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Moxifloxacin ameliorates clinical disease activity and histopathological changes of acetic acid-induced colitis model in rat possibly through its effect on proinflammatory mediators and oxidative stress

Jaffar O. Dawood, Ahmed Abu-Raghif*

Abstract

Background: Moxifloxacin (MFX), a fluoroquinolone antibiotic, was found in previous study to exert an anti-inflammatory effect including reduction of proinflammatory cytokine expression, the aim of this research was to investigate the potential anti-inflammatory effect of MFX on acetic acid (AA)-induced colitis model in rats.

Methods: About 40 males of Wistar rats were divided into four groups as following, the negative (healthy) control group, AA-colitis group, sulfasalazine (SLS)group, and MFX group. Colitis induction was done by intra-rectal injection of 2 ml AA (4% v/v). After two hours of induction, SLS (100 mg/kg/day) and MFX (8 mg/kg/day) were given by oral route for seven consecutive days.

Results: The administration of MFX substantially decreased the disease activity index (DAI), and histopathological alterations triggered by AA. Moreover, AA induced a significant rise in the production of the proinflammatory cytokine IL-1β, the adhesion molecules ICAM-1, and the oxidative stress (OS) biomarker myeloperoxidase (MPO) in colon homogenate. the MFX administration significantly reduced the production of proinflammatory cytokine IL-1 β, adhesion molecules ICAM-1, and the OS biomarker myeloperoxidase (MPO) in colon homogenate, which were elevated by AA. The influences of MFX seemed comparable to those of SLS, with no significant difference between them.

Conclusion: Consequently, MFX may possess a beneficial property in the treatment of ulcerative colitis (UC).

Introduction

Inflammatory bowel disease (IBD) is characterized by idiopathic, recurrent, and usually extensive inflammation of the colon and rectum mucosa. The two most common forms of IBD were ulcerative colitis (UC) and Crohn's disease (CD)[1]. UC is a chronic inflammation of colon and rectum. The UC disease frequently causes diarrhea, diminished weight, nausea, and stomach pain, all of which have a negative impact on quality of life [2]. Though the exact cause of UC remains not entirely understood, numerous inherited, environmental, and immune variables hypothesized to be implicated in the pathogenesis of UC [1]. The pathological characteristics of UC encompass the invasion and stimulation of inflammatory cells, subsequent release of nuclear factor kappa-B (NF-κB) reliant proinflammatory chemicals including tumor necrosis factor alpha (TNF-α) and interleukin 6 (IL-6), enhanced production of free radicals like reactive oxygen species (ROS) and reactive nitrogen species (RNS), reduction in the colon's antioxidant defenses, and compromised integrity of the mucosal lining [2]. Typically, the initial approach to managing UC involves the administration of anti-inflammatory medicines. Aminosalicylates and glucocorticoids are commonly employed in the management of mild to moderate cases, whereas immunosuppressants, such as azathioprine and cyclosporine A, are regularly prescribed for serious conditions. Nevertheless, the suboptimal results of therapy and the prevalence of negative consequences have generated a demand for innovative and efficacious therapeutic strategies [3]. Despite significant advancements in the therapy of UC, the presence of negative consequences and limited efficacy of current drugs remains an ongoing problem. Consequently, there is a continued necessity to find novel therapeutics that offer improved safety profiles and greater efficacy for UC [4]. Acetic acid (AA)-induced UC is a simple, inexpensive, well-known, and commonly employed approach to investigate UC and is regarded as an effective tool for evaluating the efficacy of compounds that have the potential to inhibit the pathogenesis of UC [3]. The cytokines most frequently implicated in the pathophysiology of UC include TNF-α, IL-1β, and IL-6 [5]. During colonic mucosal damage, the proinflammatory response was connected with higher mucosal levels of IL-1β. The IL-1β exerts a direct impact on the proinflammatory pathways and stimulates the generation of proinflammatory mediators including, TNF-α, IL-6 and prostaglandins [1]. Myeloperoxidase (MPO), a lysosomal enzyme identified in neutrophils, could be a significant diagnostic and predictive measure for determining the state of IBD. A number of investigations indicate that individuals with active IBD have a greater percentage of neutrophils and MPO than individuals with inert or no IBD [6]. Substantial rises in colonic MPO levels (that indicate the invasion of neutrophil and inflammatory response abnormality) have been documented not just in individuals with IBD, but as well in animal studies [7]. Intercellular adhesion molecule-1 (ICAM-1) is crucial for neutrophil recruitment to colonic mucosa, and gene investigations have shown polymorphisms in the gene that produces ICAM1, implying that variations in ICAM-1 functionality may be associated with the pathophysiology of UC [8]. Intercellular adhesion molecule-1 (ICAM-1) levels was shown to be elevated in colonic samples from those suffering from UC compared to healthy individuals. Moreover, the level of ICAM-1 seems to rise as endoscopic disease activity rises. Several kinds of cells can elevate ICAM-1levels in reaction to proinflammatory triggers, like TNF-α and IL-1β. In fact, just one anti-TNF antibody therapy can reduce the number of ICAM-1-producing mononuclear cells in colonic mucosa [9]. Moxifloxacin (MFX) is a fluoroquinolone of the 4th generation with a wide range of activity versus both Gram-positive and Gram-negative aerobic organisms as well as anaerobes [10]. Previous study proved that MFX have anti-inflammatory effects with significant inhibition of proinflammatory cytokine production including production of TNF-α, IL-1β and IL-8 cytokines [11]. So, the objective of this research is to study the probable therapeutic effects of MFX against AA model of UC by evaluation the probable beneficial activity of MFX on disease activity and histopathological changes of AA induced UC in rats, in addition to evaluation of MFX on the colonic concentrations of inflammatory mediators (IL-1β and ICAM-1) and oxidative stress marker MPO on this model of UC.

