Research Square

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A Steroid-Sparing Combination Regimen of Low-Dose Atorvastatin and Reduced-Dose Prednisolone Maximizes Healing in Experimental Ulcerative Colitis

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Abstract

Long-term corticosteroid treatment for ulcerative colitis (UC) is often limited by severe side effects that depend on the dosage. This makes it necessary to find new ways to reduce steroid use. This study looked at how well a combination of half-doses of Atorvastatin and Prednisolone worked in rats with acetic acid-induced colitis. Male Wistar rats were divided into several groups: a normal control group, an untreated UC group, groups receiving full doses of either Atorvastatin (10 mg/kg) or Prednisolone (10 mg/kg), and a group receiving the half-dose combination therapy. After 10 days of oral treatment, researchers analyzed the severity of the disease, colon swelling, tissue damage, pro-inflammatory cytokines, markers of oxidative/nitrosative stress, and related signaling pathways. The half-dose combination treatment was significantly more effective than either full-dose monotherapy. It resulted in the lowest disease severity and almost completely prevented immune cell infiltration in the colon lining. Biochemically, the combination worked together to suppress the NF-κB signaling pathway. This completely normalized levels of tumor necrosis factor-alpha (TNF-α), malondialdehyde (a marker of lipid damage), and NF-κB to healthy levels. It also greatly reduced interleukin-1-beta (IL-1β), interleukin-6 (IL-6), myeloperoxidase activity, and nitric oxide production. Additionally, this combined approach maximized the activity of the protective Nrf2/HO-1 antioxidant pathway more than either monotherapy alone. These results show that combining low-dose Atorvastatin with reduced-dose Prednisolone provides superior anti-inflammatory, immune-modulating, and antioxidant effects through complementary mechanisms. Ultimately, this steroid-sparing treatment offers a very effective strategy to promote mucosal healing in UC while minimizing the severe risks associated with high-dose corticosteroid management.

Introduction

Intrarectal administration of acetic acid in animals such as rats induces a complex inflammatory response and is widely used as an experimental model for studying inflammatory bowel disease (IBD), particularly ulcerative colitis (UC)[1–3]. The acetic acid-induced colitis model is characterized by mucosal inflammation, ulceration, hemorrhage, and weight loss features that closely resemble those observed in human UC[1, 4]. The mucosal erosion triggered by acetic acid is associated with the activation of inflammatory cells, such as neutrophils, and the subsequent release of inflammatory mediators[2]. In this context, disruption of the mucosal barrier allows the animal's intestinal microbiota to access the lamina propria of the colon, thereby promoting a multifaceted inflammatory cascade[1, 3].

Consequently, this model serves as a valuable tool for investigating the effects of various therapeutic interventions on inflammation, oxidative/nitrosative stress, and apoptosis in the setting of UC[3, 2]. Currently, multiple compounds with anti-inflammatory and immunomodulatory properties are employed in the management of IBD[5]. However, these agents are often associated with significant side effects, and many patients exhibit suboptimal responses owing to the complex and heterogeneous nature of inflammation in UC. Long-term use further elevates the risk of adverse effects[6, 5]. For example, prolonged corticosteroid therapy in ulcerative colitis is associated with a broad range of serious adverse

effects, including osteoporosis and bone loss, avascular necrosis (particularly of the femoral head), an increased risk of infections, diabetes mellitus, hypertension, as well as less common complications like myopathy, psychosis, and cataracts[7].In such cases, rational combination therapies using existing or novel agents may improve efficacy while minimizing the dose-dependent toxicities of individual drugs[6].

Statins, exemplified by Atorvastatin, were originally developed for their lipid-lowering effects. Subsequent research has uncovered pleiotropic anti-inflammatory and immunomodulatory properties independent of cholesterol reduction[8, 9]. For instance, Atorvastatin has demonstrated rapid reductions in C-reactive protein (CRP) levels in patients with acute coronary syndromes, highlighting its anti-inflammatory actions alongside its primary effects[10]. Beneficial effects of statins have also been reported in other inflammatory conditions, including multiple sclerosis and the prevention of chronic graft rejection[8, 9].

Prednisolone, a potent glucocorticoid, exerts its anti-inflammatory and immunosuppressive effects by binding to the glucocorticoid receptor, a ligand-dependent transcription factor that modulates gene expression[11, 7]. Despite its efficacy in controlling acute inflammation, prolonged use is associated with numerous adverse effects, including diabetes, hypertension, opportunistic infections, gastrointestinal complications, visual disturbances, and osteoporosis[7].

Nevertheless, limited data exist regarding the specific roles of Atorvastatin and Prednisolone—alone or in combination—in mitigating inflammation in UC or its experimental models. Given the established individual benefits of these agents, evaluating their combination at suboptimal (e.g., half) doses is a logical approach to potentially enhance therapeutic outcomes while reducing side-effect profiles[8]. Accordingly, the present study examined whether the combination of half-doses of standard Atorvastatin and Prednisolone could yield superior outcomes in an experimental acetic acid-induced UC model in rats.

