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Vitamin C modulates oxidative stress-induced colitis in rats

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Aim: Free radicals are an important factor in the etiopathogenesis of colitis and may increase oxidative damage. The antioxidant vitamin C efficiently scavenges free oxygen radicals. The present study aimed to investigate the probable protective effects of vitamin C on oxidative injury in rats in which colitis was experimentally induced with acetic acid.

Materials and methods: This study was conducted with rats for a period of 7 days. Group 1 intrarectally received a placebo (0.9% NaCl) and group 2 intrarectally received 2 mL of 5% acetic acid (AA) and the placebo. Group 3 intrarectally received 2 mL of 5% AA and vitamin C (100 mg/kg of body weight) via gastric gavage. Myeloperoxidase (MPO), catalase (CAT), prolidase (PRS), and arylesterase (ARE) activity, and total thiol (T-SH), total antioxidant capacity (TAC), total oxidant status (TOS), lipid hydroperoxide (LOOH), and oxidative stress index (OSI) values were analyzed in blood and intestinal samples.

Results: While CAT and PRS activity, and plasma TOS, LOOH, and OSI increased following the administration of AA, TAC decreased. TAC increased, whereas LOOH and OSI decreased in response to vitamin C treatment. While MPO and CAT activity, and TOS, LOOH, and OSI values in the colon increased in response to AA treatment, PRS, ARE, T-SH, and TAC decreased. TAC increased in response to vitamin C, whereas MPO, PRS and ARE activity, and TOS, LOOH, and OSI values decreased. While histopathologic colonic injury scores increased ($P < 0.001$) in response to AA, they decreased in response to vitamin C.

Conclusion: Histopathological damage scores, MPO, TOS, LOOH, and OSI decreased significantly in response to vitamin C treatment, whereas TAC increased. Based on these results, we think that vitamin C might play an important role in preventing oxidative stress and colonic tissue injury produced by acetic acid.

Key words: Vitamin C, colitis, histopathology, oxidative stress, rats

Vitamin C'nin sıçanlarda kolit uyarımlı oksidatif stresi hafifletmesi

Amaç: Kolit etyopatogenezinde önemli faktörlerden biri olan serbest radikaller oksidatif hasarı artırabilir. Vitamin C ise serbest oksijen radikallerini etkili biçimde temizleyebilir. Bu çalışma, sıçanlarda asetik asit ile oluşturulan deneysel kolitte oluşan oksidatif hasar üzerinde vitamin C'nin olası koruyucu etkilerini araştırmak amacı ile yürütüldü.

Yöntem ve gereç: Çalışma sıçanlar üzerinde yürütüldü. Birinci gruba rektal yolla sadece 2 mL serum fizyolojik; ikinci gruba 2 mL serum fizyolojik ve 2 mL % 5'lik asetik asit (AA) verildi. Üçüncü gruba ise rektal yolla 2 mL AA (% 5) ve gastrik gavajla 100 mg/kg vücut ağırlığı olarak vitamin C uygulandı. Çalışmada oksidatif ve kolonik doku hasarını değerlendirmek için miyeloperoxidaz (MPO), katalaz (CAT), prolidaz (PRS), arilesteraz (ARE) aktiviteleri ile total thiol (T-SH), total antioksidan kapasite (TAC), total oksidan statü (TOS), lipid hidroperoksit (LOOH) ve oxidative stress indeksi (OSI) değerleri ölçüldü.

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Bulgular: Asetik asit uygulaması ile plazmada CAT, PRS aktiviteleri ile TOS, LOOH ve OSI değerleri anlamlı olarak artarken, TAC değeri önemli düzeyde azaldı. Vitamin C uygulandığında TOS ve OSI değerleri azalırken, TAC değeri arttı. Asetik asit grubu kolon doku örneklerinde MPO ve katalaz aktiviteleri ile TOS, LOOH ve OSI değerleri artarken, PRS, ARE, T-SH and TAC değerleri azaldı. Vitamin C verilen grupta MPO, PRS and ARE aktiviteleri ile TOS, LOOH ve OSI değerleri azaldı, TAC düzeyi yükseldi. Kolon hasarının histopatolojik skorları asetik asit ile artarken, vitamin C verilmesi ile azaldı.

