended to detach spontaneously over time. This histological feature was formerly described by Bell et al [25] as an unstainable acellular layer still retaining gross mucosal architecture and referred to as “pseudomembrane”. TNBS-induced colonic damage decreased gradually thereafter, so that at 3 weeks only residual fibrosis remained. This is in agreement with the original description of the model [14], in that colonic lesions do not heal completely but lead to fibrosis and scarring of the tissue.

Colonic MPO activity, a marker of neutrophil infiltration, was significantly increased as a consequence of inflammation (Table II). This effect peaked at 1 week (30-fold increase from baseline) and diminished with time, being absent at 3 weeks. The oxidative status of the colon was evaluated by the total glutathione levels and the basal concentration of MDA. TNBS colitis was characterized by a 56% decrease in colonic glutathione concentration at 1 week, but no further change was observed from that time point on (Table II). No significant differences were observed in the reduced/oxidized glutathione ratio between colitic and non-colitic rats (6.2 ± 1.8 vs. 4.2 ± 1.2, respectively, p > 0.1) at one week. MDA concentration was also increased 2-fold during the first week of colitis compared to the control group (0.64 ± 0.23 vs. 0.34 ± 0.22 nmol/mg protein respectively, p < 0.05). The effect of chronic colitis on glutathione depletion was investigated further by determining the colonic activities of glutathione-related enzymes. Glutathione reductase activity was severely inhibited at 1 week (54%), (Table II), but it was normalized thereafter. No significant changes in either glutathione peroxidase or glutathione transferase activity were observed at any time point (data not shown). Interleukin-1β levels were markedly increased 1 week after TNBS administration (Fig. 1).

Table II: Effect of vitamin E dietary supplementation (300 mg/kg) on myeloperoxidase (MPO) activity, colonic glutathione content and glutathione reductase (GRase) activity in TNBS chronic colitis.

<table>
<thead>
<tr>
<th>Group</th>
<th>MPO (U/g)</th>
<th>Glutathione (nmol/g)</th>
<th>GRase (mU/mg prot)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>6.2 ± 0.6</td>
<td>1950 ± 32</td>
<td>102.2 ± 3.3</td>
</tr>
<tr>
<td>One week</td>
<td>TNBS</td>
<td>188.7 ± 11.5*</td>
<td>890 ± 109*</td>
</tr>
<tr>
<td></td>
<td>Vitamin E</td>
<td>166.4 ± 14.1*</td>
<td>1628 ± 113*</td>
</tr>
<tr>
<td>Two weeks</td>
<td>TNBS</td>
<td>96.6 ± 20.2*</td>
<td>1859 ± 132</td>
</tr>
<tr>
<td></td>
<td>Vitamin E</td>
<td>70.4 ± 8.5*</td>
<td>1964 ± 64</td>
</tr>
<tr>
<td>Three weeks</td>
<td>TNBS</td>
<td>18.8 ± 1.0</td>
<td>2097 ± 71</td>
</tr>
<tr>
<td></td>
<td>Vitamin E</td>
<td>11.4 ± 4.9</td>
<td>2259 ± 16</td>
</tr>
<tr>
<td>Four weeks</td>
<td>TNBS</td>
<td>21.8 ± 2.5</td>
<td>2026 ± 81</td>
</tr>
<tr>
<td></td>
<td>Vitamin E</td>
<td>6.5 ± 1.0</td>
<td>2167 ± 71</td>
</tr>
</tbody>
</table>

Data are expressed as mean ± SEM. *p < 0.05 vs. control group; *p < 0.05 vs. TNBS group.

Figure 1: Immunoblot of colonic samples separated by 15% SDS-PAGE. +: positive control (see Materials and methods). C: control group. T: TNBS group. E: vitamin E supplemented group. Std: electrophoresis standard (the band shown crossreacts with the interleukin-1β antibody).
Effect of vitamin E supplementation on TNBS chronic colitis. Rats subjected to dietary vitamin E supplementation showed an overall lower impact of TNBS-induced colonic damage at 1 week, as evidenced by a decrease, compared to the TNBS group, in the incidence of diarrhea, colonic weight and damage score (Table I). Furthermore, vitamin E treatment completely suppressed glutathione depletion, without showing any modification in the reduced/oxidized glutathione ratio (5.1 ± 0.9), normalized MDA levels (0.42 ± 0.20 nmol/mg protein) and prevented glutathione reductase inhibition (Table II). In addition, it reduced colonic interleukin-1β levels (Fig. 1). Nevertheless, MPO activity was unaffected (Table II). No effect on the incidence of adhesions, food intake or body weight were observed (not shown), and no effect whatsoever was apparent at the second week, at which time the colitis status was almost normalized in the TNBS group.