Materials and Methods

Animals:

Male Wistar rats weighing 195 ± 5 g were housed in a controlled environment with a 12-hour light/dark cycle and a temperature of 25°C upon arrival from the animal facility. The rats had unrestricted access to standard laboratory food and water. The study followed ethical guidelines and complied with the Helsinki Convention regarding the care and use of laboratory animals. Approval for the research procedures was obtained from the faculty's ethics committee.

UC induction, treatment and monitor

To induce the disease, the rats were anesthetized with ether following a 24-hour fasting period. A rubber cannula, 8 cm in length, was used to administer 2 ml of a 4% acetic acid solution into the rectum of each rat. Subsequently, the rats were kept in this position for 20 seconds and then flushed with 5 ml of normal

saline before removing the catheter to ensure complete diffusion of the acetic acid into the colon [12, 3]. In the following, acetic acid-instilled rats were accidentally allocated into five equal groups (n = 7): PBS-treated rats, Atorvastatin-treated rats (10 mg/kg-orally), Prednisolone-treated rats (10 mg/kg- orally), and rats treated with combined Atorvastatin and Prednisolone (half doses with each one-orally). Rats in the normal group received intrarectal PBS in an equal volume and were treated with PBS at the same schedule and volume of UC-induced rats. Therapies were continued for ten days.

The body weight, gross bleeding, and stool consistency of the rats were recorded on a daily basis. The Disease Activity Index (DSI) was calculated as the total of the levels of weight loss, stool consistency, and bloody stool, following the criteria outlined in Table 1.

Determination of colonic edema:

Following a 10-day study period, the colon was isolated, and longitudinal cuts were made to open the samples. Subsequently, the extracted samples were thoroughly rinsed with normal saline, and the weight and length of the colon samples were meticulously measured. The ratio of colon weight to length was utilized as an indicator of intestinal edema[13].

Homogenization of colonic tissues

The distal colons from each rat were weighed, and the specimens were gently rinsed with ice-cold saline to remove any fecal material. An equal amount of specimen was then dissected and homogenized with 0.01 M Tris-HCl solution at pH 7.4. The homogenate was centrifuged at 3000 rpm to separate the cellular debris and nuclei from the supernatant (cytosolic fraction). The protein concentration in the cytosolic fraction was determined using the standard Bradford assay[14, 4]. The obtained supernatant was utilized for further analysis.

Evaluation of Nrf2/HO-1, NF-κB, and pro-inflammatory cytokine levels in colon samples

Nrf2/HO-1 Antioxidant signaling pathway, NF-κB, and pro-inflammatory cytokine levels, including IL-1β, and TNF-α, in colon sample homogenates were assessed using commercial ELISA kits following the manufacturer's guidelines.

Measurement of nitrosativatie and oxidative stress indicators in the colon homogenate

Grice's method was utilized to assess the nitric oxide production intensity in intestinal tissue homogenates [4]. To measure myeloperoxidase (MPO) activity in colonic samples, 10 µl of colon homogenate was mixed with 110 µl of TMB solution (2.9 mM TMB in 14.5% DMSO plus 150 mM sodium

phosphate buffer at pH 5.4) and 80 µl of 0.75 mM hydrogen peroxide. Following a 5-minute incubation, 50 µL of 2 M sulfuric acid was added to stop the reaction. The optical density was measured at 450 nm, with Horseradish peroxidase (HRP) serving as the standard. MPO activity was calculated based on the variance in optical density relative to the HRP standard curve [3].

To determine the malondialdehyde levels in colon samples, 2.5 ml of reaction solution (0.25 M HCl, 15% trichloroacetic acid, and 0.37% thiobarbituric acid in a 1:1:1 ratio) was mixed with 100 microliters of colon homogenate. The mixture was heated at 95°C for 60 minutes, then cooled and centrifuged for 15 minutes at 3500 rpm. The optical density of the supernatant was measured at a wavelength of 540 nm[12, 4].

Statistical data evaluation

The nonparametric disease activity index was assessed using the Kruskal-Wallis test followed by the Mann-Whitney U test with Bonferroni adjustment. The remaining parametric data were analyzed using the one-way ANOVA followed by Tukey's range test. The values were reported as means ± SD. P-values less than 0.05 were considered statistically significant.

Results

As shown in Fig. 1, intrarectal administration of acetic acid caused colon damage. This damage was characterized by significantly higher Disease Severity Index (DSI) and colonic edema compared to the normal control animals, which showed no disease activity. The untreated ulcerative colitis (UC) group showed a rapid increase in the severity of their disease, reaching its worst point around day 6 with a Disease Severity Index (DSI) of approximately 8. Their symptoms remained very high until day 10. All treatments began to reduce this severity around days 3–4. The group receiving combination therapy experienced the most significant and fastest improvement by last day (Fig. 1A).

