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Moxifloxacin's Therapeutic Effects in AA-Induced Colitis: Anti-Inflammatory Action through NF-κB Pathway Inhibition, Including TNF-α Pathway and Downstream Inflammatory Processes

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Abstract

Objectives: The aim of the present study was to evaluate possible therapeutic effects of moxifloxacin against acetic acid-induced colitis in a rat model.

Methods: Forty adult Wistar rats were separated into 4 groups, including the negative control group, acetic acid (AA) group, AA + sulfasalazine (100 mg/kg/day) group, and AA+ moxifloxacin (MFX) (8 mg/kg/day) group. Experimental colitis was induced in rats by rectal administration of (4%v/v) acetic acid. Rats with colitis were received either MFX 8mg/kg/day or sulfasalazine 100mg/kg orally for 7days.The parameters measured are macroscopical assessment, colon weight (indicator of edema) and the measurement of concentrations of the proinflammatory cytokine TNF-α, oxidative stress marker malondialdehyde (MDA), adhesion molecule selectin and the inflammatory mediator NF-κB in colonic tissue homogenate.

Results: This study had shown that AA elevate macroscopical scores, colon weight and biochemical homogenate parameters and all measured parameters were significantly reduced by both moxifloxacin and sulfasalazine with nonsignificant difference between them.

Conclusion: The study explain that moxifloxacin possesses therapeutic potential in in AA induced colitis and its anti-inflammatory actions may involve inhibition of NF-κB inflammatory pathways including TNF-α pathway with its downstream inflammatory process such as elevation of adhesion molecule synthesis and oxidative species overproduction in the colonic tissues.

Keywords: Colitis, ulcerative, moxifloxacin, sulfasalazine, NF-κB, TNF-α

Introduction

Inflammatory bowel disease (IBD) encompasses a group of heterogeneous diseases that entail chronic, relapsing gastrointestinal tract inflammation of inexactly known etiology and pathogenesis. IBD etiology may involve the host immune system, genetic variability, and environmental factors. IBD is clinically classified as Crohn's disease (CD) or ulcerative colitis (UC). Ulcerative colitis causes long-lasting inflammation and superficial ulcerative disease in the colon, whereas CD is a transmural disease often associated with granuloma formation and can appear in any part of the gastrointestinal tract.[1] Inflammation in UC involves a rise in proinflammatory cytokines which are the consequence of both the innate and adaptive immunity with interleukin (IL)- 1β, IL-6 and tumor necrosis factor (TNF)-α, as initial perpetrators of inflammation. Another simultaneous contributor of this inflammatory process is oxidative stress which is due to lipid peroxidation leading to elevated reactive oxygen species (ROS) and metabolites. The consequent increase in neutrophil infiltration leads to mucosal edema and further deterioration of mucosa which subsequently disrupts the mucosal defense mechanism and eventually prominent intestinal inflammation.[2] For the management of UC, the most commonly used drugs are sulfasalazine, 5-aminosalicylic acid, corticosteroids, azathioprine, 6 mercaptopurine, calcineurin inhibitors (cyclosporine and tacrolimus), and anti-TNF-α antibodies, which aim to control inflammation of the mucosa, complications, and disease relapse. Regrettably, the available drugs are not much effective as they need prolonged use and also result in drug intolerance, adverse drug reactions, and allergic reactions,[3] so there is still need for development of new more effective and safer agents against UC.

Acetic acid (AA) experimentally induced colitis is a well-known model for the study of IBD. The animal AA-induced UC and human disease share common pathophysiological features as well as sensitivity to drug treatment. For instances, colonic changes such as mucosal inflammation, ulceration, hemorrhage, and weight loss, which occur following intrarectal administration of AA in rats are also common in human UC. Therefore, AA colitis in rats was used in the present study.[4]

The most common cytokines involved in UC pathogenesis are TNF-α, interleukin (IL-1β) and IL-6.[5] TNF-α plays a prudent role in the development of UC by augmenting the inflammatory response through activation of a cascade of immune responses. TNF-α also activates the production of other chemical mediators, proteases, and pro-inflammatory markers, activating chemotaxis and infiltration of inflammatory cells, leading to ulceration and hemorrhage, by generation of cytotoxic reactive oxygen species.[6]