Sonuç: Asetik asitin MPO ve CAT aktiviteleri ile TOS, LOOH ve OSI değerlerinde ve kolonik doku hasarında artışa, TAC değerinde ise azalmaya neden olduğu saptandı. Vitamin C verildiğinde, histopatolojik hasar skorları, MPO aktivitesi ile TOS, LOOH ve OSI değerleri azalırken, TAC düzeyi yükseldi. Bu sonuçlara gore vitamin C, asetik asit ile oluşturulan deneysel kolitte oksidatif strese bağlı doku hasarının koruyucu endikasyonunda önemli bir rol oynayabilir.

Anahtar sözcükler: Vitamin C, kolit, histopatoloji, oksidatif stres, sıçan

Introduction

Ulcerative colitis, diffuse inflammation of the mucous membranes of the colon, can be characterized as mild, moderate, and severe, and leads to extensive ulceration. The inflamed colon is exposed to oxidative stress produced by infiltrating macrophages and neutrophils within colonic tissue. It is thought that an exaggerated intestinal immune response and generation of reactive oxygen metabolites may play a key role in the pathophysiology of colitis (1-5). Reactive oxygen metabolites (ROS) are potent inflammatory mediators likely to be involved in tissue injury in inflammatory bowel disease (IBD). These mediators may play an important role in the course of IBD and its acute attacks. ROS in early-stage IBD may increase and may be an important factor in the etiopathogenesis of colitis. In addition, ROS, such as superoxide ($O_2^{\cdot-}$), hydroxyl radical ($OH^{\cdot}$), hydrogen peroxide (H2O2), and other free oxygen radicals, may increase in colitis (2-6).

It has been reported that antioxidants decrease the harmful effects of oxidative damage caused by ROS, as well as the production of injury in the bowel (1,2,7). Vitamin C is a well-known antioxidant that has been shown to efficiently scavenge free oxygen radicals, including superoxide, hydrogen peroxide, hypochlorite, hydroxyl, peroxyl, and singlet oxygen. Thus, vitamin C may diminish certain types of lipid peroxidation.

Insufficient levels of vitamin C inhibit collagen synthesis in cellular basal membranes and cause the destruction of mucosal epithelium (8). Acetic acid is also an important agent in colitis experimentally induced in animals (1-3,7). Several antioxidant treatments have been used to protect against oxidative damage caused by free oxygen radicals in colitis in experimental animals (2,7,9). The aim of the present study was to investigate the relationships between the levels of CAT, PRS, MPO, TAC, TOS, OSI, T-SH, and the possible protective role of vitamin C on colonic injury in rats in which colitis was experimentally induced with acetic acid.

Materials and methods

Animals and treatments

This study was conducted with rats aged 2-2.5 months that weighed 150-200 g. All the rats were fed rodent pellets and water ad-libitum, and were housed in cages at room temperature with a light-day cycle. Animal housing and the experiments were conducted in accordance with the *Guide for the Care and Use of Laboratory Animals*. All experimental animals were housed in cages at room temperature during the study. Group 1 (control) intrarectally received only 2 mL of physiologic saline (0.9% NaCl), group 2 received 2 mL of 5% acetic acid administered into the rectum and 2 mL of physiologic saline via gastric gavage, and group 3 was administrated 2 mL of 5% acetic acid plus vitamin C (100 mg/kg of body weight) via gastric gavage. Acetic acid was purchased from Merck AG (Darmstadt, Germany) and vitamin C was obtained from Sigma Chemical Co. (St. Louis, MO, USA). All treatments were applied 3 times per week. Twenty-four hours after the last dose was administered on the 7th day blood samples were obtained under ether sedation. The abdomen of each animal was opened and colon specimens were removed from the abdominal opening via laparotomy.

Preparation of blood samples

Blood samples were obtained via cardiac puncture 12 h after the last application of acetic acid and vitamin C. Whole blood was collected into heparinized tubes (Beckon Dickinson Vacuntainer System, Cedex, France) and subsequently centrifuged at 1500 × *g* for 15 min in a Heraeus Megafuge 10. Plasma samples were placed in disposable pipettes and stored at −80 °C for further biochemical analysis.