Analysis of cumulative disease severity index over 10 days demonstrated that the untreated UC group experienced a much higher total disease burden than the control group (Fig. 1B). Treating with either Atorvastatin or Prednisolone alone significantly lowered the total disease burden, and there was no significant difference between these two treatments. Importantly, the combination therapy (half-doses of Atorvastatin and Prednisolone) was the most effective, reducing the cumulative DSI more than both the untreated UC group and the monotherapy groups(Fig. 1B).

On the last day of the experiment, we assessed the severity of the disease and found clear differences in treatment effectiveness (Fig. 1C). Rats with untreated ulcerative colitis (UC) were still very sick, with a Disease Severity Index (DSI) of about 6. Atorvastatin alone significantly reduced this severity. Prednisolone alone was even more effective than Atorvastatin. However, the most impressive results were seen with the combination therapy, which brought the DSI closest to 1. This was a significant

improvement compared to the untreated group, Atorvastatin monotherapy, and Prednisolone monotherapy (Fig. 1C).

Figure 1D shows that acetic acid caused a significant increase in colon swelling (edema index) in rats with UC compared to healthy rats. This indicates severe inflammation and leaky blood vessels. Treating the rats with either Atorvastatin or Prednisolone alone reduced this swelling. Prednisolone was more effective than Atorvastatin by itself. Interestingly, combining half-doses of both Atorvastatin and Prednisolone was very effective at reducing inflammation, even better than Atorvastatin alone and much better than no treatment. While the combination therapy resulted in slightly less swelling than Prednisolone alone, this difference was not statistically significant (Fig. 1D).

Histopathological changes in colon tissues are shown in Fig. 2. Quantitative histopathological assessment of the colon sections, measured as the total infiltration across 5 microscopic fields, revealed a massive immune cell influx following colitis induction. This was lessened by the different treatments, as seen in Fig. 2F. Looking at detailed pictures of the colon (histopathology) showed a huge rush of immune cells after causing colitis. This was lessened by the different treatments, as seen in Fig. 2.Rats with untreated colitis had serious damage to their colon lining and a very high number of inflammatory cells, with the highest score for immune cell buildup at about 66.Atorvastatin significantly reduced the movement of immune cells into the colon tissue compared to the untreated colitis group, lowering the cell buildup score to about 31.Prednisolone by itself provided an even stronger protective effect, bringing immune cell counts down to about 18. This reduction by Prednisolone was significantly better than with Atorvastatin alone. The most impressive improvement in the colon's appearance was seen in the group that received both Atorvastatin and Prednisolone at half doses. This combination treatment almost completely stopped cell infiltration, bringing the score down to nearly the normal level (about 5). This strong reduction was very significant compared to the untreated colitis group, and importantly, it worked significantly better than both full-dose treatments used alone (Fig. 2F).

As detailed in Fig. 3, The instillation of acetic acid into the colon of Wistar rats led to a noticeable increase in the activity of MPO, MDA, and NO in the colonic homogenate samples. All treatments helped reduce these harmful increases. However, the two monotherapies worked differently for each problem. Atorvastatin was much better than Prednisolone at lowering lipid peroxidation (MDA). On the other hand, Prednisolone was better at reducing nitrosative stress (NO). In comparing individual treatments, Prednisolone alone was much better at lowering NO than Atorvastatin alone. For neutrophil infiltration marker (MPO), both monotherapy treatments worked similarly to reduce it. Remarkably, the combination therapy with half doses consistently showed significant therapeutic benefits for MPO and MDA parameters. It was significantly better than both single treatments at reducing MPO activity. For MDA, it worked as well as Atorvastatin alone. For NO, it worked as well as full-dose Prednisolone. Interestingly, even with lower doses, the combination therapy worked exceptionally well. The combination treatment also brought MDA levels all the way back to normal, like in healthy animals. MPO activity and NO levels were only slightly higher than normal after this treatment (Fig. 3).

The ELISA results showed that pro-inflammatory cytokines TNF-α, IL-1β, and IL-6 levels were significantly higher in UC rats compared to normal control animals (Fig. 4). Monotherapy with either Atorvastatin or Prednisolone significantly attenuated this pathological surge across all three markers. While both monotherapies exhibited comparable suppression profiles for TNF-α and IL-6, Prednisolone monotherapy demonstrated a significantly greater reduction in IL-1β levels than Atorvastatin monotherapy. The combination therapy, using half the usual amount of both medicines, worked best to reduce inflammation. It lowered all three inflammation signals much more than in the group with no treatment. This treatment was clearly better than using either medicine alone. However, this two-medicine approach didn't bring everything back to normal for every signal. It only fully normalized TNF-α, bringing its levels down to what's seen in healthy animals. The levels of IL-1β and IL-6 in the colon were still a bit high and didn't fully return to their starting levels. (Fig. 4).