Increased levels of plasma malondialdehyde (MDA) in IBD is an important indication of oxidative stress.[7] AA rectal administration was demonstrated to induce an elevation in malondialdehyde (MDA) levels in the colonic tissue. Previous studies have also shown that MDA levels are increased in IBD patients. 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.[8]

The selectin family of adhesion molecules, which comprises E-selectin, P-selectin, and L-selectin, predominantly mediates the first steps of cellular adhesion and previous studies have shown upregulation of E-selectin and P-selectin in the gut of patients with IBD.[9] Also, L-selectin ligands were expressed at sites of chronic inflammation, including the small intestine of patients with CD.[10]

Nuclear factor-kappa B NF-κB is a kind of multi-functional nuclear transcription factor involved in regulating gene transcription to influence pathological evolution of inflammatory and immune diseases. Numerous literatures suggest that nuclear factor-kappa B (NF-κB) pathway plays a central role in regulating the release of cytokines in patients with UC and participates in the inflammation and immune response in the intestinal tract of UC.[11]

Moxifloxacin (MXF) is a fluoroquinolone with activities against both gram-positive and gram-negative bacteria.[12] Previous study concluded that MXF exerts anti-inflammatory effects by suppresses the secretion of proinflammatory cytokines and by inhibiting NF-κB, ERK, and JNK activation.[13] The objective of the present study is to investigate the effects of MFX on AA colitis in rat model including probable beneficial effects on macroscopical features and inflammatory mediators' profile of the disease.

Materials and Methods

Animals

Forty (40) Male Wistar rats (200–250 g) were purchased from animal house of college of pharmacy, Babylon university. The animals were kept under normal environmental conditions with a 12 h light/dark cycle and had free access to water and standard pellet diet. Animals were separately housed in their cages (five per cage) and were acclimatized for at least one week before experiment.[14] Study protocol were approved by institutional animal ethics committee of college of medicine/ Al-Nahrain university.

Drugs and Chemicals

Moxifloxacin hydrochloride powder (Hangzhou Hyper Chemicals Limited/China); Sulfasalazine powder (Hangzhou Hyper Chemicals Limited/China); Glacial acetic acid (Loba chemie/India); Normal saline (Gulf inject/UAE); Diethyl ether (Thomas Baker/ India); PBS (phosphate buffered saline) (Medicago/ Sweden); Rat MDA ELISA Kit (ELK Biotechnology/China); Rat NF-κB ELISA Kit (ELK Biotechnology/ China); Rat Selectin ELISA Kit (ELK Biotechnology/China); Rat TNF-α ELISA Kit (ELK Biotechnology/China).

Induction of AA ulcerative colitis

Colitis was induced by the method used by Manna et al.,2017 and Mohan et al., 2022. The animals were starved 24 h and then anaesthetized by inhalation of diethyl ether. The animals were administered AA intrarectally (2 mL 4%, v/v,) by insertion of pediatric catheter up to 8 cm deep in rectum. The animals were then kept in a head-down position for 2 minutes and then returned to their cages to recover from anesthesia. The rats in the non-colitis group (negative control group) received 2 ml of normal saline by the same procedure. 7 days later, the animals were sacrificed by inhalation over dose of diethyl ether, colon tissue were removed to assess the macroscopic damage and biochemical parameters.[14,15]

Experimental Design

Rats were randomly divided into four groups (n = 10/group). Group 1: negative control group: received 2 ml/rat normal saline intra-rectally at the first day of the experiment and distilled water as a vehicle orally for 7 days;[4] group 2: AA colitis model group: received distilled water as a vehicle orally for 7 days with induction of UC by AA on the first day. Group 3: MFX +AA group: received MFX 8 mg/kg orally for 7 days, dissolved in distilled water,[16] with induction of UC by AA on the first day. Group 4: standard group: sulfasalazine +AA group: received sulfasalazine 100 mg/kg orally for 7 days, dissolved in distilled water[4] with induction of UC by AA on the first day.

The selection of MFX dose of 8 mg/kg was because this dose is equivalent to therapeutic dose in rat dependent on previous study.[17]

Samples collection and preparation

After 24 hours from the final oral dose of the treatments, rats were euthanized with diethyl ether overdose inhalation. The colons were collected from all animals and cleaned with chilled normal saline during the abdomen dissection. The excised colon of all rats was assessed macroscopically and part of sample was used for tissue homogenization for biochemical parameters measurement.[18] Estimation of colonic biochemical parameter levels including estimation the concentrations of each of TNF-α, MDA, Selectin adhesion molecules and the inflammatory mediator NF-κB in tissue homogenate were assayed using ELISA kits, according to the manufacturer's instructions[5] of (ELK Biotechnology/China). Colonic tissue homogenate preparation was done according to the manufacturer's instructions of ELK Biotechnology ELISA kits and kept at – 80°C[5] until use for estimation procedure.