Collection and examination of colon specimens

At postmortem laparotomy, 6 cm of colon, extending from approximately 2 cm above the anal margin, was removed and opened longitudinally. Then the colon specimens were divided into 2 sections. The first section was stored at −80 °C for further biochemical analysis. The other section was used for histopathological examination. The macroscopic appearance of the colon specimens was scored on a scale adapted by Morris et al. (10), ranging from 0-4: (0) no macroscopic change, (1) mucosal erythema only, (2) mild mucosal edema, slight bleeding, or small erosions, (3) moderate edema, bleeding, and ulcers, and (4) edema, severe ulceration, and tissue necrosis. Mean macroscopic scores were calculated according to the 4 criteria mentioned above. Then the colon specimens were fixed in formaldehyde (10% v/v), embedded in paraffin for ordinary histological examination, and sectioned into 3-5-μ serial sections using a rotary microtome. The sections were stained with hematoxylin and eosin (H&E) for histological evaluation. The histological sections were evaluated according to the following 8 criteria previously described by Keshavarzian et al. (1): vascular dilatation, edema, epithelial cell denudation, cellular mucin depletion, neutrophilic infiltration, eosinophilic infiltration, lymphocytic infiltration, and necrosis/ulceration. The scores of the above 8 criteria were calculated using a colon tissue injury scale of 0-4: 0 (normal), 1 (mild), 2 (moderate), 3 (severe), and 4 (most severe). The total possible score for the 8 criteria is 32.

Biochemical analysis

Catalase (CAT) activity was assayed according to Goth (11). Serum T-SH was measured in accordance with the method described by Kelly (12). Total oxidant status (TOS) and total antioxidant capacity (TAC) were measured using the methods described by Erel (13,14). The oxidative stress index was calculated as follows: OSI (arbitrary unit) = TOS [μmol H_2O_2 equivalent/L)/TAC (mmol Trolox equivalent/l) × 100]. Prolidase and arylesterase activity were measured using the methods described by Myra et al. (15) and Aslan et al. (16). Myeloperoxidase activity in the colon specimens was analyzed according to Krawisz et al. (17).

Statistical analysis

Statistical analysis was performed using SPSS v.11.5 (SPSS, Inc., Chicago, IL, USA). For the analysis of macroscopic and microscopic scores, and biochemical parameters, the Kruskal Wallis test and Mann-Whitney U-test were used for analysis of variance (ANOVA) and post hoc testing, respectively. The data are expressed as mean ± standard deviation (SD), and differences were considered significant at $P < 0.05$.

Results

Macroscopic and microscopic scores for colonic injury are shown in Table 1. Plasma levels of CAT, PRS, ARE, T-SH, TAC, TOS, LOOH, and OSI are presented in Tables 2 and 3. Histopathological appearance of the colon specimens is shown in Figures 1 and 2.

Macroscopic findings

The colon specimens taken from the rats in the control group usually demonstrated a normal aspect. Localized hyperemia was observed in only 2 sections. In the acetic acid group there was gross colonic damage characterized by marked mucosal thickening, edema, bleeding, linear ulceration in large areas, and tissue necrosis. In the vitamin C group, minimal mucosal erythema-edema, and slight bleeding or erosion were usually observed. Moderate edema, bleeding, and small ulcers were observed in some of the specimens. Macroscopic change scores increased significantly ($P < 0.001$) in response to intrarectal injection of acetic acid and decreased ($P < 0.01$) in response to vitamin C treatment (Table 1).

Table 1. Macroscopic and microscopic scores for colons in all groups*.

	Macroscopic and microscopic scores of colonic injury		
Scores/Groups	**Control**	**Acetic acid**	**Acetic acid +Vit C**
Total macroscopic scores	0.1 ± 0.01	3.20 ± 0.13[a]	1.40 ± 0.51[b]
Minimum-maximum scores	0-1	2-4	1-4
Mean microscopic scores	1.90 ± 0.16	25.10 ± 5.30[a]	15.30 ± 9.17[b]
Minimum-maximum scores	1-3	15-29	3-28

*Data are means and standard deviations.
Statistical significance versus control group, [a]$P < 0.001$.
Statistical significance versus control and acetic acid group group, [b]$P < 0.01$.

Table 2. The activities of CAT and PRS and the values of total SH, TAC, TOS, and OSI in plasma of all groups*.

