THE EFFECTS OF ALLOPURINOL ON REDOX SENSITIVE TRANSCRIPTION FACTORS, PRO-INFLAMMATORY CYTOKINES AND HEME OXYGENASE-1 IN ACETIC ACID-INDUCED COLITIS IN RATS

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ABSTRACT

Introduction*: Oxidative stress plays an important role in pathophysiology of inflammatory bowel diseases. We aimed to investigate the effects of antioxidant allopurinol (AP) and the potential mechanisms leading amelioration upon acetic acid induced colitis.*

Materials and methods*: Twenty-eight rats were divided in four groups. Control rats received 1 mL of NaCl (0.9%). In the AA group rats received 1 mL of 5% (v/v) acetic acid. The rats in the AAAP group received 1 mL of 5% (v/v) acetic acid. Additionally these rats received 100 mg/kg AP. AP group rats received only 100 mg/kg AP. Histopathological, biochemical and western blot analysis were done.*

Results*: In the AA group, colonic injury scores, malondialdehyde (MDA), NF-κB p65, TNFα, COX2, AP-1 and IL-6 levels were significantly higher than in the control group (P<0.05). In the AAAP treatment group the colonic injury scores, MDA, NF-κB p65, TNFα, COX2, AP-1 and IL-6 levels significantly decreased when compared to the AA treated rats (P<0.05). The expression of HO-1 in the AAAP group significantly increased when compared to the AA treated rats (P<0.05).*

Conclusion*: Intra colonic acetic acid instillation causes severe intestinal injury accompanied to increased oxidative stress. AP treatment ameliorates this injury by overcoming oxidative stress and regulating cellular redox balance in favor of antioxidant defense mechanisms probably via manipulation of redox sensitive transcription factors.*

Keywords*: Oxidative Stress, Allopurinol, Acetic Acid, Colitis, Redox Sensitive Transcription Factors.*

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Introduction

Ulcerative colitis (UC) and Crohn's disease (CD) are chronic and idiopathic intestinal inflammatory disorders. Although the pathophysiology of these disorders is not completely understood, accumulated experimental and clinical data suggests that increased oxidative stress plays important role in these diseases initiation and progression[(1)]. Xanthine oxidase (XO) enzyme is one of the major pathways for excessive reactive oxygen species (ROS) generation, and this enzyme is present at the highest level in the intestine. XO-derived ROS causes tissue injury in various ways such as direct damaging cellular components, activation of nuclear factor-kappa B (NF-κB) signaling pathway, and upregulation of inducible nitric oxide synthase (iNOS)[(2, 3)]. Allopurinol (AP) is a competitive inhibitor of XO enzyme and has dose dependent free radical scavenging ability[(4)].

Additionally, AP decreases tumor necrosis factor-alpha (TNF-α) and the expression of intercellular adhesion molecules-1 (ICAM-1) production. It has been also reported that AP can down-regulate ROS dependent activation of NF-κB which is a very important mediator of tissue inflammation. Useful effects of XO inhibition have been reported with AP and amflutizole treatment in different colitis models[5-7].

Heme oxygenase-1 (HO-1) is considered as a major cellular defense system against oxidative insults. In increased oxidative stress conditions HO-1 production is upregulated by redox sensitive transcription factors, including NF-κB, activator protein-1 (AP-1), and nuclear factor E2-related factor-2 (Nrf2)[8]. Cyclooxygenase-2 (COX-2) is also induced mainly at sites of inflammation by above factors like HO-1 enzyme. It has been demonstrated that intestinal inflammation can be prevented by inhibition of COX-2 expression[9]. At the present day, it is well known that synthesis pro-inflammatory mediators and antioxidant defense enzymes are strictly controlled at the nuclear level by aforementioned redox-sensitive transcription factors. Therefore, development of the new treatments targeting the manipulation of these redox sensitive signaling is considered as ideal strategies to prevent or treat oxidative stress-related diseases.