Methods

Animals

About 40 males of Wistar rats (weighing 200-250 g) were brought from animal house of college of pharmacy, Babylon university. Rats were kept under normal environmental conditions including a 12-hour light/dark cycle and unrestricted accessibility to normal pellet food and water. Animals were separately housed in their cages (five per cage) and were acclimatized for at least one week before experiment [12]. Research protocol was authorized 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); Formaldehyde

(formalin)37% solution (PanReac AppliChem/Spain); Normal saline (Gulf inject/UAE); Diethyl ether (Thomas Baker/ India); PBS (phosphate buffered saline) (Medicago/ Sweden); Rat ICAM1 ELISA Kit (ELK Biotechnology/China); Rat IL-1β ELISA Kit (ELK Biotechnology/China); Rat MPO ELISA Kit (ELK Biotechnology/China).

Induction of AA ulcerative colitis

Colitis induction was done according to the technique 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 rats then kept in a head-down position for 2 minutes and then returned to their cages to recover from anesthesia. The rats in the noncolitis group (negative control group) received 2ml of normal saline by the same procedure. About 7 days later, the rats were euthanized by inhalation excessive dose of diethyl ether, colon tissue were removed to assess the macroscopic damage, histological and biochemical parameters [4,12].

Experimental Design

The animals were allocated into 4 distinct groups by random selection (n=10/group). Group 1: negative(healthy) control group: received 2 ml/rat normal saline intrarectally at the first day of the experiment and distilled water (DW) as a vehicle by oral route for 7 days [13]. Group 2: Colitis control group: administered DW as a vehicle by oral route for 7 days, with AA colitis triggering on the first day of experiment. Group 3: MFX group: administered MFX 8 mg/kg dissolved in DW by oral route for 7 days [14], with AA colitis triggering on the first day of experiment. Group 4: Sulfasalazine (standard) group: administered SLS 100 mg/kg dissolved in DW by oral route for 7 days [13] with AA colitis triggering on the first day of experiment. The selection of MFX dose of 8mg/kg was because this dose is equivalent to therapeutic dose in rat dependent on previous study [15].

