Effects of dexpanthenol on acetic acid-induced colitis in rats

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Abstract. While the pathogenesis of acetic acid (AA)-induced colitis is unclear, reactive oxygen species are considered to have a significant effect. The aim of the present study was to elucidate the therapeutic potential of dexpanthenol (Dxp) on the amelioration of colitis in rats. Group I (n=8; control group) was intrarectally administered 1 ml saline solution (0.9%); group II [n=8; AA] was administered 4% AA into the colon via the rectum as a single dose for three consecutive days; group III (n=8; AA + Dxp) was administered AA at the same dosage as group II from day 4, and a single dose of Dxp was administered intraperitoneally; and group IV (n=8; Dxp) was administered Dxp similarly to Group III. Oxidative stress and colonic damage were assessed via biochemical and histologic examination methods. AA treatment led to an increase in oxidative parameters and a decrease in antioxidant systems. Histopathological examination showed that AA treatment caused tissue injury and increased caspase-3 activity in the distal colon and triggered apoptosis. Dxp treatment caused biochemical and histopathological improvements, indicating that Dxp may have an anti-oxidant effect in colitis; therefore, Dxp may be a potential therapeutic agent for the amelioration of IBD.

Introduction

Inflammatory bowel disease (IBD) describes a group of chronic diseases, including ulcerative colitis and Crohn's disease, which have a significant effect on life quality (1). While the pathogeneses of these diseases have not been fully identified, there is evidence to suggest that the immune system may have a causative role (2). Tissue injury occurs not only as a result of uncontrolled activation of the immune response, but also due to an increase in oxygen and nitrogen metabolites (3). Within this mechanism, reactive oxygen species (ROS) have been demonstrated to have a significant propagation effect on the pathogenesis of IBD, and has a dominant role in oxidative stress and apoptosis development (4). Oxidative stress, and the subsequent increase in apoptosis, have constituted the basis of the treatment strategy for the past 50 years; therefore, the majority of research into therapeutic agents in this field has aimed to eliminate ROS (5).

Acetic acid (AA)-induced colitis is one of several models of experimental colitis used to investigate IBD, and is propagated by intrarectal administration of AA to induce inflammation and ulceration in the rectum and the colon in rats. Oxidative destruction is thought to be the pathogenetic factor in this scenario, and this damage is also observed in the gross morphology of the colon (6). It is assumed that the same result occurs in colitis in humans (7).

Dexpanthenol (Dxp) is a biologically active alcohol-analog of pantothenic acid (PA) that is converted into PA inside the cell (8). PA exerts its anti-oxidative effects by increasing the synthesis of reduced glutathione (GSH) and associated peroxidase enzymes, which serve as the most important protective systems against oxidative stress and lipid peroxidation (9). Additionally, PA is incorporated into the structure of coenzyme A, stimulates epithelization, and exerts anti-inflammatory effects (10). The present study aimed to investigate the effects of Dxp on tissue damage and oxidative stress in a rat model of acetic acid-induced colitis, using biochemical and histopathological analyses. This will elucidate whether or not Dxp is a potential therapeutic agent for IBD.

Materials and methods

Animals. The present study was approved by the Institutional Ethics Committee for Animal Experiments (2014/A-41). All experiments were performed at the Inonu University Experimental Animals Production Center (IUEAPC; Malatya, Turkey). The study protocol was executed in accordance with the United States Animal Welfare Act and the Guide for the Care and Use of Laboratory Animals (National Institutes of Health, publication no. 5377-3, 1996), on the principles of animal research. A total of 32 Wistar albino female rats (3-4 months old; weight, 200±20 g) were used. Rats were purchased from the IUEAPC and randomly divided into groups (n=8/group). Each rat was kept in a separate cage. All rats were fed a standard rat pellet and tap water diet, with 12-h fasting employed prior to the experiment. Rats were

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maintained in an environment with a 12:12 light-dark cycle at 21±2˚C room temperature and 60±5% humidity.