In contrast to drugs that have been already used in inflammatory bowel diseases (IBD) treatment including 5-ASA, corticosteroids and biological agents, allopurinol is cheap, and has been generally regarded as a safe drug. All these data about AP suggest that AP may be a considerably proper candidate for prevention of intestinal inflammation. In this study we aimed to investigate the effects of AP treatment on redox sensitive transcription factors (AP-1, NF-κB, Nrf2, and TNF-α) and these factors mediated synthesis of pro-inflammatory COX-2 interleukin-6 (IL-6) and antioxidant HO-1 enzyme in acetic acid-induced colitis in rats.

Material and methods

Animals

Healthy, male, Albino, Wistar strain rats, weighing 250-300g, were purchased from the Firat University Laboratory Animal Research Center (Elazig, Turkey). The animals were housed at temperature-controlled (24±1 °C), humidity of 55 ±5%, alternating 12 h light/12 h dark cycles, with normal rat chow and fresh water ad libitum. Rats were deprived of not water for 24 h prior to the induction of chemical colitis. The study protocol was in accordance with the guidelines for animal research and approved by the Ethics Committee of Firat University Medical Faculty.

Experimental Design and Induction of Colitis

Twenty eight rats were divided four group: Control group (C), acetic acid group (AA), acetic acid+100 mg/kg allopurinol (AAAP) group, and only 100 mg/kg allopurinol (AP) group. Each group consisted of seven rats. Fasted animals were lightly anesthetized with ether. Control rats received 1 mL of NaCl (0.9%) by intracolonic with a flexible plastic rubber catheter with an outside diameter of 2 mm which was inserted 8 cm into the colon via the anus. In the AA group rats received 1 mL of 5% (v/v) acetic acid in 0.9% NaCl by intracolonic with the same tip of cannula in the control group (10). The rats in the AAAP group were subjected to the same procedure in the AA group rats. Additionally, these rats received 100 mg/kg AP (Ürikoliz® 300 mg tablet, obtained from Sandoz, Turkey) by intraperitoneal (ip) route at the same day of the colitis induction and AP treatment was continued for seven consecutive days. To avoid chemical, infectious peritonitis and achieve optimal dissolution, the AP was dissolved in sterile water and then titrated to pH 11 with NaOH under strict sterile conditions[7].

Allopurinol treatment solutions were prepared and used daily. In the AP group rats were subjected to the same procedure in the control group rats. Additionally, in this group rats received AP treatment at the same dose, route and duration in the AAAP group rats. At the end of the seventh day all rats were decapitated. The colon was removed at the level of 8 cm from the anus. The colon were opened longitudinally, and rinsed with saline solution. Then, colon samples were cut in two parts. One part fixed in 10% neutral buffered formalin for histopathological examination. Other tissue samples were stored at -70°C for subsequent measurement of tissue malondialdehyde (MDA), NF-κB, AP-1, Nrf2, and TNF-α, HO-1, COX2 and IL-6 levels.

Histopathological Examination of Colon

Distal colon segments which were fixed in 10% phosphate-buffered formaldehyde, embedded in paraffin blocks and sliced in 5 μm thickness sections. These sections were stained with hematoxylin

and eosin for light microscopic examination. The tissue slices were scanned and scored by two expert pathologists who were unaware of sample assignment to the experimental groups. Histological scoring was determined by examining each specimen for following features of damage: submucosal edema, vasodilation, inflammatory cell infiltration and necrosis. The degree of intestinal injury was scored to above features using a scale of 0-3 (normal: 0, mild: 1, moderate: 2, severe: 3) like previously described[11].

Determination of Colonic Malondialdehyde Levels

The colonic samples (100 mg) were homogenized in 1 mL of 0.05M Trizma base buffer (pH 7.4). The homogenates were centrifuged (16,000g, 4 ºC) for 30 min. The supernatant was collected and stored at -80 ºC. The colonic MDA level was measured using the fully automatic High Performance Liquid Chromatography (HPLC) (Shimadzu, Kyoto, Japan) equipped with a pump (LC-20AD), an ultraviolet-visible detector (SPD-20A), an inertsil ODS-3 C18 column (250x4.6 mm, 5m), a column oven (CTO-10ASVP), an autosampler (SIL-20A), a degasser unit (DGU-20A5), and a computer system with LC solution Software (Shimadzu, Kyoto, Japan)[12].