Colonic segment Weight Assay

Assessment of the edema degree and the severity of colitis was conducted by recording the weight of the distal 8 cm segment of the colon of rats[5].

Macroscopic colonic score (MAC) Assessment

The macroscopic findings of the colonic mucosa were counted on a scale ranging from 0 to 6.[18] Briefly, the grading system is as follows: no macroscopic change (grade 0); no ulcer, mucosal hyperemia only (grade 1); hyperemia and mild edema with little erosion, no ulcers (grade 2); one ulcer or inflammation at one site (grade 3); ulceration or inflammation at two or more site (grade 4); severe ulceration extending > l cm along the entire colon and tissue necrosis (grade 5); and damage covered > 2 cm along the whole colon and tissue necrosis (grade 6).

Biochemical analysis

The content of TNF-α in the colon homogenate done according to ELK biotechnology manufacturer instructions of rat TNF-α ELISA kit.[19] The content of selectin in the colon homogenate done according to ELK biotechnology manufacturer instructions of rat selectin ELISA kit.[20] Malondialdehyde (MDA) content in the colon homogenate done according to ELK biotechnology manufacturer instructions of rat MDA ELISA kit.[21] The content of NF-κB in the colon homogenate done according to ELK biotechnology manufacturer instructions of rat NF-κB ELISA kit.[22]

Statistical Analysis

Data analysis was performed using IBM SPSS version 26.[6] Data were expressed as mean ± standard deviation (SD).[6] The

normality of the data was checked by Kolmogorov Smirnov test[15] and the Shapiro-Wilk test.[6] Data with normal distribution (colon weight, colonic TNF-α, colonic selectins, colonic MDA and colonic NF-κB levels) were analyzed by one-way ANOVA with Tukey's post-hoc test for pairwise intergroup comparisons.[8] Non-parametric data (macroscopical scores) were analyzed by the Kruskal-Wallis H test for multiple groups, when significant differences were determined, comparisons between different groups were carried out using the Mann Whitney U test.[8] Statistical significance was fixed at a value of < 0.05.[6]

Results

Effect on colonic segment Weight

Acetic acid (AA) significantly increased the colon segment weight as compared with the negative control group ($P < 0.05$). Treatment with either sulfasalazine or MFX significantly decreased the colon segment weight compared with the AA colitis group ($P < 0.05$). Although sulfasalazine slightly exerts higher lowering effect on colon segment weight than MFX, the difference between sulfasalazine and MFX on colon segment weight was insignificant ($P < 0.05$). See Table 1.

Effect on Macroscopic score

In comparison to the negative control rats, AA caused severe edematous inflammation in the colon, with a much higher macroscopic score of colonic damage ($P < 0.05$) (Figure 1: A and B). The mucosa was ulcerated, edematous and hemorrhagic in appearance. Meanwhile, the MFX (8 mg/kg/day) and sulfasalazine (100 mg/kg/day) treated groups significantly alleviated the severity of the gross lesion score as compared to the AA group with insignificant difference between them in macroscopical scores ($P < 0.05$) but sulfasalazine was slightly more potent than MFX regarding the effect on these scores. See (Figure 1: C and D) and Table 2.

