Original Article

Anti-Inflammatory Effect of Atorvastatin on Colitis Induced in Male Mice

Zeena Hadi Mageed, Majid K. Abbas, Ahmed Raji[1]

Departments of Pharmacology, [1]Pathology, College of Medicine, University of Babylon, Babylon, Iraq

Abstract

Background: Statins are approved for cholesterol reduction and may also be beneficial in the management of inflammatory diseases. Another essential pleiotropic effect of β-hydroxy β-methylglutaryl-CoA (HMG-CoA) reductase inhibitors is their anti-inflammatory properties. Statins lower C-reactive protein (CRP) levels while also inhibiting inflammatory mediators like tumor necrosis factor-alpha and interleukins including interleukin-6 (IL-6). **Objective:** The purpose of this study was to determine the relationship between the severity index and other inflammatory markers in animals with colitis and to investigate the possible effects of atorvastatin on disease activity index and the inflammatory markers in experimentally induced colitis. **Materials and Methods:** Mice were given acetic acid to induce colitis. For 7 days, mice were divided into four groups (control group, induction group, standard group treated with prednisolone, and atorvastatin-treated group) and monitored daily for weight loss, feces consistency, and rectum bleeding for measuring the disease activity index. At last, we killed the animals and took blood samples for IL-6. **Results:** Acetic acid caused significant colonic inflammatory response and shrinking, as well as a reduction in body weight. The use of atorvastatin to treat acetic acid-induced colitis resulted in weight-loss recovery and decrease in expression concentration of IL-6 in the treated group compared with the colitis group when using in high dose. **Conclusion:** These findings indicate that atorvastatin preserves intestinal integrity in colitis, most likely by modifying the Th cell-mediated immune response independently of innate immunity.

Keywords: Atorvastatin, inflammatory bowel disease, interleukin-6 factor, mice, the disease activity index, ulcerative colitis

Introduction

Inflammatory bowel disease (IBD) refers to a group of disorders that have a propensity for persistent relapsing-remitting inflammation of the large intestine.[1,2]

Progressive inflammatory intestinal disease known as "inflammatory bowel disease" is characterized by colon tissue ulceration, increased colon epithelial permeability, and significant leukocyte infiltration.[3]

Crohn's disease (CD) and ulcerative colitis (UC), the two primary forms of IBD, have different histological and clinical characteristics.[4,5]

Inflammation of the gastrointestinal tract (GIT) can affect any portion of the GIT, from the mouth to the anus, whereas CD mostly causes inflammation of the colon in UC. A chronic IBD called UC causes ulceration and swelling of the rectum that can reach the cecum.[6,7]

Because UC patients frequently undergo a relapsing and remitting illness course, the aim of treatment was to induce and sustain long-term remission.[6,8] This clinical course occurs either spontaneously or in response to treatment,[9] as a result, evaluations of new treatments for UC also take into account assessments of the symptoms and more accurate markers of the underlying disease process, such as endoscopy. Clinical trials in UC require both these disease activity (total Mayo score) and endoscopy.[6,10] UC is caused by a variety of pathogenic factors, including genetic predisposition, environmental factors, microbial factors, immunological factors, and cytokines.

Address for correspondence: Dr. Zeena Hadi Mageed, Department of Pharmacology, College of Medicine, University of Babylon, Babylon, Iraq.
E-mail: hadizena429@gmail.com

Submission: 02-Jan-2023 **Accepted:** 08-Feb-2023 **Published:** 17-Jul-2024

How to cite this article: Mageed ZH, Abbas MK, Raji A. Anti-inflammatory effect of atorvastatin on colitis induced in male mice. Med J Babylon 2024;21:240-4.