Discussion

The involvement of free radicals in inflammatory injury in general, and in IBD in particular, is well-known [7, 11]. These agents have critical cytotoxic effects on invading microorganisms (bacteria, protozoa), but also on neighboring tissue that suffers injury as an innocent bystander. In addition, these reactive species have been shown to directly upregulate the inflammatory response mainly via activation of NF-κB, a regulatory transcription factor linked to the synthesis of several cytokines and adhesion molecules such as P-selectin [7, 8]. However, when inflammation extends in time (i.e. when it becomes chronic), free radicals only exert a largely deleterious effect on the affected organ, and therefore free radical formation should be limited.

Vitamin E has a unique profile as a candidate compound for the treatment of IBD, because: first, it is a powerful free radical scavenger [12], and particularly the most important one in biological membranes; and second, it is practically devoid of adverse effects even when pharmacological doses are used [13]. Intestinal oxidative stress has been demonstrated in IBD, although it is not clear whether it acts as a causative agent in this condition or if it simply represents a product of inflammatory injury [26]. Considering the above, we used a well-established animal model of intestinal inflammation to test the putative beneficial effect of α-tocopherol, the most abundant form of vitamin E, on IBD.

Dietary supplementation was preferred over oral dosage because in order to use the latter approach, vitamin E would have had to be interposed in a lipid vehicle. Since dietary lipids have been shown to significantly affect the course of chronic inflammation [27], this would have made the interpretation of results difficult, even with the use of appropriate controls. On the other hand, dietary supplementation has some advantages such as being a more physiological (and convenient) way of administration, and can be considered adequate as long as local enrichment of vitamin E in the colonic tissue is achieved. Indeed, our data demonstrate that supplementation for 4 weeks with 300 mg/kg chow of α-tocopherol resulted in a 3-fold increase in vitamin E levels in colonic tissue. Given the very low toxicity of vitamin E, it is feasible that even higher levels of supplementation could be used to treat intestinal inflammation. No signs of toxicity were detected in the rats receiving the vitamin E supplement. In fact, body weight gain in this group was higher than that of the TNBS group from the third day on. This was not caused by a difference in the caloric content of the diets because no lipids were added to facilitate vitamin E incorporation into the chow mix. In turn, average dietary intake (per cage) was also significantly increased in the rats of the vitamin E group compared with the TNBS group, possibly because of a change in the organoleptic characteristics of the supplemented diet. The fact that the TNBS and vitamin E groups differed in body weight at the time of TNBS administration is irrelevant however, since we have never observed an impact of this variable in the animals response to the phlogogen agent (unpublished observations). Indeed, TNBS behaves as a contact sensitizer and so the severity of the inflammatory response is a function of the local concentration in the colonic lumen rather than of the dose:body weight ratio. As a consequence, TNBS dosage is fixed rather than adjusted to body weight [14]. On the other hand, the fact that fat intake was concomitantly increased in the rats receiving the supplemented diet should not influence the outcome of the experiment, since only the administration of unsaturated fatty acids has been reported to have an effect on colonic eicosanoid production and inflammation [27].

The TNBS-induced experimental colitis is probably one of the most widely used models of colonic inflammation. It is a convenient, reproducible and relatively inexpensive model which has an acute phase, followed by a prolonged period of progressive healing with some features of chronic inflammation. It shares some characteristics of IBD, such as transmural inflammation of the colon, granuloma formation, scarring and fibrosis of intestinal tissue, fecal impaction, stenosis, diarrhea, and oxidative stress [14]. The technique described here differs slightly from the one originally described by Morris et al in that a lower dose of TNBS was used, in an attempt to make inflammation more amenable to treatment, as has been suggested previously [4]. In fact, the colonic damage induced by 10 mg of TNBS was qualitatively similar.
to that observed with 30 mg of the compound, but quantitatively lower with regard to both the macroscopic score and the biochemical markers of inflammation, such as MPO activity, as reported previously [3, 5].