Parameters/Groups	Control	Acetic acid	Acetic acid +Vit C
Catalase *kU/L*	50.73 ± 6.69	55.19 ± 8.27[a]	54.62 ± 6.76
Prolidase *kU/L*	0.46 ± 0.03	0.51 ± 0.10[a]	0.50 ± 0.13
Arylesterase *U/dL*	3.38 ± 0.53	3.40 ± 0.78	4.10 ± 0.94
Total SH *mmol/L*	0.43 ± 0.06	0.42 ± 0.07	0.37 ± 0.03
TAC *mmol Trolox eqv/L*	0.76 ± 0.08	0.66 ± 0.11[a]	0.77 ± 0.03[a]
TOS *mmol H_2O_2 Eqv/L*	16.91 ± 2.88	18.04 ± 2.64[a]	17.79 ± 1.09
LOOH *mmol/L*	7.87 ± 1.22	9.12 ± 0.55[a]	8.88 ± 0.36[a]
OSI *arbitrary unit*	22.46 ± 3.65	28.06 ± 6.87[b]	23.09 ± 1.94[a]

*Data are means and standard deviations.
Statistical significance versus control group, [a]$P < 0.05$, [b]$P < 0.01$.
Statistical significance versus acetic acid group, [c]$P < 0.05$.

Table 3. The activities of CAT, PRS and MPO and the values of total SH, TAC, TOS and OSI for colon of all groups*.

Parameters/Groups	Control	Acetic acid	Acetic acid +Vit C
Catalase *kU/g protein*	0.20 ± 0.001	1.16 ± 0.13[b]	1.21 ± 0.09
Prolidase *IU/g protein*	11.04 ± 1.38	5.09 ± 0.14[b]	4.74 ± 0.21[c]
Arylesterase *IU/g protein*	47.31 ± 11.17	40.85 ± 15.46[a]	33.04 ± 8.73[c]
Total SH *mmol/g protein*	18.312 ± 2.11	7.52 ± 0.32[b]	7.47 ± 0.09
TAC *mmol trolox eqv/g protein*	24.70 ± 0.95	21.13 ± 0.94[b]	23.78 ± 1.56[d]
TOS *μmol H_2O_2 eqv./g protein*	394.21 ± 18.47	501.30 ± 14.38[b]	483.603 ± 55.03[c]
LOOH *μmol H_2O_2 eqv./g protein*	211.87 ± 48.33	249.81 ± 27.26[a]	228.28 ± 17.96[c]
OSI *arbitrary unit*	15.871 ± 0.93	23.48 ± 1.47[b]	20.38 ± 2.48[d]

*Data are means and standard deviations.
Statistical significance versus control group, [a]$P < 0.05$, [b]$P < 0.01$.
Statistical significance versus acetic acid group, [c]$P < 0.05$, [d]$P < 0.01$.

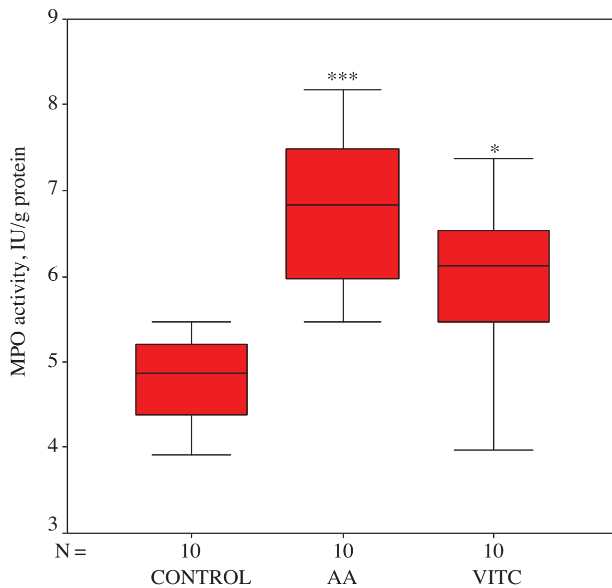

Figure 1. MPO activity (IU/g protein) in colonic tissue. Statistical significance versus control group, ***P < 0.01. Statistical significance versus acetic acid group, *P < 0.05.