Western Blot Analyses

Protein extraction was performed by homogenizing the rat colonic tissue samples in 1 mL ice-cold hypotonic buffer A, containing 10 mM HEPES (pH 7.8), 10 mM KCL, 2 mM MgCl2, 1 mM DTT, 0.1 mM EDTA, and 0.1 mM phenylmethylsulfonylfluoride (PMSF). To the homogenates was added 80 μL of 10% Nonidet P-40 (NP-40) solution, and the mixture was centrifuged for 2 min at 14,000g. The supernatant was collected as a cytosolic fraction for HO-1. The precipitate, containing nuclei, were washed once with 500 μL of buffer A plus 40 μL of 10% NP-40, centrifuged, suspended in 200 μL of buffer C [50 mM HEPES (pH7.8), 50 mM KCl, 300 mM NaCl, 0.1 mM EDTA, 1 mM DTT, 0.1 mM PMSF, 20% glycerol], and centrifuged for 5 min at 14,800g.

The supernatant containing nuclear proteins was collected for other proteins[13]. Concentration of the protein was determined using a protein assay kit supplied by Sigma (St. Louis, MO, USA). Sodium dodecyl sulfate-polyacrylamide gel electrophoresis sample buffer containing 2% β-mercaptoethanol was added to the supernatant. Equal amounts of protein (50 μg) were electrophoresed and subsequently transferred to nitrocellulose membrane (Schleicher and Schuell Inc., Keene, NH, USA). Blots on nitrocellulose membrane were washed twice for 5 min each in PBS and blocked with 1% bovine serum albumin in PBS for 1 h prior to application of the primary antibody. The antibody against Nrf-2, TNFα, COX2, AP-1, and IL-6 was the purchased from Santa Cruz Biotechnology, Inc. (Santa Cruz, CA, USA). Antibodies against HO-1 and NF-ϰB p65 were purchased from Abcam (Cambridge, UK). Primary antibody was diluted (1:1000) in the same buffer containing 0.05% Tween-20. The nitrocellulose membrane was incubated overnight at 4 °C with protein antibody. The blots were washed and incubated with horseradish peroxidase-conjugated goat anti-mouse IgG (Abcam, Cambridge, UK). Specific binding was detected using diaminobenzidine and H_2O_2 as substrates. Protein loading was controlled using a monoclonal mouse antibody against β-actin antibody (A5316; Sigma). Bands were analyzed densitometrically using an image analysis system (Image J; National Institute of Health, Bethesda, USA).

Statistical Analyses

Data are given as means. Sample size was calculated based on a power of 85% and P value of 0.05. Given that assumption, a sample size of seven per treatment was calculated. The data were analyzed using the GLM procedure of SAS (2002). The treatments were compared using ANOVA and student's unpaired t test; $P<0.05$ was considered statistically significant. Between group differences in latencies were analyzed by the analysis of variance for repeated measurements (ANOVAR) followed by Fisher's post hoc test for all groups.

Results

Colonic Malondialdehyde Levels

Colonic MDA levels were significantly different among the groups ($P<0.05$). In the AA group, colonic MDA levels were significantly higher than in the control group ($P<0.05$). In the AAAP group, MDA levels were significantly decreased compared to the AA group ($P<0.05$). In the AP group, colonic MDA levels are the same in the control group. Mean ± SD values of colonic MDA levels are summarized in Table 1.

Groups (n)	Intraperitoneal saline	Intraperitoneal TAA*	Intraperitoneal AP**
Control (7)	+	-	-
TAA (7)	-	+	-
TAA+25AP (7)	-	+	+
TAA+50AP (7)	-	+	+
TAA+100AP (7)	-	+	+

Table 1: Experimental protocol of the study.
***: TAA: 300 mg/kg intraperitoneally for 2 days.*
****: AP: allopurinol was started at the same day with the first dose of TAA and continued for seven consecutive days with 24 hours intervals.*
Abbreviations: AP: Allopurinol, TAA: thioacetamide.
•Group TAA+25AP: dose of allopurinol was 25 mg/kg
•Group TAA+50AP: dose of allopurinol was 50 mg/kg.
•Group TAA+100AP: dose of allopurinol was 100 mg/kg.