Induction of colitis. AA was used to induce acute colitis in rats. Rats were administered 4% AA at 24-h intervals for three days with a 6G nelaton catheter, under mild anesthesia [ketamine hydrochloride (50 mg/kg) and xylazine (5 mg/kg) mixture]. A catheter was inserted 6 cm into the anus, and 1 ml AA was administered followed by 1 ml air, after which rats were maintained in the Trendelenburg position for 15 min. Dxp was administered as a single dose of 500 mg/kg for three days, under mild anesthesia (as described above). An insulin injector (Beybi Plastik Fabrikasi Sanayi A.Ş, Istanbul, Turkey) was used to deliver Dxp into the peritoneum from the right lower quadrant of the abdomen. Dosages of AA and Dxp were adjusted according to previous dose-response studies (4,11).

Experimental protocol. A total of 32 rats were randomly divided into four equal groups (n=8); i) group I, the control group, 1 ml saline solution (0.9%) was administered intrarectally; ii) group II, rats received 4% AA (1 ml/day into the colon intrarectally) as a single dose for three consecutive days; iii) group III, rats received 4% AA (1 ml/day into the colon intrarectally) as a single dose for three consecutive days, and from day four, a single dose of Dxp (500 mg/kg; Bepanthene ampule®; Bayer AG, Leverkusen, Germany) was administered intraperitoneally (IP) for three days; and iv) group IV, 500 mg/kg Dxp was administered IP as a single dose for three consecutive days.

On day 7, laparotomy was performed under high-dose anesthesia (70 mg/kg ketamine and 8 mg/kg xylazine; i.m.), and tissue specimens were subsequently collected from the area 10-cm distal from the colon. Oxidative stress markers, including malondialdehyde (MDA), total oxidant status (TOS), oxidative stress index (OSI), and anti-oxidant system markers, including superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPX), glutathione (GSH) and total anti-oxidant capacity (TAC) levels, were analyzed. Histopathological examination was performed under light microscopy.

Histological analyses. For light microscopic analysis, samples harvested from the distal colon were fixed in 10% formalin for 48 h, dehydrated in an ascending alcohol series, and subsequently embedded in paraffin. Using a microtome, paraffin blocks were prepared for sectioning at 5-μm thickness. Sections were stained with hematoxylin and eosin to assess the general morphology and Periodic acid-Schiff was employed for goblet cell secretion assessment.

Assessment of colonic injury was performed using the following criteria: Mucosal epithelium (ulceration); lamina propria (polymorphonuclear cell infiltrate); crypts loss, submucosa (hemorrhage, edema, infiltration of inflammatory cells); and caspase-3 immuno-reactivity (according to the extent of cell staining in all layers of the colon). Colonic mucosal damage was evaluated on a 0-4 scale by two histologists, who were blinded to the experimental group. Grading criteria were as follows: Grade 0, normal appearance; grade 1, slight injury (0-25% involvement); grade 2, moderate injury (25-50% involvement); grade 3, severe injury (50-75% involvement); and grade 4, extensive full thickness injury (>75%) (12). The microscopic grade of each tissue was calculated as the sum of the scores given to each criteria; therefore, the maximum score that could be obtained was 28. Surface goblet cells and mitosis figures in crypts were counted using a Leica Q Win image analysis system (Leica Microsystems Ltd., Milton Keynes, UK) in 20 areas under X40 objective.

For immunohistochemical analysis, sections of 4 μm thickness were placed on polylysine-coated slides. Following rehydration, samples were immersed in a citrate buffer (pH 7.6), heated in a microwave oven for 20 min, cooled for 20 min at room temperature, and subsequently washed with phosphate-buffered saline (PBS; three 2 min washes). Washed sections were immersed in 0.3% H_2O_2 for 7 min prior to washing again with PBS. Sections were incubated with a primary caspase-3 (CPP32) Ab-4 rabbit polyclonal antibody (cat no. RB-1197-R7; ready-to-use; Thermo Fisher Scientific, Inc.) for 30 min at room temperature, rinsed in PBS and subsequently incubated with biotinylated goat anti-polyvalent (cat. no. TP-125-BN; ready-to-use) antibody and streptavidin peroxidase (cat no. TS-125-HR) (both Thermo Fisher Scientific, Inc., Waltham, MA, USA) for 10 min at room temperature. Staining was completed with chromogen substrate for 15 min and slides were counter-stained with Mayer's hematoxylin for 1 min, rinsed in tap water and dehydrated. Sections were examined by a histopathologist unaware of the experimental protocol using a Leica DFC 280 light microscope (Leica Microsystems, Ltd.). Caspase-3-positive cells in the cytoplasm were stained brown.