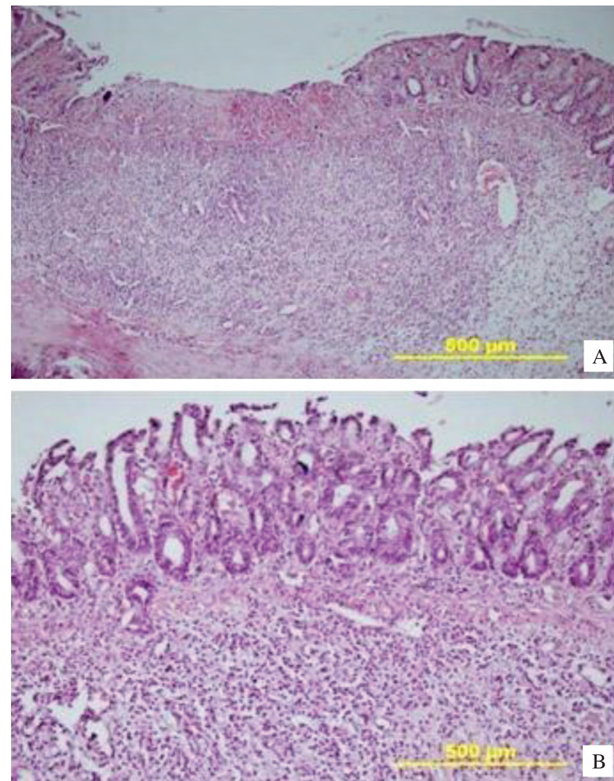

Figure 2. Microscopic appearances of colonic lesions; **A)** in acetic acid-induced group, mucosal epithelial denudation and submucosal ulceration, predominantly neutrophilic infiltration, much glandular destruction, and some scattered glands in the top right are seen. These histological features are described an ulcerous lesions in colonic mucosa and submucosa, (H&E stain; original magnification, ×100); **B)** in acetic acid+vitamin C treated group, cellular mucin depletion, focal luminal epithelial denudation, vascular dilatation, edema, and moderate degree neutrophilic infiltration are seen. However, colonic glands in the lamina propria appear to be better protected in compared with the acetic acid group (H&E stain; original magnification, ×100).

Histopathologic findings

In the acetic acid group hemorrhage, edema, massive mucosal and submucosal inflammatory infiltration, glandular destruction, and ulcerous areas were observed in all sections. Neutrophilic infiltration was observed predominantly in the mucosa, submucosa, and occasionally in the muscularis propria. Eosinophilic and lymphocytic cell infiltration occurred around the ulcerous areas. The above histopathological scores in the acetic acid group were significantly higher ($P < 0.001$) than those in the control group. Colonic injury scores in the vitamin C group were significantly lower ($P < 0.01$) than those in the acetic acid group (Table 1). Histological slides from the acetic acid and vitamin C groups are shown in Figure 2.

Biochemical findings

CAT and PRS activity in plasma significantly increased ($P < 0.05$) in response to acetic acid, but were not affected by vitamin C treatment. While TAC decreased significantly ($P < 0.01$) in response to acetic acid treatment, it increased significantly ($P < 0.05$) (almost to the level observed in the control group) after the administration of vitamin C. TOS and OSI increased significantly ($P < 0.01$) after intrarectal injection of acetic acid. While LOOH and OSI levels decreased ($P < 0.05$) (almost to the levels in the control group), TOS was not affected by the administration of vitamin C (Table 2).

While PRS, ARE, T-SH, and TAC activity in colon tissue decreased significantly ($P < 0.01$) in response to intrarectal injection of acetic acid, TOS, LOOH, and OSI increased ($P < 0.01$). TAC increased ($P < 0.01$), whereas PRS, ARE, TOS, LOOH, and OSI decreased ($P < 0.05$-0.01) in response to vitamin C

treatment. CAT activity increased significantly (P < 0.01) following acetic acid treatment, whereas T-SH and CAT were not significantly affected by the administration of vitamin C (Table 3).

Discussion

Many factors have been implicated in the pathogenesis of colitis, including overproduction of proinflammatory mediators (including reactive oxygen mediators, cytokines, and arachidonate metabolites), and neutrophil infiltration (6,7). ROS in colonic mucosa have been described in colorectal specimens obtained from patients with IBD. These results increased interest in the role of free oxygen radicals in the pathogenesis of colitis (7,18). Increased ROS in IBD may be an important factor in the etiopathogenesis of colitis (2,5,19). The inflamed colon is exposed to oxidative stress produced by infiltrating macrophages and neutrophils within the lamina propria of the colon (2). Respiratory burst by neutrophils and macrophages is characterized by marked changes in oxygen metabolism, which results in an increase in the production of superoxide and hydrogen peroxide (H_2O_2) radicals (20). The tissue damage produced by neutrophils and macrophages has been attributed to their ability to release ROS, nitrogen metabolites, cytotoxic proteins, lytic enzymes, and cytokines, as well as their negative effects on epithelial integrity (2,5-7,18,19).