Access this article online

Quick Response Code:

Website: https://journals.lww.com/mjby

DOI: 10.4103/MJBL.MJBL_3_23

Several drug classes are used to treat UC. These include corticosteroids, immunomodulators, calcineurin inhibitors, aminosalicylic acid agents, prebiotics, probiotics, and antibiotic therapy.[11] Statin also possesses antioxidant and anti-inflammatory properties. The most often given medications worldwide are statins. The primary and secondary prevention of cardiovascular illnesses as well as the treatment of hypercholesterolemia are indications for their usage.[12,13] Based on their immunomodulatory properties, statins may have impacts beyond decreasing cholesterol levels.[12] Statins have previously been shown to reduce oral steroids used during the acute phase of IBD, as well as the disease activity index (DAI) and inflammatory mediators in IBD patients.[14] Other research has linked statins to a lower risk of developing new-onset IBD, increasing the likelihood that these medications also have anti-inflammatory properties and control molecules required for immunomodulation. The synthesis of interleukin-6 (IL-6) and inducible nitric oxide synthetase by microglia and astrocytes was discovered to be inhibited by statin.[15]

Materials and Methods

Experimental animals

The Experimental Animal Center provided 40 healthy adult male albino-Wister mice weighing (25–30 g) for this study (Baghdad, Iraq). They were housed in standard plastic cages in the animal house under good conditions, including a room temperature of 23°C, a relative humidity of 55%, and a 12-h light/dark cycle. Mice were fed standard chow food with free access to water for 1 week prior to the experiment.

Preparation of drugs

Solutions of atorvastatin were freshly prepared in a sterile condition prior to administration each day of the experiment by dissolving their powders in distilled water.

Induction of colitis

Mice that had fasted for 24 h were briefly given a light chloroform anesthesia. The tip of a 6-Eng. catheter was then cautiously placed into the colon at a distance of 4 cm from the anus. A solution of 2 mL of acetic acid (4%, v/v) in 0.9% sterile water was injected for 30 s into the colon's lumen to induce colitis. This was done to stop the intracolonic infusion from leaking. Using the same method, mice were given 0.9% saline alone in normal control tests.[16]

Experimental protocol

After acclimatization, mice were randomly divided into four groups (n = 10/group) and were given a single daily dose of the following for 10 consecutive days (3 days before induction and 7 days after induction of acetic acid-induced colitis):

Group I (normal control group): received normal saline (2 mL/day) rectally.[17]

Group II (colitis control group): received only 4% v/v acetic acid rectally.[16,18]

Group III (positive control group): received prednisolone (1 mg/kg/day) orally.[19,20]

Group IV (high-dose atorvastatin group): received atorvastatin (50 mg/kg/day) orally.[21,22]

On the 4th day of the experiment, all animals (except group 1) were given acetic acid (4% v/v) intrarectally for the induction of colitis.

So at end of the study, the mice were sacrificed after 24 h of starvation by an overdose of chloroform inhalation, the colon was removed, and the abdomen was quickly dissected. Then blood samples were taken from each mouse's heart, and serum was prepared and stored at −80°C to measure biochemical parameters.

Assessment of colitis severity

The disease activity index

The disease was clinically assessed using scores for body weight loss (0 = weight gain or no loss, 1 = 1%-5% decrease, 2 = 6%-10% decrease, 3 = 11%-15% decrease, and 4 = more than 15% decrease), feces consistency (0 = normal, 2 = loose feces, and 4 = diarrhea), and rectum bleeding (0 = normal, 2 = mild bleeding, and 4 = severe bleeding). The total DIA scores were combined to arrive at the calculation. Normal stool consisted of well-formed pellets, loose stool consisted of pasty stools that did not stick to the anus, and diarrhea consisted of liquid stools that adhered to the anus.[23,24]

Interleukin-6

The IL-6 levels in the tissues were determined using an enzyme-linked immune-sorbent assay (ELISA) kit and the instructions provided by the manufacturer.[25,26]

Statistical analysis

To summarize, analyze, and present the data, the Statistical Package for Social Science 23 software program was used (Microsoft Iraq). We used the mean and standard deviation of quantitative (numerical) variables. To investigate the difference in the mean of quantitative variables between groups, a one-way analysis of variance (ANOVA) was used, followed by a post hoc least significant difference test to assess the mean difference within groups. P values of less than 0.05 were considered significant.[27]

Results

Clinical parameters

High dose of atorvastatin treatment produce significant decreased in DAI when compared colitis group. Colon weight /length ratio was highly significant decreased with high dose atorvastatin treatment as compared with colitis group.

Effect of on cytokines (IL6)

High dose of atorvastatin treatment produce highly significant decrease expression concentration of (IL-6) as compared to colitis group.