Biochemical analysis. A total of 200 mg frozen colonic tissue was cut into sections using liquid nitrogen and homogenized in 10 volumes of ice-cold Tris-HCl buffer with respect to tissue weight (50 mmol/l; pH 7.4) using a homogenizer (Ultra Turrax IKAT18 basic homogenization; Werke, Staufen, Germany) for 3 min at 7,500 x *g* and 4˚C. The supernatant solution was extracted with an equal volume of ethanol/chloroform mixture (3/5; v/v). Following centrifugation at 3,000 x *g* for 30 min at 4˚C, the upper layer was used to analyze total tissue protein levels.

Determination of MDA. MDA content in the homogenates was determined spectrophotometrically by measuring the presence of thiobarbituric acid reactive substances (TBARS) (13). A total of 3 ml 1% phosphoric acid and 1 ml 0.6% thiobarbituric acid solution were added to 0.5 ml homogenate and pipetted into a tube. The mixture was heated in boiling water for 45 min and, following cooling, the colored part was supplemented with 4 ml *n*-butanol. Absorbance was measured using a spectrophotometer (UV-1601; Shimadzu Corporation, Kyoto, Japan) at 532 and 520 nm. Amount of lipid peroxide was calculated as TBARS of lipid peroxidation. Results were expressed in nmol/g tissue according to a prepared standard graph, prepared using the measurements of the standard solutions (1,1,3,3-tetramethoxypropane).

Determination of protein content. Protein content of the samples was determined by the method outlined by Lowry *et al* (14), using bovine serum albumin as a standard.

Determination of SOD activity. Total SOD activity was determined according to the method used by Sun *et al* (15), which is based upon the principle of the inhibition of nitro-blue-tetrazolium (NBT) reduction by the xanthine-xanthine oxidase system as a superoxide generator. One unit of SOD was defined as the enzyme amount required to induce a 50% reduction in the NBT reduction rate. SOD activity was calculated as U/mg protein.

Determination of CAT activity. CAT activity was determined according to the method outlined by Aebi and Suter (16). The principle of the assay is based on the determination of the rate constant (k, s^{-1}) or H_2O_2 decomposition rate at 240 nm. Results are provided as k/g protein.

Determination of GPX activity. GPX activity was measured according to the method used by Paglia and Valentine (17). Briefly, in a tube containing NADPH, GSH, sodiumazide and glutathione reductase, an enzymatic reaction was initiated by the addition of H_2O_2, and the change in absorbance at 340 nm was recorded using a spectrophotometer. Activity was expressed in U/g protein.

Determination of GSH content. Concentration of GSH in the homogenate was measured spectrophotometrically according to Ellman's method (17). Briefly, aliquots of tissue homogenate were mixed with distilled water and 50% trichloroacetic acid in glass tubes and centrifuged at 2,000 x *g* for 15 min at 4˚C. Supernatants were mixed with 0.4 mol Tris buffer (pH 8.9) and 0.01 mol 5,5'-dithiobis (2-nitrobenzoic acid) (DTNB) was added. Following agitation of the reaction mixture, absorbance was measured at 412 nm within 5 min of the addition of DTNB against a blank sample that contained no homogenate. Absorbance values were extrapolated from a glutathione standard curve and provided as GSH (μM/g tissue).

Determination of TAC. TAC levels were determined using a novel automated colorimetric measurement method developed by Erel (18). In this method, a hydroxyl radical is produced by the Fenton reaction, which reacts with the colorless substrate, *O*-dianisidine, to produce a dianisyl radical that is bright yellow-brown in color. Following the addition of the sample, the oxidative reactions initiated by the hydroxyl radicals present in the reaction mix are suppressed by the antioxidant components of the sample. In turn, this prevents the color change and thereby provides an effective measurement of the total antioxidant capacity of the sample. This assay has been demonstrated to have excellent precision values (<3%). Results were presented as mmol Trolox equivalent/l.