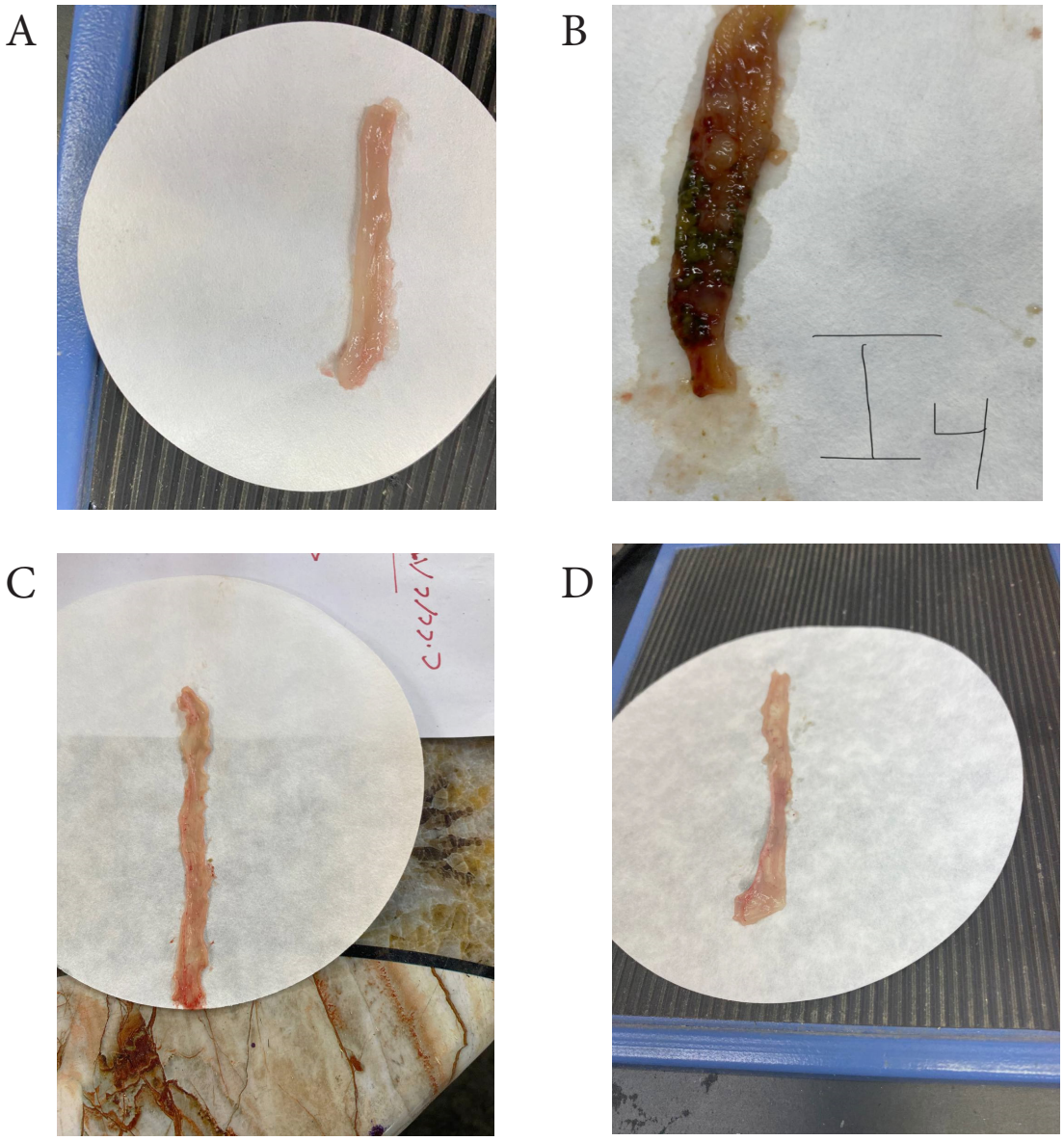

Fig. 1 **Gross features of A: normal colon showing shiny serosa and healthy mucosa in negative control group; (B) colitis group colon with marked edema, congestion, ulceration and necrosis. (C) colon of sulfasalazine treated group animals showing nearly normal mucosa with no (or mild) edema, congestion, or ulceration;(D) colon of MFX treated group animals showing nearly normal mucosa with no (or mild) edema, congestion, or ulceration.**

Table 1. **Biochemical parameters and colon segment weight (CW) in colonic homogenate of different groups in the study.**

Variable	Negative control (healthy) group†	Acetic Acid induced colitis group†	Sulfasalazine treated group†	MFX treated group†
CW(g)	1.30 ± 0.06 A	3.38 ± 0.27 B	1.54 ± 0.04 AC	1.67 ± 0.10 C
Colonic TNF-α pg/ml	88.41 ± 8.78 A	449.58 ± 17.52 B	150.58 ± 17.95 C	164.01 ± 13.41 C
Colonic Selectin pg/ml	143.36 ± 13.29 A	493.72 ± 12.44 B	175. 70 ± 19.41 C	182.42 ± 12.73 C
Colonic MDA pg/ml	81.02 ± 10.53 A	267.07 ± 19.13 B	123.45 ± 13.09 C	127.23 ± 12.10 C
Colonic NF-κB ng/ml	1.51 ± 0.13 A	8.84 ± 0.26 B	1.97 ± 0.19 C	2.14 ± 0.18 C