Discussion

Effect of acetic acid on colonic mucosa in mice

Excessive oxidative stress, increased microvascular permeability, sustained immunocyte infiltration, and increased inflammatory mediator output were all major causative factors in the initiation of human UC in this animal model. As a result, it is appropriate for studying UC pathogenesis and evaluating novel therapeutic options for this disease.[28,29]

Acetic acid caused an increase in mean DAI in the untreated colitis rats. Our findings were in agreement with other previous results elevation DAI scores in the colitis group evidenced by reduce body weight, decrease in food intake, and increase in diarrhea with or without blood.[30]

Our findings support the work by Bertevello *et al.*[31] who described the increased level of expression of pro-inflammatory cytokine (IL-6), and these outcomes support the concept of the efficacy of this model in reproducing observations made on UC in humans, where the increase of these pro-inflammatory cytokines is related to disease severity.[32] In addition, their presence in UC had previously been linked to colonic inflammation. IL-6 is important in the pathogenesis of UC because it stimulates neutrophil infiltration and chemotaxis, resulting in tissue necrosis and destruction. It can also produce different mediators that are responsible for severe inflammation, which contributes to the development of UC.[33,34]

All of this proved that the level of IL-6 in UC was significantly elevated, and it can also produce various mediators responsible for severe inflammation, which contributes to the development of UC.[35]

Effect of atorvastatin in acetic acid-induced colitis in mice

Our findings showed that atorvastatin significantly reduced the severity of murine colitis as measured by body weight loss, diarrhea, and rectal bleeding, all of which resulted in a lower DAI.[21,36]

Treatment with atorvastatin significantly reduced IL-17 levels, as we also saw with IL-6 (a critical cytokine). IL-17 is a pro-inflammatory cytokine that stimulates the production of a variety of pro-inflammatory cytokines, including IL-6, by fibroblasts, endothelial cells, macrophages, and epithelial cells.[22,37]

Effect of prednisolone in acetic acid-induced colitis in mice

The prednisolone was significant decrease DAI score by more lowering (weight loss, rectal bleeding, and diarrhea).[38] A loose stool was observed in the induction group on day 3 and had returned to normal by day 6.[39] Prednisolone treatment was effective in lowering levels of pro-inflammatory mediators such as IL-6. When IL-6 was used instead of UC, the mediator levels were significantly repaired [Figures 1 and 2].[35,40]

Conclusion

In summary, atorvastatin's anti-inflammatory and antioxidant properties have been shown to effectively treat acetic acid-induced colitis in mice. Biochemical parameters like cytokine (IL-6) have a positive correlation with DAI [Table 1-3].

Ethical approval statement

The research was approved by local ethics committee in the Collage of Medicine with document number 2114 on November 1, 2020.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

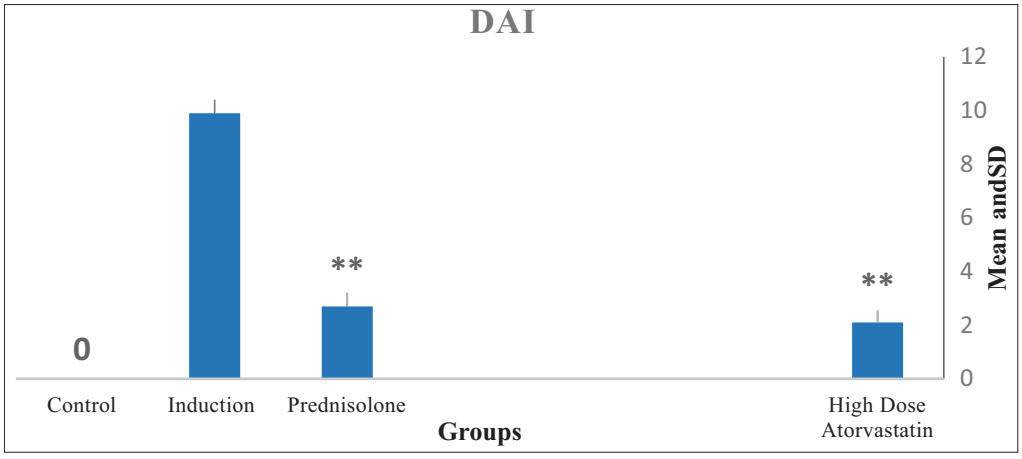

Figure 1: Disease activity index (DAI) score in control and study groups. Comparison between means of DAI in all groups ($N = 10$), $^{**}P < 0.01$, SD: standard deviation