As detailed in the Fig. 5A and B, the instillation of acetic acid resulted in a profound suppression of the colonic antioxidant defense mechanism, characterized by a sharp, statistically significant decrease in both Nrf2 and HO-1 expression levels compared to healthy controls. While treatments tried to fix this pathway, the single medicines worked differently. Atorvastatin alone greatly increased the levels of both Nrf2 and HO-1. It worked better than Prednisolone, which did not show any significant improvement in HO-1 levels compared to the untreated colitis group. The half-dose combination therapy was consistently the most effective treatment. It significantly improved both pathway components, performing much better than the untreated group and either both individual monotherapies (Fig. 5A and B).

Figure 5C showed that rats with colitis had significantly higher levels of NF-κB in their colon homogenates compared to normal rats. In contrast, all treatment regimens led to a significant decrease in colonic homogenate NF-κB levels compared to untreated UC rats. Atorvastatin reduced NFκb expression moderately, whereas Prednisolone monotherapy and the combination therapy performed a much more pronounced suppression. Interestingly, while Prednisolone alone was significantly more effective than Atorvastatin alone, there was no statistically significant difference between the Prednisolone monotherapy and the combination therapy. Importantly, only the combination therapy brought NFκb levels down to where they were like those in healthy animals (Fig. 5C).

Discussion

The primary objective of combination therapy in inflammation is to suppress the inflammatory process and enhance clinical outcomes more effectively than treatment with individual drugs[15, 16]. The findings of the current study demonstrated that combining Atorvastatin and Prednisolone at half the optimal dose resulted in superior outcomes in the inflammation intensity index, colonic edema index, histopathological manifestation, and biochemical indices in the ulcerative colitis model compared to using the full dose of each drug. Our data strongly suggests that Atorvastatin may significantly reduce the need for steroids. This approach may effectively reduce the severe side effects and risks associated with long-term, high-dose steroid treatments.

The rational combination of medications should consider several key points. The primary consideration is to utilize medications with distinct mechanisms of action[17, 18, 8]. It appears that the anti-inflammatory and immunomodulatory impacts of Atorvastatin primarily arise from its inhibition of isoprenoid compound synthesis in the mevalonate pathway. This pathway plays a crucial role in the isoprenylation of various key cell signaling proteins involved in the inflammatory process[8]. Atorvastatin has been shown to inhibit MyD88/NF-κB signaling in human coronary arteries in humans and various experimental animal models[19]. This process is believed to result in the inhibition of leukocyte adhesion to intercellular adhesion molecule-1 (ICAM-1) [20]. Moreover, Atorvastatin has been discovered to reduce the expression and activation of the NLRP3 inflammasome, along with lowering the plasma levels of IL-1β and IL-18 in Cardiovascular Disease [19]. The anti-inflammatory effects of Prednisolone are mediated by genomic and non-genomic mechanisms[7, 21]. Genomic-dependent mechanisms involve the suppression or activation of specific genes encoding pro-inflammatory and anti-inflammatory proteins[11, 21]. The non-genomic mechanisms of Prednisolone action are less understood. It has been proposed that this medication directly affects the physicochemical properties of cell membranes or binds to intracellular or membrane-bound receptors, leading to the inhibition of inflammatory cell function[22, 21]. Another hypothesis suggests that glucocorticoids, when binding to their receptors, release associated chaperone proteins. The release of signaling molecules from the multiprotein complex is believed to be responsible for the rapid glucocorticoid effects[21].

Oxidative stress plays a crucial role in the development of UC and acetic acid-induced colitis[23].

In the acetic acid-induced colitis model, oxidative stress markers like myeloperoxidase (MPO) activity and malondialdehyde (MDA) levels are utilized to evaluate the level of oxidative stress in the colonic tissue[4, 3]. The overproduction of reactive oxygen metabolites (ROS) leads to lipid peroxidation, causing the oxidative damage of lipids and cells. MDA, the final product of lipid peroxidation in cells[3], serves as a marker of oxidative stress in the control colitis group, significantly higher than the other experimental groups. Statins, like Atorvastatin, possess antioxidant properties that are separate from their cholesterol-lowering abilities[24, 25]. Statins can effectively decrease oxidative stress by inhibiting the production of reactive oxygen species (ROS) and adjusting antioxidant enzymes such as Nrf2 and Nrf2/HO-1[25]. It has been found to decrease Thiobarbituric acid reactive substances levels and increase non-protein associated sulfhydryl activity in streptozotocin-induced oxidative damage [26]. Additionally, Atorvastatin has been shown to reduce oxidative stress markers such as 8-isoprostane levels in patients with hyperlipidemia [27]. Although Prednisolone is not directly classified as an antioxidant, its anti-inflammatory properties can indirectly help protect cells from oxidative damage[28]. Our findings also showed that Atorvastatin treatment was more effective than Prednisolone in reducing MDA levels in rats with colitis. Furthermore, our data indicated that a combined therapy using half doses of Atorvastatin and Prednisolone was more effective in reducing MDA levels than treatment with the full dose of either drug alone.