Samples collection and preparation

Animals were sacrificed by diethyl ether excess breathing 24 hours following the last oral dosage of the medications. Following the abdomen dissection, the colons of every animal were obtained and washed with chilled normal saline. The removed colon was subsequently separated into two parts: one for histological examination and the other for tissue homogenization for biochemical parameters analysis [16]. Estimation of colonic biochemical parameter levels including estimation of pro-inflammatory cytokine IL-1β, oxidative stress biomarker MPO and adhesion molecule ICAM-1 in tissue homogenate were done by applying ELISA kits, in accordance with the manufacturer's directions [5] of (ELK Biotechnology/ China). Colonic tissue homogenate preparation was done in accordance with the manufacturer's directions of ELK Biotechnology ELISA kits and kept at – 80 °C [5] until use for estimation procedure.

Assessment of disease activity index (DAI)

The disease activity was clinically assessed using the disease activity index (DAI) scoring system utilized in El-Akabawy and ElSherif's (2019) study, which employs a scoring system to assess weight reduction, feces consistency, and hemorrhaging. The following variables were noted daily in accordance to that system: body weight reduction (0, none; 1, 1-5%; 2, 6-10%; 3, 11-20%; and 4, > 20%), diarrhea (0, normal; 1, soft feces yet remaining formed; 2, extremely loose stool; 3, mild diarrhea and 4, intense diarrhea), and rectal hemorrhage (0, normal; 1, positive hemoccult; 2, blood traces in stool noticeable; 3, mild hemorrhaging 4, intense bleeding). DAI values were determined using the mean values of these variables [3].

Histopathological study

The colon sections were immersed in a 10% formalin solution for fixation and subsequently embedded in paraffin wax. In order to conduct histological analysis, 5-µm slices were subjected to deparaffinization and rehydration using a graded ethanol series (100%, 90%, and 70%). Subsequently, these pieces had been stained by hematoxylin and eosin (H&E) to examine the overall histopathological morphology [17,18]. The histological alterations were evaluated by investigating and scoring slides. The tissue sections were examined in a blinded method by an expert histopathologist, and the findings were assessed via the scoring method applied by Manna et al. study and Attarbashee & Abu Raghif study using a scale of 0-3 (0=normal; 1=focal; 2=zonal; 3=severe), that evaluated the degree of: damage to glands and epithelial tissue, expansion of glandular crypts, lack of goblet cells, invasion of inflammatory cells, edema, dysplasia, mucosal bleeding and crypt abscesses [4,19].

Biochemical analysis

The content of IL-1β in the colon homogenate done according to ELK biotechnology manufacturer instructions of rat IL-1β ELISA kit [20]. The content of ICAM-1 in the colon homogenate done according to ELK biotechnology manufacturer instructions of rat ICAM-1 ELISA kit [21]. Myeloperoxidase (MPO) content in the colon homogenate done according to ELK biotechnology manufacturer instructions of rat MPO ELISA kit [22].

Statistical Analysis

The version 26 of IBM SPSS was utilized in the analysis of the data [7]. The data had been presented as a mean ± standard deviation (SD) [7]. The normality of the data was assessed using Kolmogorov Smirnov test [4] and the Shapiro-Wilk test [7]. The normally distributed data (colonic IL-1β, colonic ICAM-1 and colonic MPO) were analyzed by one-way ANOVA with Tukey's post-hoc test for pairwise intergroup comparisons [23]. Non-parametric data (DAI and histopathological scores) were analyzed by the Kruskal-Wallis H test for multiple groups, when significant differences were determined, comparisons between different groups were carried out utilizing the Mann Whitney U test [23]. Statistical significance was considered at a value of < 0.05[7].

Results

Effect on DAI

DAI values in the colitis control group (group 2) were significantly greater than that of the healthy control group (group 1) (p0.05). On the other hand, oral MFX or SLS therapy significantly reduced DAI values compared to the colitis control group (group 2) ($p < 0.05$) with insignificant difference between the two drugs (MFX and SLS) in DAI lowering effects, although SLS was slightly more potent than MFX in this regard. See table (1).