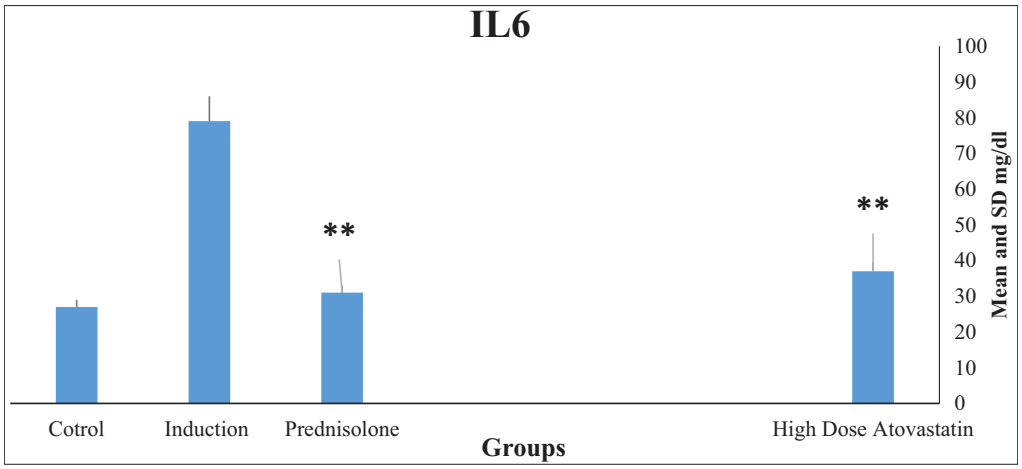

Figure 2: Interleukins-6 (IL-6) concentration in control and study groups. Comparison between means of IL-6 in all groups ($N = 10$), $^{**}P < 0.01$, SD: standard deviation

Table 1: Compares clinical and biochemical parameters between healthy controls and acetic acid-induced colitis groups.

Variable	Acetic acid-induced colitis group	Healthy control group	*P* value
DIA	9.89±0.5	0.00±0.0	<0.01**
IL-6	79.15±7	27±2	<0.01**

**More significantly increase

Table 2: compares clinical and biochemical parameters in the acetic acid-induced colitis and atorvastatin groups.

Variable	Acetic acid-induced colitis group	Atorvastatin group	*P* value
DIA	9.89±0.5	2.1±0.4	<0.01**
IL-6	79.15±7	32±2	<0.01**

**More significantly increase

Table 3: Compares clinical and biochemical parameters between the acetic acid-induced colitis and prednisolone groups.

Variable	Acetic acid-induced colitis group	Prednisolone group	*P* value
DIA	9.89±0.5	2.7±0.0	<0.01**
IL-6	79.15±7	30±4.9	<0.01**