Myeloperoxidase (MPO), an enzyme concentrated in neutrophil azurophilic granules, is used as a reliable biochemical indicator of neutrophil infiltration in colon tissue from rats with acetic acid-induced

ulcerative colitis[29, 12]. Its activity in tissue homogenates correlates directly with the degree of local inflammation[29]. Infiltrating neutrophils exacerbate mucosal injury by generating reactive oxygen species via MPO, releasing proteolytic enzymes, and secreting pro-inflammatory mediators, leading to oxidative stress, epithelial destruction, and barrier dysfunction[12]. Consequently, MPO activity serves as a key surrogate marker of colonic inflammation severity and is widely used to assess the efficacy of anti-inflammatory treatments in preclinical studies. The decrease in serum MPO levels in patients with acute coronary syndrome and in hemodialysis patients was previously reported after receiving Atorvastatin[30, 31]. Based on our data, Prednisolone was as effective as Atorvastatin in reducing MPO levels in the acetic acid–induced colitis model. The combination of both drugs at half the optimal dose demonstrated superior efficacy in reducing MPO levels.

Nitrosative stress is a key factor in the progression of acetic acid-induced colitis. In the model of acetic acid-induced colitis, elevated nitric oxide (NO) levels are detected, leading to oxidative stress and inflammation in the colon tissue[2]. The available evidence suggests that Atorvastatin can reduce nitrosative stress, as demonstrated by its ability to decrease nitrate levels in the myocardial tissues of diabetic animals. However, the effect of Atorvastatin on nitrosative stress may not be universal and may depend on the specific experimental conditions and disease models studied[32]. The results of our study indicated that Atorvastatin treatment was not statistically significant in reducing nitric oxide levels. Prednisolone reduces nitrosative stress. It lowers the expression of iNOS, an enzyme that produces excess nitric oxide (NO) during inflammation. This happens mainly by blocking the NF-κB pathway, which stops iNOS and other inflammation-causing genes from being activated. This results in lower levels of nitrite, nitrate, and peroxynitrite, which are markers of nitrosative stress, in inflamed tissues. By limiting NO, Prednisolone also reduces the formation of harmful peroxynitrite[33]. In comparing individual treatments, Prednisolone alone was much better at lowering NO than Atorvastatin alone. Interestingly, even at lower doses, the combination therapy worked exceptionally well. It significantly outperformed Atorvastatin alone and was as effective as the full dose of Prednisolone alone. The final NO levels in the combination therapy group were only slightly higher than the initial control levels.

NF-κB is a multifunctional nuclear transcription factor that regulates the expression of genes involved in inflammatory and immune responses, including those encoding proinflammatory cytokines, cell surface receptors, adhesion molecules, and other transcription factors. In ulcerative colitis, aberrant activation of NF-κB plays a central role in disease progression by upregulating key pro-inflammatory mediators such as IL-1β, IL-6, and TNF-α [4]. Atorvastatin exerts potent anti-inflammatory and anti-proliferative effects by inhibiting NF-κB activation and its downstream signaling pathways [34]. Prednisolone treatment reduces NF-kBp65 mRNA expression. This decrease lowers pro-inflammatory cytokines like TNF-α and IL-6, which contribute to colitis[17]. Prednisolone treatment resulted in a more substantial improvement in the serum level of NF-κB compared to Atorvastatin. Importantly, only the combination therapy brought NFκb levels down to where they were like those in healthy animals.

The activation of the NF-κB pathway and the subsequent increase in inflammatory cytokines like TNF-α and IL-1 intensify the inflammatory reaction and tissue injury[35]. In UC rats, the combination of half-

dose Atorvastatin and Prednisolone produced significantly lower colonic TNF-α and IL-1 levels than full-dose monotherapy with either drug alone.

Conclusion

The findings from this study demonstrate that a rational combination therapy utilizing half-doses of Atorvastatin and Prednisolone provides superior therapeutic outcomes in experimental acetic acid-induced colitis compared to full-dose monotherapies. This combination treatment improves healing by reducing inflammation (NF-κB pathway), normalizing markers of inflammation and oxidative stress, and boosting the body's protective mechanisms (Nrf2/HO-1). This approach could help reduce the need for high doses of corticosteroids, which have severe side effects, while still promoting mucosal healing. However, there are some limitations to consider. First, the acetic acid-induced colitis model in rats, while useful for screening and mimicking some aspects of human ulcerative colitis, is an acute injury and doesn't fully represent the complex, chronic nature of inflammatory bowel disease in humans. Second, this study was short (10 days) and conducted in rodents, so more research is needed to determine the long-term safety and potential interactions of these combined drugs. Finally, while the biochemical results strongly suggest that these drugs work well together, further genomic and proteomic studies are needed to understand the exact molecular interactions between Atorvastatin and Prednisolone before this treatment can be used in humans.