Acetic acid is an important agent for experimental colitis and causes inflammation of the colon in laboratory animals. Colitis induced with acetic acid is also known to produce excess ROS, and antioxidants reduce the levels of these oxygen radicals (2-4,6,7). In the present study macroscopic and microscopic colon injury scores increased significantly (P < 0.01) following intrarectal injection of acetic acid and decreased (P < 0.05-0.01, respectively) in the group treated with vitamin C (Table 1 and Figure 2). These results are in accordance with those of studies that reported that lipid peroxidation decreased in bowel disorders (2,5-7). Thus, reactive oxygen radicals may defeat the antioxidant defense system, controlling the production of ROS during normal cellular metabolism (2,5-7,21-23). Indeed, it has been reported that ROS were responsible for acetic acid's toxicity to the colon and caused oxidative damage in unsaturated lipids in cellular components of the colon (2,6).

Plasma TOS increased significantly (P < 0.01) after intrarectal injection of acetic acid. The LOOH and OSI levels decreased (P < 0.05) (almost to the levels in the control group) following the administration of vitamin C, whereas TOS was not affected (Table 2). While TOS, LOOH, and OSI levels increased (P < 0.01) following intrarectal injection of acetic acid, TOS, LOOH, and OSI levels decreased (P < 0.05-0.01) in response to vitamin C treatment (Table 3). These results are similar to those of earlier studies that reported that lipid peroxidation decreased in bowel disorders (7,24). While TAC decreased significantly (P < 0.01) in response to acetic acid treatment, it significantly increased (P < 0.05) (almost to the level in the control group) after the administration of vitamin C (Table 2). TAC activity in colonic tissue decreased significantly (P < 0.01) following intrarectal injection of acetic acid and increased (P < 0.01) in response to vitamin C (Table 3). These findings are similar to those of studies that reported a decrease of TAC in experimental colitis (9,25).

Bregano et al. reported that experimentally induced colitis affected enzymatic and oxidative markers in rats (26). MPO, a component of neutrophil azurophilic granules, is a good marker of inflammation and injury in colonic tissue. A 3-fold increase in MPO activity was observed after 1 week of treatment with 2,4,6-trinitrobenzenesulfonic acid (TNBS). On the other hand, MPO activity decreased in colon mucosa of rats fed a diet supplemented with antioxidant substances (26-29). These results lead to the overall concept of attempting to restore antioxidant defense as a treatment for ulcerative colitis (25).

CAT activity increased in the colon tissue of rats administered TNBS, an important agent in experimentally induced colitis. A physiological function of this enzyme counteracted the oxidative stress that plays an important role in experimental colitis produced by different chemical substances (27-29). In addition, it was reported that CAT activity increased in the lamina propria of intestinal mucosae inflamed by neutrophilic and monocytic infiltrates in ulcerative colitis (28). This imbalance between CAT

activity and inflammation might be attributed to an increase in free radicals, such as HOCl, H_2O_2, and OH, contributing to oxidative damage in colonic mucosae. Thus, CAT may play an important role in scavenging H_2O_2, OH, and HOCl, or in preventing their common effects. CAT, an essential peroxisomal hydrogen peroxide-consuming enzyme, also decreased the oxidative effects of hydrogen peroxide (H_2O_2) and may play a role in scavenging oxidative radicals. This enzyme is considered the antioxidant enzyme that defends against reactive oxygen species (3,19). Several experimental treatments using antioxidants and other chemical substances have been used to protect against free radical injury in bowel diseases (2,5,7), and CAT prevented the harmful effects of free radicals and reduced the number of reactive metabolites produced in response to acetic acid. We observed that CAT activity in plasma and the colon increased significantly ($P < 0.05$) following acetic acid treatment (Table 2). These results are similar to those of other studies that reported the effects of acetic acid and other chemicals used to induce colitis on CAT (2,25,30).