Table 2. **Macroscopical (MAC) scores of different groups in the study.**

Variable	Negative control (healthy) group†	Acetic Acid induced colitis group†	Sulfasalazine treated group†	MFX treated group†
MAC Score	00.0 ± 00.0 A	5.67 ± 0.52 B	0.83 ± 0.41 C	1.33 ± 0.52 C

Biochemical parameters of colon inflammation

There was a significant increase in colonic NF-κB, TNF-α, MDA and the Selectin adhesion molecules levels in the AA-induced colitis group compared to the normal control group ($P < 0.05$). Both MFX and sulfasalazine significantly reduced all these parameters compared to AA colitis group with insignificant difference between the two drugs in this regard, but sulfasalazine was slightly more potent in lowering the above biochemical measurements than MFX ($P < 0.05$). See Table 1.

Capital letters for comparison; different letters indicate significant difference(P<0.05); similar letters indicate insignificant difference (P value ≥0.05); MAC Score: Macroscopical Score; † values expressed as mean ± Standard deviation (SD).

Capital letters for comparison; different letters indicate significant difference(P<0.05); similar letters indicate insignificant difference; (P value ≥ 0.05); g: gram; MDA: malondialdehyde; ml: milliliter; ng: nanogram; NF-κB: Nuclear factor Kappa B; pg: Picogram; TNF-α: tumor necrosis factor – alpha; † values expressed as a mean ± Standard deviation (SD).

Discussion

Ulcerative colitis is an inflammatory bowel disease with rising prevalence in developing countries which involves the inflammation in the colon and rectum and if not treated or managed appropriately could lead to colorectal cancer.[2] Failure or adverse reactions of the available medications for UC treatment leaves the patient with little choice other than colectomy. This made the research for more effective drugs with lower adverse effects very important target.[23] Acetic acid-induced UC is one of the standardized and widely used experimental models for studying inflammatory bowel disease[24] was used in the present study.

In this study, there is a significant increase in colon weight in AA induced ulcerative colitis animal compared to negative control rats. The rat colon weight was markedly elevated following the intra-rectal injection of AA, and this increase was accompanied by severe macroscopic lesions and ulceration of the colon tissue. Additionally, AA produced severe inflamed edematous colons accompanied by a significant macroscopic scoring of colon lesions as compared with the negative control. Similar results were reported by previous studies such as Rehman et al. (2022),[2] Shahid et al. (2022)[25] and Oubaid et al. (2023),[18] after AA rectal administration to rat animals. Acetic acid induced UC in the colon was attributed to the release of protons to the epithelium, thereby causing intracellular epithelial acidification that causes massive epithelial injuries.[25]

The major indicator of UC disease is the release of pro-inflammatory cytokines, such as TNF-α and IL-6, which are crucial to the start and progression of intestinal inflammation, [26]. In colitis, damage to the epithelium increases the uptake of bacterial endotoxins like LPS (lipopolysaccharide) by the epithelium. TLR4 in the intestine wall identifies these LPSs.[27] The interface of LPS with TLR4 activates a signaling flow, which leads to the activation of NF-κB. This phenomenon leads to creation of proinflammatory cytokines including TNF-α and IL-1β.[27] This mechanism may interpret the results of the present study in which AA administration significantly increased colonic TNFα concentration compared to healthy negative control rats. It is well documented in previous studies [28-30] that AA colitis induction caused marked elevation of TNF-α cytokine and these results are consistent with the present study.

Nuclear transcription factor kappa B (NF-κB) plays a key role in UC by inducing the production of inflammatory mediators, such as TNF-α, IL-1β and cyclooxygenase-2 (COX-2) in inflammatory mucosae.[31] The previous reports implicated an enhanced level of NF-κB with increased levels of oxidative stress and inflammatory cytokines (that are causes of increased colon epithelial injuries leading to colitis).[25] Ample indications have revealed that following colitis, gene expression of inflammatory cytokines increased in the colonic tissue.[27] These data

are consistent with the present study which showed that AA administration significantly elevated NF-κB in colonic tissue. Collectively these data may explain that increased levels of inflammatory cytokines and other inflammatory mediators after AA administration may be mediated by increased activity of NF- κB within inflamed colonic tissue.