In agreement with these observed features, the animals of the TNBS group presented a strong inflammatory response restricted to the distal colon with mucosal necrosis, bowel wall thickening, and fibrosis. This was associated initially with anorexia and loss of body weight and, functionally, with signs of diarrhea in the vast majority of animals. Infiltration of neutrophils and other leukocytes is a prominent feature of both IBD and TNBS experimental colitis, and was demonstrated by the use of a biochemical marker, myeloperoxidase, an enzyme found predominantly in the azurophilic granules of neutrophils [15].

Vitamin E dietary treatment had a significant antiinflammatory effect on the large bowel. Thus rats of the vitamin E group showed a decrease in the colonic weight and damage score compared to TNBS controls at 1 week (p < 0.05). Amelioration of the colonic status was accompanied by a preservation of colonic function, as suggested by the reduction in the incidence of diarrhea (p < 0.05). Therefore, the colonic mucosa seems to be protected from inflammatory injury by vitamin E.

As expected, TNBS-induced colonic inflammation was associated with increased oxidative stress, in agreement with previous studies [2–5]. This was reflected in the depletion of total glutathione and in the increase in lipoperoxidation, as determined by basal MDA levels, and was detected only at 1 week and appeared to cease by the second week. The colonic oxidative stress was associated with a decreased activity in one of the glutathione-related enzymes, glutathione reductase, in TNBS control animals at 1 week. Recently it has been reported that S-nitrosylation of proteins such as glutathione reductase, resulting in enzyme inactivation, may occur as a consequence of oxidative stress but only if the reducing potential of the cells is impaired [28]. This may be indeed the situation in the present study, since colonic oxidative stress was linked to a depletion of glutathione, thus explaining the observed decrease in colonic glutathione reductase activity. In agreement with this hypothesis, two weeks after colonic insult, when glutathione levels were normalized, glutathione reductase activity was restored.

Vitamin E treatment effectively counteracted the oxidative stress associated with colonic inflammation. Thus, the decrease in glutathione levels, an indirect estimation of oxidative damage, was completely prevented in the vitamin E group. Moreover, the colonic levels of MDA, a degradative byproduct of lipoperoxidation, did not increase in rats receiving the vitamin E supplement. This latter effect would be the logical consequence of the well-known activity of vitamin E as a free radical scavenger in biological membranes. The protection of glutathione from oxidative damage can be partly ascribed to the same effect since oxidative stress to the colonic tissue would be reduced. However, the fact that glutathione reductase activity was also preserved in animals under vitamin E treatment suggests that vitamin E may play an important role in maintaining the functional integrity of the glutathione antioxidative defense system during the course of inflammatory conditions. This observation is relevant, because the antioxidative actions of glutathione are not restricted to the lipid phase and would therefore widen the protective effects of vitamin E. Recently and in accordance with the present study, it has been shown vitamin E supplementation (500 mg/kg diet) protects against aspirin-induced gastric mucosal injury in rats by inhibiting lipid peroxidation [29].

In view of the above mentioned results, it may seem surprising that vitamin E did not reduce granulocyte infiltration (as measured by MPO activity) in the intestine. As stated previously, free radicals are considered to play a role in the amplification of the inflammatory response [7] and therefore antioxidative therapy might downregulate the recruitment of leukocytes to the inflammatory site. The lack of effect observed is probably due to the redundancy of signals driving these phenomena, in which many other mediators are involved (eicosanoids, cytokines, adhesion molecules, etc.). However, vitamin E supplementation did reduce the levels of interleukin-1β, one of the cytokines that plays a key role in TNBS colitis as well as in IBD [30] in the rat colon. Interleukin-1β is thought to be increased early in the inflammatory cascade, at least in experimental colitis, to mediate the suppression of food intake in this context [31]. While our data are insufficient to establish the mechanism of action involved in interleukin-1β downregulation, it is tempting to speculate that it may interfere with oxidative activation of transcription factors like NF-κB or AP-1, which seem to be involved in interleukin-1β induction in macrophages, the main source of this cytokine [32].

In conclusion, dietary vitamin E supplementation protects the rat colon from experimental colitis through a mechanism that involves a decrease in oxidative stress associated with inflammation and downregulation of interleukin-1β. Further studies are needed to explore the potential therapeutic association of dietary vitamin E with conventional pharmacological treatment.

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