Declarations

Author Contribution

M.Q. performed the experiments, analyzed the data, and visualized the results. S.M.A.F. conceived and supervised the study, acquired resources, and administered the project. Both authors contributed to the methodology, validation, writing (original draft, review, and editing), and have approved the final manuscript.

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Table

Table1. Scoring system to evaluate ulcerative colitis severity

Score	Weight loss	Stool consistency	Blood feces
0	Negative	Normal	Negative
1	1-9%	Soft	Red
2	10-19%	Very Soft	Dark Red
3	<20%	Diarrhea	Black

Figures

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Figure 1

Evaluation of clinical symptoms in experimental ulcerative colitis (UC). A) Trends in disease severity index, B) Cumulative disease severity index, C) Disease severity index on the last day (day 10), Colonic edema index. (**** $P < 0.00001$, *** $P < 0.0001$, ** $P < 0.001$, * $P < 0.05$, ns: non-significant ($P > 0.05$)).

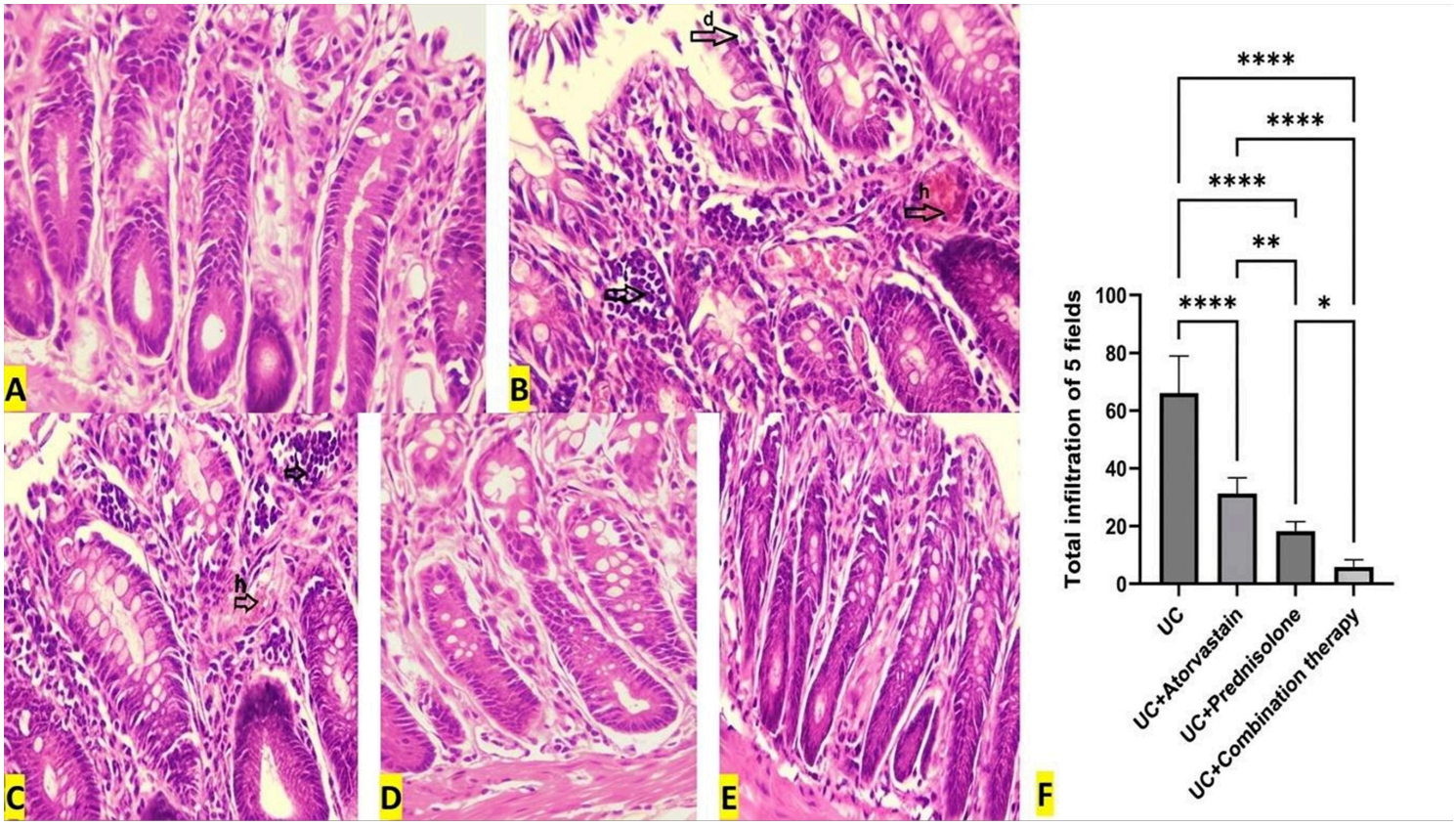


Figure 2

Histopathological changes in colon tissues (H&E x400**).** A) normal colon with parallel crypts, B) Ulcerative Colitis group. Distortion (d) of the architecture of the colonic crypts and hyperemia (h) and inflammation (i) in the epithelium, C) UC rats treated with Atorvastatin. This drug has not been able to reverse the lesions in the colon caused by ulcerative colitis and hyperemia (h) and infiltration of