Western Blot Analyses

NF-κB p65, AP-1, TNF-α, COX2 and IL-6 in the colonic tissue of AA treated group of rats was significantly higher than the control group (P<0.05) (Figure 1). In contrast, AP treatment significantly decreased the expression of NF-κB p65, AP-1, TNF-α, COX2 and IL-6 in the AAAP group when compared to the AA treated rats (P<0.05). As shown in Figure 1, AP treatment increased Nrf2 accumulation in the nuclear fraction (P<0.05), and increased the expression of HO-1 in the AAAP group when compared to the AA treated group of rats (P<0.05).

Histopathological Results

Single intracolonic 5% acetic acid application caused severe edema, vasodilation and necro-inflammation in the colon compared to the control group (All of them; P<0.05). Colonic edema, vasodilation and necro-inflammation scores of AAAP group were significantly lower than in the AA group (All of them; P<0.05). Colonic edema, vasodilation and necroinflammation scores are shown as mean ± SD values in Table 1.

Discussion

Single intracolonic 1 mL 5% acetic acid application produced severe bloody diarrhea in the AA and AAAP group rats at the end of the first day experiment. The biochemical assay of this study clearly demonstrated that intracolonic instillation of acetic acid causes intestinal damage accompanied to increased oxidative stress, demonstrated by high MDA levels in the AA group. Also significantly increased levels of pro-inflammatory mediators (NF-κB, AP-1, TNF-α, IL-6 and COX2) were ascertained in this group. Histopathological examination of colon samples revealed obvious high intestinal damage scores in the same group.

Inflammatory bowel diseases (including CD and UC) are chronic idiopathic inflammatory disorders of the intestine. The pathophysiology of these diseases is not yet completely understood[1]. However, at present it is well known that chronic intestinal inflammation is associated with over production of ROS[7, 14]. It have been demonstrated that increased ROS and presence an imbalance between ROS and antioxidant defense mechanisms in favor of oxidative stress contribute to the onset and progression of tissue damage in IBD patients and experimental colitis models[15-17].

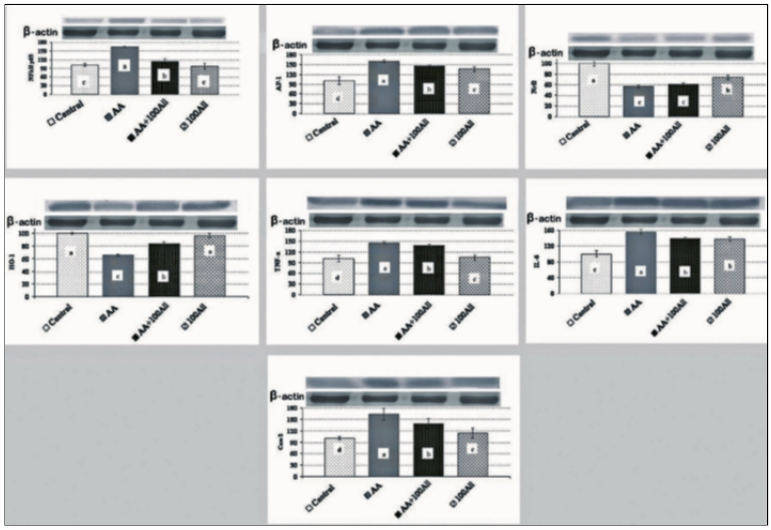

Fig. 1: Effect of AP on colonic NF-κB-p65 (Panel A), activator protein-1 (AP-1; Panel B), nuclear factor E2-related factor-2 (Nrf2; Panel C), heme oxygenase-1 (HO-1; Panel D), tumor necrosis factor-alpha (TNF-α; Panel E), interleukin-6 (IL-6, Panel F) and cyclooxygenase-2 (COX-2; Panel G) expressions in rats. Values are means ± standard deviation of the mean. Western blot analysis was repeated at least 3 times (n=3) and a representative blot is shown. Data points with different superscripts are significantly different at the level of P<0.05 by Fisher's multiple comparison test. Control: control rats, AA: intra colonic acetic acid instilled rats, AAAP: intra colonic acetic acid instillation plus 100 mg/kg allopurinol treated rats.