**More significantly increase

References

1. Safarpour AR, Hosseini SV, Mehrabani D. Epidemiology of inflammatory bowel diseases in Iran and Asia: A mini review. ran J Med Sci 2013;38(2 Suppl):140-9.
2. Kayal M, Shah S. Ulcerative colitis: Current and emerging treatment strategies. J Clin Med 2020;9:1-12.
3. Xiao HT, Lin CY, Ho DHH, Peng J, Chen Y, Tsang SW, *et al*. Inhibitory effect of the gallotannin corilagin on dextran sulfate sodium-induced murine ulcerative colitis. J Nat Prod 2013;76:2120-5.
4. Ko J, Lam F-Y-L, Cheung A-P-L. Amelioration of experimental colitis by *Astragalus membranaceus* through anti-oxidation and inhibition of adhesion molecule synthesis. World J Gastroenterol 2005;11:5787-94.
5. Aleisa AM, Al-Rejaie SS, Abuohashish HM, Ola MS, Parmar MY, Ahmed MM. Pretreatment of *Gymnema sylvestre* revealed the protection against acetic acid-induced ulcerative colitis in rats. BMC Complem Altern Med 2014;153:11.
6. Head K, Jurenka J. Inflammatory bowel disease. Part I: Ulcerative colitis—Pathophysiology and conventional and alternative treatment options. A J Clin Ther 2019;8:1-6.
7. Md BA, Dabd M, Frcpc BSM, Al E. Fecal calprotectin use in inflammatory bowel disease and beyond: A mini-review. Gastroenterol Hepatol 2015;163:1-163.
8. Stange EF, Travis SPL, Vermeire S, Reinisch W, Geboes K, Barakauskiene A, *et al*. European evidence-based Consensus on the diagnosis and management of ulcerative colitis: Definitions and diagnosis. J Crohns Colitis 2008;2:1-23.
9. Kornbluth A, Sachar DB. Ulcerative colitis practice guidelines in adults: American College of Gastroenterology, Practice Parameters Committee. Am J Gastroenterol 2010;105:501-23.
10. Christensen B, Hanauer SB, Erlich J, Kassim O, Gibson PR, Turner JR, *et al*. Histologic normalization occurs in ulcerative colitis and is associated with improved clinical outcomes. Clin Gastroenterol Hepatol 2017;15:1557-64.e1.
11. Alridha AMA, Al-Gburi KM, Abbood SK. A review of pharmacogenetics of anticoagulant therapy: Heparins, rivaroxaban, apixaban, and dabigatran. Med J Babylon 2022;19:332-40.
12. Peppas S, Piovani D, Peyrin-biroulet L, Danese S, Bonovas S. Statins and inflammatory bowel disease: Where do we stand? Eur J Intern Med 2020;11:48-56.
13. Madloom BM, Umran HH. Evaluation of risk factor in Iraqi patients with angiographically documented peripheral vascular disease and the effect of specific risk factor on specific site or vessel. Med J Babylon 2022;17:347-52.
14. Crockett SD, Hansen RA, Stürmer T, Schectman R, Darter J, Sandler RS, *et al*. Statins are associated with reduced use of steroids in inflammatory bowel disease: A retrospective cohort study. Inflamm Bowel Dis 2012;18:1048-56.
15. Ungaro R, Chang HL, Cote-Daigneaut J, Mehandru S, Atreja A, Colombel JF. Statins associated with decreased risk of new onset inflammatory bowel disease. Am J Gastroenterol 2016;111:1416-23.
16. Niu X, Fan T, Li W, Huang H, Zhang Y, Xing W. Protective effect of sanguinarine against acetic acid-induced ulcerative colitis in mice. Toxicol Appl Pharmacol 2013;267:256-65.