Determination of TOS. TOS was determined using a novel automated measurement method, developed by Erel (18). Oxidants present in the sample oxidize the ferrous ion-O-dianisidine complex to a ferric ion. The oxidation reaction is enhanced by glycerol molecules, which are abundantly present in the reaction medium. The ferric ion forms a colored complex with xylenol orange in an acidic medium. Color intensity, which can be measured spectrophotometrically, is correlated to the total amount of oxidant molecules present in the sample. The assay was calibrated with H_2O_2 and the results were expressed in terms of μmol H_2O_2 equivalent/l.

Measurement of OSI. The TOS to TAC ratio was accepted as the OSI, which is an indicator of the degree of the oxidative stress (20). The OSI value was calculated using the following formula: OSI (arbitrary unit)=TOS/TAC. The OSI value of the distal colon samples was also calculated as OSI (arbitrary unit).

Statistical analysis. Data were expressed as median (min-max) values or mean ± standard deviation depending upon the overall variable distribution. Normality was assessed using the Shapiro-Wilk test. Normally distributed data were analyzed by one-way analysis of variance, followed by Tukey's *post-hoc* tests. Non-normally distributed data were compared by Kruskal Wallis H test among the groups. When significant differences were determined, multiple comparisons were carried out using the Mann Whitney U test with Bonferroni correction. Statistical analyses were performed using SPSS version 22.0 for Windows (IBM SPSS, Armonk, NY, USA). $P<0.05$ was considered to indicate a statistically significant difference.

Results

Body weight. No animal mortality occurred during the experimental period. A decrease in the body weights was observed among the groups prior to and following the experiments (data not shown), however, this difference was not significant.

Biochemical assessment. As demonstrated in Table I, a significant difference was detected in the MDA levels between group AA and the control group ($P<0.05$), and between the MDA levels in Group AA and Group AA + Dxp ($P<0.05$). A significant decrease was detected in SOD, CAT and GPX levels in Group AA, compared with the control group ($P<0.05$). When Group AA + Dxp and Group AA were compared, significant improvements were observed in SOD, CAT, GPX, and GSH parameters in Group AA + Dxp ($P<0.05$). Significant differences in TOS and OSI levels were also detected between Group AA and the control group ($P<0.05$). Furthermore, there were marked differences in TOS, TAC, and OSI levels, and a significant difference in TOS levels between Group AA and Group AA + Dxp ($P<0.05$).

Histological results. Tissue sections of rats in the control and Dxp groups exhibited normal large bowel structures, and the colonic mucosa was observed as intact (Fig. 1A and B). Numerous goblet cells were identified on the surface epithelium and crypt in the control and Dxp groups (Fig. 2A and B). Notable histological damage occurred in the AA group (score, 12.5±2.3). The lamina epithelialis, lamina propria, muscularis mucosae, and the submucosa of the colon layers could not be distinguished from each other due to extensive inflammatory cell infiltration and ulceration (Fig. 3A). Diffuse inflammatory cells in the mucosa were predominantly composed of neutrophils. In addition to severe goblet cell depletion, an absence of crypts was observed (Fig. 3B).

The microscopic score of the AA + Dxp group was significantly reduced compared to the AA group ($P<0.01$); however, Dxp

Table I. Comparison of tissue biochemical parameters among the study groups (n=8).

Group	MDA (nmol/g)	SOD (U/mg protein)	CAT (k/g protein)	GPX (U/g protein)	GSH (micromol/g)	TOS (micromol/g)	TAC (mmol troloxEq/l)	OSI (arbitrary unit)
Control	3.18 (2.52-5.20)	0.68 (0.61-0.97)	1.36 (0.86-1.95)	285.43 (198.53-395.44)	2.69 (2.13-3.20)	1.78 (1.38-2.15)	0.95 (0.81-1.12)	1.86 (1.25-2.42)
AA	5.89 (4.97-7.52)[a]	0.41 (0.24-0.59)[a]	0.49 (0.25-0.84)[a]	143.51 (92.54-150.7)[a]	1.91 (1.15-2.81)	2.56 (1.58-4.14)[a]	0.87 (0.63-1.05)	2.76 (2.09-6.47)[a]
AA+Dxp	4.04 (3.42-6.06)[b]	0.69 (0.58-0.97)[b]	1.12 (1.05-1.92)[b]	290.91 (204.62-293.59)[b]	2.81 (2.47-3.08)[b]	1.80 (1.34-2.41)[b]	0.98 (0.81-1.07)	1.96 (1.40-2.42)
Dxp	4.16 (3.13-6.06)	0.68 (0.51-0.93)[b]	1.15 (0.77-1.92)[b]	250.30 (201.00-294.57)[b]	2.86 (2.00-3.27)[b]	2.09 (1.38-2.26)	0.91 (0.73-0.98)	2.03 (1.40-2.69)
P-value	<0.05	< 0.01	<0.01	<0.001	<0.01	<0.05	>0.05	<0.05