Irrespective source of execessive producing ROS, they can lead to tissue damage via direct react with cellular components such as lipids, proteins, and DNA. In addition, ROS can activate many pro-inflammatory mediators (NF-kB, TNF-α, IL-6), causes over production of cytokines, chemokines and adhesion molecules. The efficacy of current

standard IBD treatment substances is depends on their antioxidant actions and anti-inflammatory effects such as blocking TNF-α and inhibition of neutrophil function[6]. But these treatment modalities have many adverse effects. The lack of curative treatments with limited side effects necessitate developing new effective therapeutic options. AP is a competitive inhibitor of XO enzyme and has dose dependent free radical scavenging ability[4].

Variables	Groups				
	Control	TAA	TAA+25AP	TAA+50AP	TAA+100AP
AST (U/L)	255.2±26*	982.0±178.6**	564.4±87.9	433.0±67.9	312.8±52.2
ALT (U/L)	101.7±15.0*	558.1±278.0**	244.0±90.2	182.5±38.2	124.1±29.6
Necrosis	.00±.00*	2.2±0.7**	1.2±0.4	0.57±0.53	0.53±0.42
Inflammation	.00±.00*	2.14±0.8**	1.1±0.3	0.48±0.28	0.48±0.28
MDA (μg/ml)	0.079±0.007*	0.176±0.010***	0.139±0.009	0.135±0.009	0.124±0.008

Table 2: Serum AST, ALT, liver necrosis, and inflammation scores and liver MDA levels in the groups.
Each result represents the mean value of experiments performed in duplicate ± SE.
** :p <0.05 between control and TAA group.*
*** : p <0.05 among TAA group and TAA + 25AP, TAA + 50AP, TAA + 100AP groups.*
****: p <0.05 among TAA group and TAA + 50AP, TAA + 100AP groups.*
Abbreviations: AP: Allopurinol, AST; Aspartate Transaminase, ALT; Alanine Transaminase, MDA; Malondialdehyde, TAA: thioacetamide .

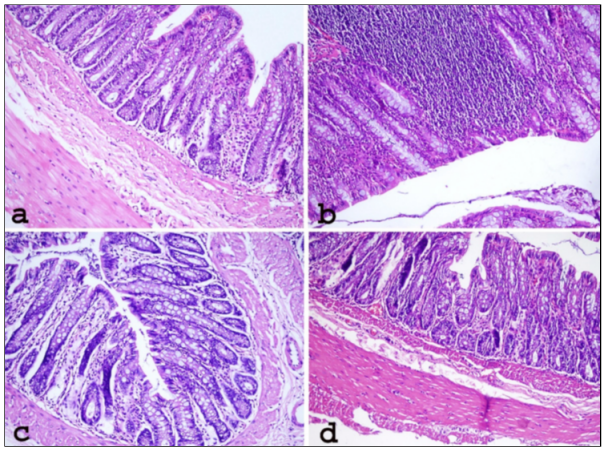

Fig. 2: Hematoxylin and eosin staining of representative colon sections in acetic acid induced colitis and the effect of allopurinol treatment on this intestinal damage. Control (Panel a) showing normal colonic architecture (hematoxylin and eosin (H&E), 200x). AA (Panel b) shows severe intestinal edema, vasodilation and necroinflammation (H&E, 200x). AAAP (Panel c) shows mild intestinal inflammatory cell infiltration (H&E, 200x). AP (Panel d) shows normal architecture of the colon (H&E, 200x). Each group consists of 7 animals.