17. Naito Y, Katada K, Takagi T, Tsuboi H, Isozaki Y, Handa O, *et al*. Rosuvastatin, a new HMG-CoA reductase inhibitor, reduces the colonic inflammatory response in dextran sulfate sodium-induced colitis in mice. Int J Mol Med 2006;17:997-1004.
18. Maheshwari RA, Balaraman R, Sailor GU, Sen DB. Protective effect of simvastatin and rosuvastatin on trinitrobenzene sulfonic acid-induced colitis in rats. Indian J Pharmacol 2015;47:176.
19. Melgar S, Bjursell M, Gerdin AK, Svensson L, Michaëlsson E, Bohlooly-Y M. Mice with experimental colitis show an altered metabolism with decreased metabolic rate. Am J Physiol—Gastrointest Liver Physiol 2007;292:165-72.
20. Christensen B, Colman RJ, Micic D, Gibson PR, Goeppinger SR, Yarur A, *et al*. Vedolizumab as induction and maintenance for inflammatory bowel disease: 12-Month effectiveness and safety. Inflamm Bowel Dis 2018;24:849-60.
21. Lei A, Yang Q, Li X, Chen H, Shi M. Atorvastatin promotes the expansion of myeloid-derived suppressor cells and attenuates murine colitis. Immunology. 2016;149:432-46.
22. Aktunc E, Kayhan B, Arasli M, Gun BD, Barut F. The effect of atorvastatin and its role on systemic cytokine network in treatment of acute experimental colitis. Immunopharmacol Immunotoxicol 2011;33:667-75.
23. Gong Y, Niu W, Tang Y, Zhang Q, Liu S, Liu X, *et al*. Aggravated mucosal and immune damage in a mouse model of ulcerative colitis with stress. Exp Ther Med 2019;2341:8.
24. Kasaian MT, Page KM, Fish S, Brennan A, Cook TA, Moreira K, *et al*. Therapeutic activity of an interleukin-4/interleukin-13 dual antagonist on oxazolone-induced colitis in mice. Immunology 2014;143:416-27.
25. Xiao Y-T, Yan W-H, Cao Y, Yan J-K, Cai W. Neutralization of IL-6 and TNF-a ameliorates intestinal permeability in DSS-induced colitis. Cytokine 2016;8:189-92.
26. Lv YL, Han FF, Jia YJ, Wan ZR, Gong LL, Liu H, *et al*. Is cytomegalovirus infection related to inflammatory bowel disease, especially steroid-resistant inflammatory bowel disease? A meta-analysis. Infect Drug Resist 2017;10:511-9.
27. Gupta RA, Motiwala MN, Mahajan UN, Sabre SG. Protective effect of *Sesbania grandiflora* on acetic acid induced ulcerative colitis in mice by inhibition of TNF-α and IL-6. J Ethnopharmacol 2018;219:222-32.
28. Hagar HH, El Medany A, El Eter E, Arafa M. Ameliorative effect of pyrrolidine dithiocarbamate on acetic acid-induced colitis in rats. Eur J Pharmacol 2007;554:69-77.
29. Randhawa PK, Singh K, Singh N, Jaggi AS. A review on chemical-induced inflammatory bowel disease models in rodents. Korean J Physiol Pharmacol 2014;18:279-88.
30. Owusu G, Obiri DD, Ainooson GK, Osafo N, Antwi AO, Duduyemi BM, *et al*. Acetic acid-induced ulcerative colitis in Sprague Dawley rats is suppressed by hydroethanolic extract of *Cordia vignei* leaves through reduced serum levels of TNF-α and IL-6. Int J Chronic Dis 2020;11:35-7.
31. Bertevello PL, Logullo AF, Nonogaki S, Campos FM, Chiferi V, Alves CC, *et al*. Immunohistochemical assessment of mucosal cytokine profile in acetic acid experimental colitis. Clinics (Sao Paulo) 2005;60:277-86.
32. Gotteland M, Lopez M, Munoz C, Saez R, Altshiller H, Llorens P, *et al*. Local and systemic liberation of proinflammatory cytokines in ulcerative colitis. Dig Dis Sci 1999;44:830-5.
33. Mehesen M. Effects of statins on experimental colitis in normocholesterolemic rats. Acad Res 2015;7:43-50.
34. Adhikary M, Phukan JP, Debnandi A, Sinha A, Das S, Lath A. Prevalence of rifampicin-resistant *Mycobacterium tuberculosis* by CBNAAT in a tertiary care hospital of West Bengal, India. Med J Babylon 2022;19:362-6.
35. Gupta RA, Motiwala MN, Mahajan UN. Protective effect of *Sesbania grandiflora* on acetic acid induced ulcerative colitis in mice by inhibition of TNF-α and IL-6. J Ethnopharmacol 2018;7:11-30.
36. Soliman NA, Keshk WA, Rizk FH, Ibrahim MA. The possible ameliorative effect of simvastatin versus sulfasalazine on acetic acid induced ulcerative colitis in adult rats. Chem Biol Interact 2019;298:57-65.
37. Stedman HH, Byrne BJ. Signs of progress in gene therapy for muscular dystrophy also warrant caution. Mol Ther 2012;20:249-51.
38. Ansari MN, Rehman NU, Karim A, Soliman GA, Ganaie MA, Raish M, *et al*. Role of oxidative stress and inflammatory cytokines (TNF-α and IL-6) in acetic acid-induced ulcerative colitis in rats: Ameliorated by *Otostegia fruticosa*. Life 2021;11:1951-17.
39. Soyturk M, Saygili SM, Baskin H, Sagol O, Yilmaz O, Saygili F, *et al*. Effectiveness of *Saccharomyces boulardii* in a rat model of colitis. World J Gastroenterol 2012;18:6452-60; discussion 6459.
40. Gupta RA, Motiwala MN, Dumore NG, Danao KR, Ganjare AB. Effect of piperine on inhibition of FFA induced TLR4 mediated inflammation and amelioration of acetic acid induced ulcerative colitis in mice. J Ethnopharmacol 2015;164:239-46.