[a]P<0.05 vs. control group, [b]P<0.05 vs. AA group. Data are presented as the median (minimum-maximum). AA, acetic acid; Dxp, Dexpanthenol; MDA, malondialdehyde; SOD, superoxide dismutase; CAT, catalase; GPX, glutathione peroxidase; GSH, glutathione; TOS, total oxidant status; TAC, total anti-oxidant capacity; OSI, oxidative stress index.

Figure 1. Regular epithelial line and intact crypt in the (A) control and (B) dexpanthenol groups, as detected by hematoxylin and eosin staining (magnification, x33).

Figure 2. Abundant goblet cells in the surface and crypt epithelium, and Periodic acid-Schiff (+) reaction-induced magenta staining in the (A) control and (B) dexpanthenol groups (magnification, x33).

Figure 3. Acetic acid group. (A) Extensive epithelial loss, destruction of crypts and severe inflammatory cell infiltration, hemorrhage and sub-mucosal edema, as detected by hematoxylin and eosin staining. (B) Marked goblet cell depletion and absence of crypts, as detected by the Periodic acid-Schiff staining. Images were captured at x33 magnification.

treatment did not completely alleviate lesions, it merely restricted them. Ulceration, inflammatory cell infiltration and loss of crypt were still present in the affected areas (Fig. 4A). In some areas, the mucosa remained intact in this group. In intact fields, goblet cells were detected on the surface and crypt epithelium (Fig. 4B). Additionally, the number of mitotic figures was significantly increased when compared with the AA group (P<0.0001).

Caspase-3 immunostaining was only detected in the epithelial cells on the luminal surface in the control and Dxp groups (Fig. 5A and B). Conversely, caspase-3-positive cells were observed only in epithelial crypt cells due to superficial epithelial ulceration in the AA group (Fig. 5C). The appearance of cells stained with caspase-3 in the AA + Dxp group was consistent with the control group (Fig. 5D). Results of histological grading and the number of goblet cells and mitotic figures in all groups are shown in Tables II and III.

Discussion

IBD describes a group of chronic diseases that have marked effects on the quality of life of those affected. In recent years,

Table II. Colonic injury grades among the study groups.

Group	Control	AA	AA + Dxp	Dxp
Microscopic grade	1.1±0.1	12.5±2.3[a]	6.8±2.3[b]	1.4±0.2

[a]Statistically significant increase (P<0.001) vs. control group. [b]Statistically significant decrease (P<0.05) vs. AA group. AA, acetic acid; Dxp, Dexpanthenol.

Table III. Number of goblet cells and mitotic figures.

Group	Goblet cell	Mitotic figure
Control	62.0±17.3	1.3±1.1
AA	15.1±19.6[a]	0.7±1.0[a]
AA + Dxp	37.7±25.5[b]	1.2±1.1[b]
Dxp	61.8±17.2	1.2±1.2

[a]Statistically significant decrease (P<0.001) vs. control group. [b]Statistically significant increase (P<0.001) vs. AA group. AA, acetic acid; Dxp, dexpanthenol.