Oxidative stress is known to serve a major role in the initiation and progression of IBD. It has been demonstrated that oxidative injury results from the overproduction of free radicals, which leads to the lipid peroxidation (LPO) of membranes and the impairment of cellular proteins and nucleic acids, thereby disrupting epithelial cell integrity and hindering mucosal recovery.[32] MDA is an end-product from the oxidation of polyunsaturated fatty acids, which is a common marker of oxidative stress.[33] AA-induced model of IBD was reported to be associated with an increased production of ROS,[32] also according to wang et al. study (2019) AA caused significant rising in levels of MDA compared to negative control animals.[33] These data come in agreement with the present study in which AA colitis induction caused significant elevation of MDA which is an indicator of lipid peroxidation and used to assess the impact of oxidative stress.[2]

Endothelial selectin is particularly interesting because it is only found in the activated endothelium, in contrast with other adhesion molecules, which have a more widespread tissue distribution. The demonstration of E-selectin can thus be considered conclusive evidence of endothelial activation.[34] In present study AA intrarectal injection caused significant elevation in selectin colonic concentrations. These results come in agreement with previous study in which ulcerative colitis significantly increase the levels of endothelial selectin adhesion molecules.[35] It was reported that inflammatory cytokines including TNFα (observed in inflamed tissues of colon in AA colitis rats in the present study) induce selectin expression,[36] and these cytokines may be the base of this increase in selectin adhesion molecules found in AA animal group in the present study.

Sulfasalazine has been used frequently as refence drug against AA-induced UC model in animals, by reducing disease clinical, histopathological and biochemical inflammatory features of disease[18,33,37] and these data are compatible with results of present study were sulfasalazine significantly reduced macroscopical features and biochemical inflammatory mediators in colonic tissue compared to AA colitis group. The therapeutic benefits of sulfasalazine for UC might at least in part be attributed to its ability to inhibit NF-κB activation, resulting in the downregulation of pro-inflammatory cytokines.[38]

In the present study moxifloxacin, which is a synthetic antibacterial agent of the fluoroquinolone family,[39] exerts a significant anti-inflammatory effect against AA colitis model with effects comparable to these of sulfasalazine with no significant difference between them in all measured parameters. Really MFX reduced macroscopical scores and colon segment weight in AA colitis group animals. Furthermore, the anti-inflammatory effect of MFX was confirmed by significant reduction of colonic concentration of the inflammatory mediators NF-κB, TNF-α, selectin adhesion molecules and also oxidative stress biomarkers MDA .Suitable interpretation for results revealed by the present study about the anti-inflammatory effect of MFX and on experimental model of colitis may be found in previous studies which proved that MXF exerts anti-inflammatory effects by inhibiting proinflammatory cytokines production including TNF-α, IL-1β and interleukin-8 (IL-8) with significant inhibitory effects on the major signal transduction pathways associated with inflammation, namely, NF-κB and the mitogen-activated protein kinases ERK and c-Jun N-terminal kinase (JNK).[13,40]

As mentioned before, NF-κB reported to induce the production of inflammatory cytokines, such as TNF-α in UC[31] and also an enhanced level of NF-κB associated with increased levels of oxidative stress in UC.[25] Also, it was reported that pro-inflammatory cytokines including TNF-α are responsible for the generation of ROS, which activate the oxidative stress response in IBD.[32] In addition, it was reported that inflammatory cytokines including TNFα induce selectin expression[36]. So, by linking above data, inhibition of NF-κB inflammatory pathways by MFX in the present study might cause the inhibition of the TNF-α inflammatory pathways including TNF-α downstream inflammatory process such as elevation of selectin adhesion molecule synthesis and oxidative marker MDA overproduction in the colonic tissues.

Conclusion

The present study explains that moxifloxacin possesses therapeutic effects in AA induced colitis and its anti-inflammatory actions involve inhibition of NF-κB inflammatory pathways including TNF-α pathway with its downstream inflammatory process such as elevation of selectin adhesion molecule synthesis and oxidative marker MDA overproduction in the colonic tissues.

Conflict of Interest

None.

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