MPO activity has been widely used to detect neutrophil infiltration in mucosal inflammation of the bowel, and a reduction in this enzyme's activity can be used as an indicator of the anti-inflammatory activity of a given compound. In the present study MPO activity in colonic tissue increased significantly ($P < 0.001$) in response to intrarectal injection of acetic acid and decreased ($P < 0.05$) following administration of vitamin C (Figure 1). These results are in accordance with those of other studies that reported that MPO activity increased in experimental colitis (5,9,25,31).

Some thiol-containing compounds, such as cysteine, methionine, taurine, glutathione, and lipoic acid, are considered effective antioxidants. The reduced forms of thiol-containing compounds are known to have the greatest antioxidative activity. The number of sulfur atoms in thiol-containing compounds partially determines the activity of glutathione-related antioxidant enzymes (32). Serum SH groups act as important cellular scavengers of peroxides, and so help to protect cells from damage caused by these molecules. A decrease in the SH level not only impairs cellular response to oxidants, but also changes the functions of inflammatory cells (33). The thiol values in colonic tissue decreased ($P < 0.01$) in response to acetic acid injection and vitamin C treatment in the present study (Table 3). The decrease in thiol values may have been due to the oxidation of protein thiols such as GAPDH, or depletion of low molecular weight thiols such as glutathione. The present findings are in accordance with those of similar studies (9,34).

Prolidase, a highly specific peptidase, may play an important role in proline conservation (35). Serum prolidase activity was correlated with increased oxidative stress. Moreover, it has been reported that plasma prolidase activity might be useful in evaluating bowel disorders and fibrotic processes in chronic liver disease in humans (15,24,36). In the present study prolidase activity in colonic tissue decreased significantly ($P < 0.05$), whereas the values in plasma increased significantly ($P < 0.05$) following intrarectal injection of acetic acid and decreased ($P < 0.05$) following administration of vitamin C (Tables 2 and 3). These results are similar to those of some other studies (24,29,35,37). ARE located on HDL and free sulfhydryl (SH) groups are enzymes or proteins with antioxidant characteristics. ARE can hydrolyze LOOH products, as well as organophosphates (37).

It has been reported that human and rabbit plasma contains at least 12 different arylesterases, but only 1 is probably related to cholesterol metabolism in rabbit plasma. Plasma esterases may also be involved in cholesterol metabolism; arylesterases also catalyze the covalent coupling of fatty acids to tyrosine groups in plasma albumin. In addition, it was suggested that variation in the response of the plasma cholesterol level to dietary cholesterol was associated with a genetically determined variation in plasma esterases. (38). Arylesterase (ARE) activity varies with antioxidant vitamins. Vitamin C, an important antioxidant, may scavenge free-oxygen radicals that may depress ARE (34). The values of ARE in plasma were not affected by the acetic acid or vitamin treatments in the present study (Table 2). While ARE activity in colonic tissue decreased significantly ($P < 0.01$) following acetic acid treatment, it decreased ($P < 0.05$-0.01) in response to vitamin C treatment in colonic tissue (Table 3). These results are similar to those of other studies (29,34).

Vitamin C insufficiency inhibits collagen synthesis in cellular basal membranes and destroys mucosal epithelium. L-ascorbic acid is an important antioxidant in extracellular fluids, and inhibits the peroxidation of unsaturated lipids by scavenging or quenching free radicals, such as superoxide and singlet oxygen, and therefore may prevent the increase in free radicals produced by oxidative damage in lipids and lipoproteins during the progression of IBD. This can be attributed to the prevention of oxidation reactions via the antioxidant functions of L-ascorbic acid in cellular membranes. Moreover, vitamin C is an antioxidant that protects the basal membrane, limits destruction of mucosal epithelium (8,25), and protects surrounding tissues from oxidative attack by free radicals produced by the respiratory burst of neutrophils and macrophages (20). Thus, vitamin C may prevent certain types of oxidative damage produced by infiltrating macrophages and neutrophils within the inflamed colon. These opinions are in agreement with those of other researchers that investigated the effects of antioxidants on oxidative damage (8,25,29,34,37).

In conclusion, the present findings show that vitamin C had a beneficial effect on oxidative damage produced during the process of inflammatory response to experimentally induced colitis. Vitamin C may be used for protective purposes in animals exposed to acetic acid and as a prophylactic treatment by acting on colonic mucosa in experimental colitis; however, there is a need for more detailed studies in order to assess the possible relationships between colitis induced with acetic acid and antioxidants.

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