Histopathological changes in different study groups

The histopathological evaluation of colon samples showed that colon specimens of the negative control group possessed normal morphology of mucosa, submucosa, and muscularis (figure 1). The colonic crypts had a lining of absorptive columnar epithelium, which was accompanied by a substantial number of goblet cells. Additionally, the lamina propria displayed a scarcity of mononuclear inflammatory cells. The colitis control group demonstrated pronounced histological alterations, which indicate a classic presentation of UC (figure 2). The presence of several regions exhibiting erosion and ulceration, accompanied by the full necrosis of the mucosa, submucosa, and muscularis layers of the intestinal wall, was seen colitis control group. The colonic mucosa and submucosa that remained exhibited notable crypt deformation, accompanied with necrosis of the lining epithelium and a substantial reduction in goblet cells. Additionally, the lamina propria and submucosa displayed an extensive infiltration of neutrophils and mononuclear cells, together with evident submucosal edema and bleeding. In contrast, the experimental groups administered SLS or MFX demonstrated a notable decrease in ulcer development and mucosal injury. Additionally, these groups displayed low inflammatory response in both the mucosa and submucosa, lacking any indications of tissue loss caused by AA (figures 3 and 4). Regarding the scoring of histopathological lesions in colitis control group, it was observed that the colitis control group showed a statistically significant increase in all histopathological variables, including the total score, when compared to the negative control group. Additionally, both the SLS and MFX groups demonstrated a significant decrease in these variables when compared to the colitis control group with no significant difference between them in this regard, although sulfasalazine appears to have slightly more potent effect than MFX. See table (1).

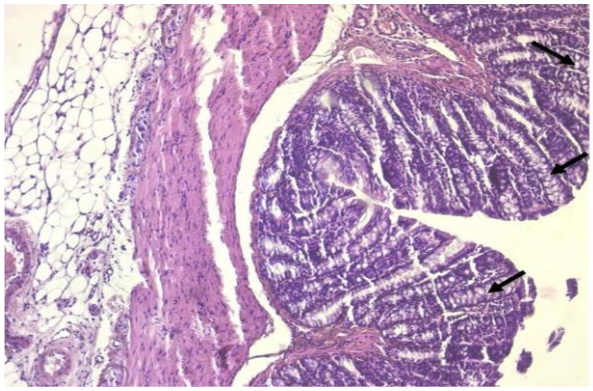

Figure 1: Histological section through colonic wall of negative (healthy) control group animal showing normal mucosal and submucosal pattern with no evidence of inflammation and preservation of colonic gland with goblet cells (arrows); 10X; H and E stain.

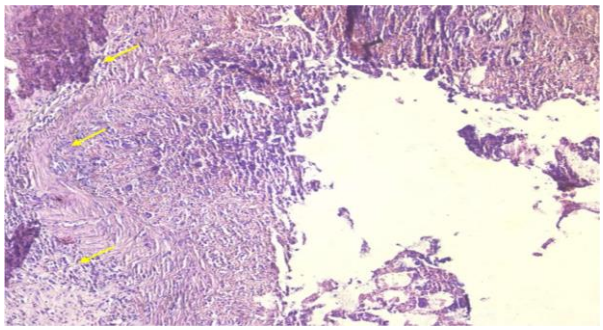

Figure 2: Histological section through colonic wall showing extensive ulceration; necrosis with no gland; and mononuclear inflammatory infiltrate (arrows) in experimentally induced colitis in rat; 10X; H and E stain.

Biochemical parameters of colon inflammation

The colitis control group exhibited significant elevation in colonic concentrations of IL-1β, ICAM-1, and MPO in comparison to the healthy control group. Furthermore, there were significant differences observed between the groups treated with MFX and SLS in terms of colonic IL-1β, colonic ICAM-1, and colonic MPO measures ($p < 0.05$) in comparison to the colitis control group with insignificant difference between MFX and SLS in lowering effects on these biochemical

parameters ($p < 0.05$), although sulfasalazine has somewhat more potent effect than MFX. See table (2).