inflammatory cells (i) were still observed, D) UC rats treated with Prednisolone. Structure of colon similar to normal group and crypts are uniformly. E) UC rats treated with half dose of combined Prednisolone plus Atorvastatin The lesions of colon greatly eliminated after administration of prednisolone and atorvastatin.Distortion of crypts and infiltration of inflammatory cells was decreased. F) Evaluation of total leukocyte infiltration of 5 microscopic fields. (**** $P < 0.00001$, *** $P < 0.0001$, ** P < 0.001, * $P < 0.05$).

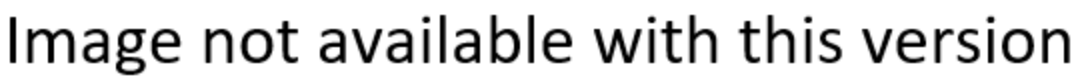


Figure 3

Biochemical analysis of colonic homogenates. A) Malondialdehyde (MDA), B) Myeloperoxidase, C) Nitric oxide. (**** $P < 0.00001$, *** $P < 0.0001$, ** P < 0.001, * $P < 0.05$, ns: non-significant ($P > 0.05$)).

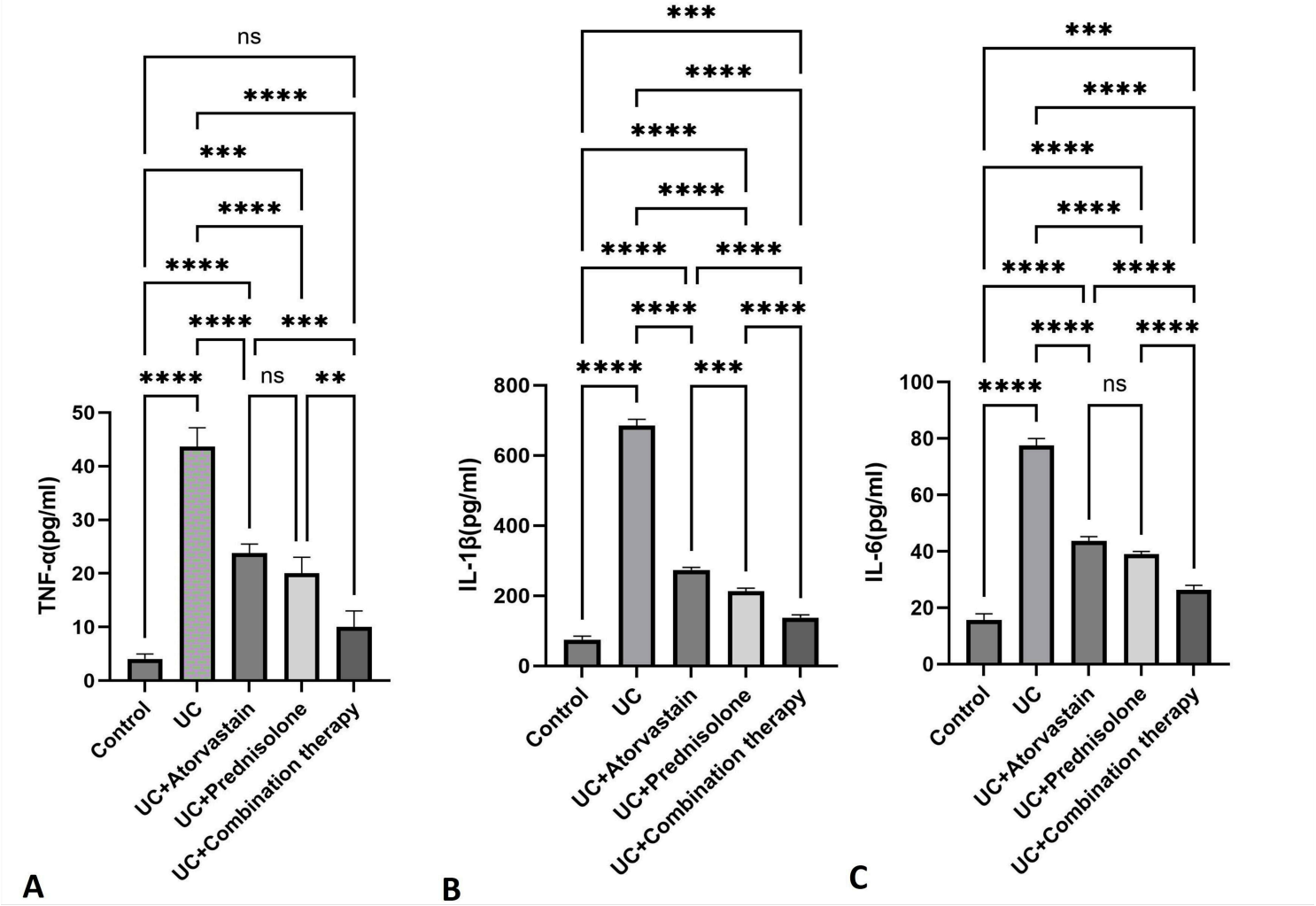


Figure 4

Evaluation of proinflammatory cytokines in colonic homogenates. A) TNF-α, B) IL-1 β, C) IL-6. (**** *P* < 0.00001, *** *P* < 0.0001, ** P < 0.001, ns: non-significant (*P* > 0.05)).