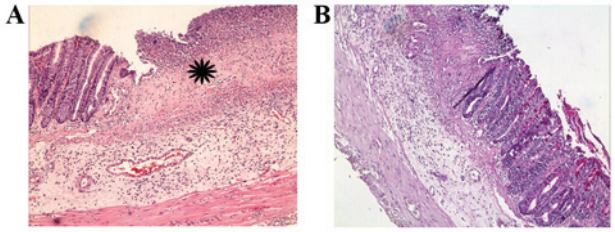

Figure 4. Acetic acid + dexpanthenol group. (A) Some mucosal areas were damaged (star), whereas other mucosal areas are intact. Degenerative changes and loss of crypts were detected in the injured field, following hematoxylin and eosin staining. (B) Goblet cells are visible, except in the affected area, following Periodic acid-Schiff staining. Images were captured at x33 magnification.

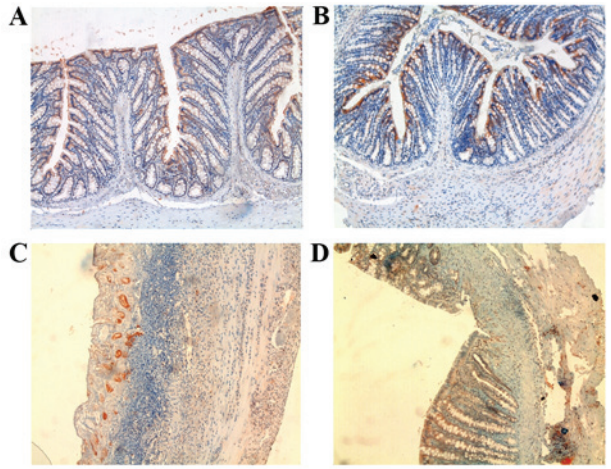

Figure 5. Diffuse caspase-3 staining in the surface epithelium of the (A) control and (B) dexpanthenol groups. (C) In the acetic acid-treated group, cells stained with caspase-3 were observed in the crypts. (D) Caspase-3-positive cells were only visible in the surface epithelium of unaffected areas. Images were captured at x33 magnification.

the incidence of IBD has gradually increased (19). Despite the potential role of genetic, immunologic, and environmental factors, the etiology of IBD remains unclear; however, free oxygen radicals are considered to be a causal factor for IBD (20). Therefore, several studies have aimed to identify potential IBD therapies. A number of previous studies have focused on oxidative stress, which is important in mucosal injury pathogenesis (21) and is an initiator of apoptosis (22,23). Apoptosis is important in tissue homeostasis (24); insufficient or excessive apoptosis disrupts equilibrium and causes various diseases (25). Apoptotic cell death also alters epithelial barrier function and allows infiltration by pathogenic microorganisms. While the details remain unclear, several studies (26-28) have suggested that apoptosis has a role in IBD pathogenesis, together with oxidative stress (29). Therefore, the majority of studies have focused on substances with anti-oxidant and anti-inflammatory properties.

In the present study, the oxidative stress, apoptosis and anti-oxidant properties of Dxp were investigated in a rat model of AA-induced colitis. The AA-induced colitis model used, is beneficial for the investigation of IBD pathogenesis and novel treatment options for ulcerative colitis, since it induces inflammation and ulceration (7).

Colon tissue injury was detected in the colitis model with a significant increase in oxidative stress markers and histopathological changes. Oxidative stress is considered to be a causative factor of AA-associated alterations in the colon tissue. Experimental studies have indicated that oxidative stress results from the shift of equilibrium between the pro-oxidant and anti-oxidant systems in favor of the pro-oxidant system as a result of excessive production of free oxygen radicals (30).

In the present study, distal colitis was induced via AA treatment, which revealed that AA treatment caused prominent tissue injury. When the results of the colonic tissue in the control group and Group AA were compared, AA treatment was demonstrated to induce an increase in MDA levels in the tissue. Therefore, it was determined that AA may have caused oxidative stress by leading to a marked increase in MDA levels in the colon tissue. MDA is the toxic end-product of lipid peroxidation, and reflects the level of lipid peroxidation in the tissue; hence its common usage as a marker of lipid peroxidation (31). MDA is secreted due to the toxic effects of active free oxygen radicals. While ROS affect all biomolecules in the organic environment, their major targets are membrane lipids, other lipids, proteins and DNA (32). ROS initiate lipid peroxidation by removing a hydrogen atom (33). Previous studies have shown that MDA levels are elevated in IBD (34-36). Furthermore increased lipid peroxidation has been reported in AA-associated tissue injuries, which is consistent with the findings of the present study. The present study demonstrated that Dxp treatment caused a significant decrease in AA-associated oxidative stress.