Variable	Negative control (healthy) group†	Acetic Acid induced colitis group†	Sulfasalazine treated group†	MFX treated group†
DAI	00.0±00.0 A	11.00 ± 0.89 B	1.17 ± 0.41 C	1.50±0.55 C
Histo Score	00.0±00.0 A	3.00±00.0 B	0.67±0.51 AC	0.83±0.40 CD

**Capital letters for comparison; different letters indicate significant difference (P<0.05); similar letters indicate insignificant difference (P value ≥0.05); DAI: Disease Activity Index; Histo score: Histopathological Score; † values expressed as mean ± Standard deviation (SD).

Table 1: DAI scores and histopathologic scores in different groups of the study.

Variable	Negative control (healthy) group†	Acetic Acid induced colitis group†	Sulfasalazine treated group†	MFX treated group†
Colonic IL-1β pg/ml	149.21±15.47 A	654.17±12.35 B	181.54±14.68 C	196.83±9.17 C
Colonic ICAM1 ng/ml	1.51±0.14 A	7.67±0.38 B	2.02±0.15 C	2.13±0.19 C
Colonic MPO ng/ml	1.42±0.15 A	7.78±0.35 B	1.85±0.13 C	2.07±0.15 C

**Capital letters for comparison; different letters indicate significant difference(P<0.05); similar letters indicate insignificant difference; P value ≥ 0.05); g: gram; ICAM-1: intercellular adhesion molecule; IL-1β: interleukin-1 beta; ml: milliliter; MPO: myeloperoxidase; ng: nanogram; pg: Picogram; † values expressed as a mean ± Standard deviation (SD).

Table 2: Biochemical parameters and colonic homogenates of different groups of the study.

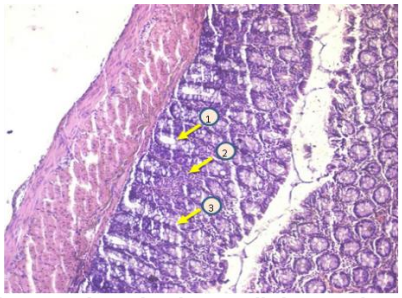

Figure 3: Histological section through colonic wall showing drug (sulfasalazine) effects after 7 days treatment in which there is evidence of mucosal regeneration and glandular formation (1); mild inflammation (2); and goblet cells regeneration (3); 10X; H and E stain.

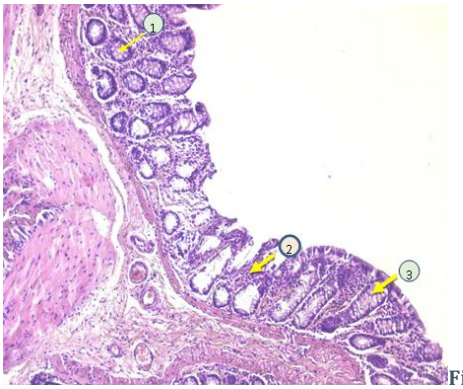

Figure 4: Histological section through colonic wall showing drug (MFX) effects after 7 days treatment in which there is evidence of mucosal regeneration and glandular formation (1); mild inflammation (2); and goblet cells regeneration (3); 10X; H and E stain.