Additionally direct free radical scavenging ability, AP decreases TNF-α and intercellular adhesion molecules-1 (ICAM-1) production. Also AP can down-regulate ROS dependent activation of NF-κB[18]. In contrast to drugs that have been already used in IBD treatment including 5-ASA, corticosteroids and biological agents, AP is cheap, and has been generally regarded as a safe drug. All these favorable effects of AP suggested that it may be a good candidate for prevention of intestinal damage. In this study we used AP at very high dose (100 mg/kg) especially for evaluation the effects of its direct free radical scavenging feature beyond its XO inhibitor effect on intestinal damage. It have been reported that AP has direct free radical scavenger effect with intrinsic antioxidant properties especially at up to 50 mg/kg doses[7, 19].

In the present study although hemorrhagic diarrhea continued throughout experiment in the AA group rats, in the AAAP group hemorrhagic diarrhea ceased at the end of the third day of experiment. Significantly reduced MDA levels and a concomitant lower intestinal damage scores in the AAAP group suggest high dose AP treatment can successfully prevent oxidative stress-induced intestinal damage itself.

Recently, it is demonstrated that AP has a protective effect against ischemia/reperfusion injury in several experimental models. In one study, the administration of AP decreased the free oxygen radical production and damage during ischemia/reperfusion of the small intestine[20]. Our results are consistent with the results of mentioned study.

NF-κB is a redox sensitive transcription factor and activation this pathway is believed a key phenomenon in both the initiation and progression of the inflammatory reactions. In increased oxidative stress conditions, oxidants can degrade NF-κB in the cytoplasm and leads to translocate to the nucleus of activated NF-κB. Activated NF-κB induces the expression of many pro-inflammatory cytokines, chemokines and adhesion molecules[18, 21].

In our study, the results of AA group demonstrated that intracolonic acetic acid instillation markedly increased MDA levels. Also remarkably increased levels of pro-inflammatory cytokines including TNF-α, IL-6 and COX2 accompanied to this increased oxidative stress in the same group. This finding supports the idea that activation of the NF-κB signaling pathway plays key role in the expression of the many pro-inflammatory cytokines and enzymes in response to presence of excess ROS[10].

In the AAAP group, AP treatment significantly reduced the expression levels of NF-κB almost at the levels in the control group. Superoxide ($O2^{-}$•) anion is a primary ROS and generated in both physiological and pathological conditions by neutrophils or colonic epithelial cells in the intestine. It has been reported that $O2^{-}$• anion cause tissue damage by converted other more potent free radicals and scavenging this anion significantly improves intestinal injury in acetic acid induced colitis models[6, 7]. In the present study, we did not measure $O2^{-}$• level.

Significant decreased MDA levels in the AAAP group suggested that high dose of AP treatment effectively scavenged $O2^{-}$• anion and consequently prevented generation of new more potent free radicals. Thus, absence free radicals in the milieu might be resulted down regulation of NF-κB activation in the AAAP group. Neutrophils are also another important $O2^{-}$• source at inflammation site[6]. Histopathological results revealed significant reduced colonic inflammatory cell infiltration in the AAAP group. These beneficial effects of AP treatment might be due to inhibition expression of inflammatory enhancer molecules (chemokines and ICAM-1) via inactivation of NF-κB pathway.

AP-1 is another major redox sensitive transcription factor that up regulates many pro-inflammatory mediator genes in response to oxidative insults. Beneficial effects of AP-1 inhibition have been reported in various intestinal damage models related to increased oxidative stress[22, 23]. Increased AP-1 levels accompanied to increased intestinal oxidative stress in the AA group. In contrary to NFκB, although AP treatment significantly decreased oxidative stress, it could not effectively inhibit AP-1 activation. The lack of study about the effects of AP treatment on AP-1 regulation in colitis model in the literature makes interpretation difficult our results.

Nrf2 is a member of redox sensitive transcription factors and plays central role in regulation of cellular antioxidant genes expression. Under normal conditions, Nrf2 is found in the cytoplasm by Ketch-like ECH-associated protein 1 (Keep 1), which stimulates proteasome Nrf2 degradation. In increased oxidative stress conditions, Nrf2 are dissociated from Keep 1 and translocate into the nucleus to activation of many antioxidant defense genes. The up regulation of these antioxidant defense mechanisms at the level of their genes implicated in many inflammatory signaling pathways for neutralizing ROS and attenuation inflammation[24].