Another important finding of the present study was that AA treatment leads to a decrease in SOD, CAT and GPX levels in the tissue. It is well-known that enzymatic and non-enzymatic anti-oxidant systems exist to protect tissues from pro-oxidants and, as there is a balance between these systems, the imbal-

ance has a role in the development of various diseases (37). SOD, CAT and GPX are endogenous enzymatic anti-oxidants, whereas GSH is a non-enzymatic anti-oxidant (38). These molecules protect cells and organisms from cytotoxic free oxygen radicals. SOD is among the most important enzymatic anti-oxidants. Oxygen radicals are converted into H_2O_2 by SOD. H_2O_2 is subsequently detoxified by CAT and GPX, and is converted into H_2O and O_2 molecules. The release of ROS in IBD disrupts the anti-oxidant system during mucosal inflammation and leads to oxidative damage. Decreased anti-oxidant levels have been reported in patients with ulcerative colitis (39,40). Kruidenier *et al* (41) demonstrated that Cu/Zn SOD and MnSOD levels were elevated in patients with IBD, compared with the control group. Kuralay *et al* (35) established an experimental model of colitis and indicated that SOD levels increased as a response to oxidative stress, and this increase was successfully reduced after treatment with anti-oxidant agents. The findings of the present study are consistent with the existing hypothesis of the mechanism of colitis tissue injury, which occurs as a result of ROS that weaken the anti-oxidant system. Furthermore, the results of the present study are consistent with previous studies, which have demonstrated increased lipid peroxidation and decreased anti-oxidant systems in AA-induced colitis (42).

The GSH cycle is the other important intracellular anti-oxidant defense system. It is used as a substrate for the activity of several anti-oxidant enzymes. In particular, GPX is a glutathione-dependent enzyme. In the presence of GSH, GPX detoxifies H_2O_2 into H_2O and O_2 molecules. GSH subsequently loses a hydrogen atom and oxidized glutathione (GSSG) is formed (43). Glutathione reductase forms GSH from GSSG. A decrease in GSH activity increases oxidative stress and leads to the accumulation of toxic products (44). In the present study, AA led to a decrease in GSH levels.

In the present study, increased levels of TOS and OSI, and decreased TAC levels were detected in Group AA. TOS and OSI levels in Group AA + Dxp were reduced compared with Group AA; however, no significant increase in TAC levels was detected. These findings are consistent with the results of a previous study (45), suggesting that AA increases oxidative stress, whereas Dxp decreases it.

Macroscopic and histopathological examinations are the gold standards for evaluating inflammatory injury in the colon. The present study demonstrated that caspase-3 activity in the colon was considerably increased in Group AA compared with the control group. When Group AA + Dxp and Group AA were compared, Dxp treatment significantly decreased caspase-3 activity. This effect can be attributed to the anti-inflammatory and anti-apoptotic effects of Dxp. Altintas *et al* (39) have previously demonstrated that Dxp treatment significantly decreases tissue injury and apoptosis. The findings of the present study are consistent with this study. Despite these effects of Dxp, it is still unclear how Dxp exerts these effects at the molecular level.

The present study indicated that when Dxp was IP administered to rats with AA-induced colitis can reduce the extent of colonic mucosal damage, abate the increase in MDA, TOS and OSI levels, and restore diminished antioxidant enzymes and substances such as SOD, CAT, GPX, GSH and TAC. These effects can be attributed to the anti-oxidant, anti-inflammatory and anti-apoptotic effects of Dxp. These data also suggest that Dxp is more effective when administered after the induction of colitis.

In conclusion, the present study employed biochemical and histopathological analysis to demonstrate that oxidative stress and apoptosis are elevated in IBD. This, in turn, showed that oxidative stress and apoptosis may have a major role in IBD pathogenesis. These findings indicate that studies on IBD treatment will continue to aim to reduce the effects of the ROS. The beneficial effects of Dxp on tissue lipid peroxidation, protein oxidation, and anti-oxidant systems suggested that it may represent a treatment option to alleviate the spread of IBD.

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