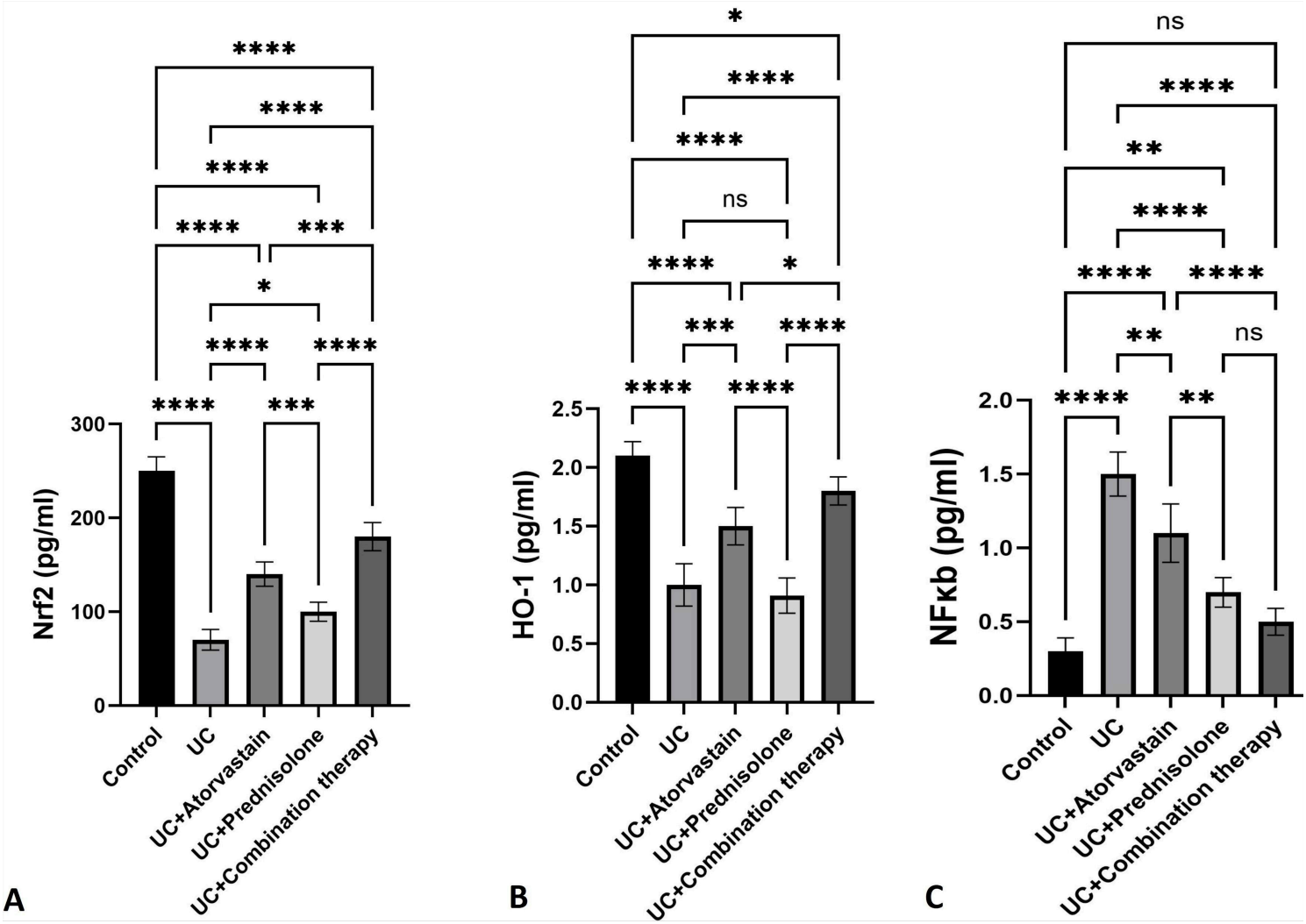


Figure 5

Analysis of Nrf2/HO-1, and NF-κB in colonic homogenates. A) Nrf2, B) HO-1, C) NF-κB. (**** $P < 0.00001$, *** $P < 0.0001$, ** $P < 0.001$, * $P < 0.05$, ns: non-significant ($P > 0.05$)).