In the present study, in contrast to the NFκB and AP-1 expression, intracolonic acetic acid application caused significant depletion of colonic Nrf2 levels. Despite AP treatment successfully reduced oxidative stress, it could not upregulate of Nrf2 expression. This is the first study that evaluates the effects of AP treatment on the regulation of Nrf2 signaling pathway. In spite of marked decreased oxidative stress by AP, the lack of Nrf2 upregulation may be related to oxidative stress intensity in this model. We used 5% acetic acid for inducing colitis. At this high concentration of acetic acid causes severe oxidative stress induced intestinal damage[7]. Our histopathological and biochemical results confirmed significant increased MDA levels and intestinal damage scores. In this context, the lack of Nrf2 signaling pathway activation may be expect, because it has been reported that Nrf2 comes to active state in case of the presence low oxidative stress intensity. In contrast to Nrf2, the NF-κB and AP-1 signaling pathways activation develop at the higher levels of oxidative stress intensity. At the higher level of oxidative stress conditions NF-κB and AP-1 pathways are mainly responsible for the cellular antioxidant systems regulation.

Heme oxygenase-1 is an important antioxidant mechanism and inducible in response to various noxious stimuli. Under increased oxidative stress condition HO-1 production can be up regulated by redox sensitive transcription factors, including NF-κB, AP-1, Nrf-2[8]. Beneficial effects of HO-1 induction by conventional therapies and other substances have been reported in different colitis models[25, 26]. In our study, intestinal HO-1 levels decreased to half of in the control HO-1 levels by intracolonic acetic acid administration. AP treatment clearly increased HO-1 levels and a concurrent decreased MDA level and NF-κB activation in the AAAP group. These findings demonstrate that AP can also effectively upregulate HO-1 enzyme by the mediation of prevention of oxidative stress and NF-κB activation like conventional therapies and other substance. Upregulation HO-1 enzyme and its metabolites such as carbon monoxide, biliverdin, and iron may be other factors that contributed favorable effects of AP treatment. In this study we did not measure level of mitogen-activated protein kinase (MAPK). MAPK signaling pathway also involves in sensing and cellular response, leading to

enhanced antioxidant defense mechanisms in higher increased oxidative stress conditions like NF-κB signaling pathway[24]. MAPK pathway might be another factor that mediated this upregulation of HO-1 expression.

Cyclooxygenase-2 (COX-2) production is also upregulated by redox sensitive transcription factors at inflammation sites. In the presence of oxidative stress it has been shown that increased NF-κB pathway activation results upregulation of COX2 expression. Useful effects of NF-κB dependent COX2 inhibition have been reported in various inflammatory models with N-Acetylcysteine plus mesalamine and curcumin treatments[27, 28]. This is the first study that evaluates the effects of AP treatment on COX2 expression in this colitis model. The colonic levels of COX2 increased at almost two fold in the control group levels by intracolonic acetic acid instillation. AP treatment clearly decreased COX2 level accompanied to reduced NF-κB levels like previously reported. This decreased COX2 levels may be another factor that contributed beneficial effects of AP treatment on colonic inflammation in the AAAP group.

The addition of AP treatment to the immunomodulators resulted in improved disease activity in CD and UC patients who nonresponders to thiopurine drugs. In these exist studies, although the shifting thiopurine metabolism by AP treatment have been responsible from favorable results, the exact contribution mechanism of AP treatment was not[29, 30]. On the light of the present study results, it may be expect that antioxidant and anti-inflammatory effects of AP may be also provide important contributions on improved disease activity in above studies.

In summary, intracolonic 5% acetic acid instillation develops severe colitis. Significant increased oxidative stress, pro-inflammatory mediators and decreased cellular antioxidant enzymes accompany to this colitis. As a cheap and safe molecule, AP effectively prevents development of oxidative stress, inhibits activation of pro-inflammatory mediators and activates cellular antioxidant defense mechanisms.

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