Discussion

Ulcerative colitis (UC) represents a worldwide health issue that affects individuals of any age. Furthermore, the available UC therapy has various side effects, as well as decreased efficacy with continuous use, necessitating the development of novel more efficient and safer drugs [16]. Acetic acid-induced colitis has been extensively studied as a means of establishing an animal model of UC. This model effectively mimics the pathological characteristics observed in humans with UC, including elevated levels of inflammatory mediators, localized inflammation, and damage to the colonic mucosa [24]. In this research, there was a significant elevation in DAI value in AA colitis animals in comparison to negative control rats. Additionally, As confirmed by histopathological examination, AA administration caused substantial damage, ulcerations, erosions, congestion, invasion of inflammatory cells, necrosis, and hyperplasia of goblet cells throughout the colonic mucosa. Similar results were reported by previous studies such as Rehman et al. [18], Shahid et al. [25] and Oubaid et al. [16], after AA rectal administration to rat animals. The UC model triggered by AA injection inside the colon was believed to be caused by the passage of protons into the epithelium, resulting in intracellular epithelial acidity and severe epithelial damage [25]. The primary determinant of UC is the generation of proinflammatory cytokines, including TNF-α, IL-1β, and IL-6, which play a pivotal role in initiating and advancing colonic inflammation [26]. In this current study, AA administration significantly increased colonic concentration of IL-1β proinflammatory cytokine. These findings align with other studies in which AA caused marked elevation of IL-1β cytokine concentration [25-27]. In the context of colitis, the integrity of the epithelium is compromised, leading to an enhanced absorption of bacterial endotoxins such as lipopolysaccharide (LPS) by the epithelial cells. The Toll-like receptor 4 (TLR4) present in the intestinal wall is responsible for the identification of these (LPSs) [27]. The interaction between LPS and TLR4 initiates a signaling cascade that ultimately results in the activation of nuclear factor kappa B (NF-κB). This situation results in the production of proinflammatory cytokines, such as TNF-α and IL-1β [27]. This interpretation come in agreement with the present study in which AA colitis induction induce significant raising in colonic concentration of IL-1β cytokine. The involvement of NF-κB is crucial in the pathogenesis of UC since it triggers the synthesis of pro-inflammatory molecules, including TNF-α, IL-1β, and cyclooxygenase-2 (COX-2), inside the inflamed mucosal tissues [3]. Oxidative stress (OS) is a significant contributing factor to tissue damage in individuals with UC. Reactive oxygen and

nitrogen species (ROS and RNS), which are generated as a consequence of OS, play a crucial role in the stimulation of lipid peroxidation. This process negatively impacts the organization of cellular membranes, as well as the structural integrity of proteins and DNA bases [28]. In the current study colitis induction by AA caused significant elevation of MPO level (a popular biomarker of OS). These results come in agreement with wang et al. study (2019) in which AA group have significantly higher level of MPO compared to negative control animals [28]. The intercellular adhesion molecule (ICAM-1) facilitates the interaction between leukocytes and endothelial cells, hence facilitating leukocyte infiltration into the inflammatory region [19]. In present study AA intrarectal injection caused significant elevation in ICAM-1 colonic concentration. These findings are consistent with prior researches in which AA colitis induction significantly increase the levels of ICAM-1 adhesion molecules [19,29]. It was reported that inflammatory cytokines including TNFα and IL-1β induce ICAM1 and other adhesion molecules expression [30], and these cytokines may be the base of this increase in 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 [16,19,28,31]and these data are compatible with results of present study were sulfasalazine significantly reduced DAI, histopathological scores and biochemical inflammatory mediators in colonic tissue compared to AA colitis group. The therapeutic benefits of sulfasalazine for UC may be partially attributable to its capacity to hinder the stimulation of NF-κB, leading to the inhibition of pro-inflammatory cytokines [32]. In the present study MFX, which is a synthetic antibacterial agent of the fluoroquinolone family [33], 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 DAI and histopathological scores in AA colitis. Furthermore, the anti-inflammatory effect of MFX was confirmed by significant reduction of colonic concentrations of the inflammatory mediators IL-1β, ICAM-1 and also oxidative stress biomarker MPO. Previous studies confirmed the immunomodulatory effects of the fluroquinolones including MFX and explain that the mechanism of these anti-inflammatory effects is due to the impact of these substances on intracellular cyclic AMP and phosphodiesterases, as well as their influence on transcription factors like NF-κB and activator protein 1 (AP-1), and their ability to initiate a response similar to the bacterial SOS response in eukaryotes [33,34]. These data are compatible with and may give suitable interpretation for results revealed by this current study about the anti-inflammatory activity of MFX on experimental model of colitis.

The present study suggests that moxifloxacin has a therapeutic effect on AA induced colitis in rat model through reduction of clinical disease activity and histopathological features in addition to reduction of inflammatory and oxidative stress biomarkers and these effects possibly comparable to the effects produced by sulfasalazine as reference drug on AA colitis.

Author Contributions

Dawood: Conceptualization, writing-original draft, data curation and editing
Abu-Raghif: Supervision, methodology and review.

Competing Interests

The authors declared that there were no conflictsof interest.

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