

Blocking the Apelin Receptor (APJ) Attenuates TNBS-Induced Colitis in Rats

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ABSTRACT

Objective: The apelinergic system, consisting of apelin, ELABELA, and the apelin receptor (APJ), has a wide range of roles in physiological and pathophysiological processes in tissues. The effects of increased apelin and APJ as an indicator of damage in inflammatory conditions or as a compensatory mechanism are not fully clear in inflammatory bowel disease (IBD). This study was designed to assess the role of APJ in 2,4,6-trinitrobenzene sulfonic acid (TNBS)-induced colitis model.

Methods: Colitis in adult male Wistar rats were induced by intrarectally administered TNBS (30 mg b.w. in 50% ethanol). While the control group was treated with only saline to the colon, the TNBS+F13A and F13A groups received the APJ antagonist F13A (30 µg/kg/day, i.v.) for 3 days, starting immediately after TNBS or saline administration, respectively.

Results: A decrease in body weight and an increase in colon weight/length ratio and stool consistency score were observed in the TNBS group. TNBS caused an increase in the myeloperoxidase (MPO) activity and the number of proinflammatory cytokines (TNF-α, IL-1β, and IL-6), as well as apelin production, leading to mucosal ulceration, necrosis, and submucosal edema in the colon. While F13A administration to the control did not cause any change in the colon, F13A administration immediately after TNBS greatly reduced the effects of TNBS.

Conclusion: APJ is involved in the development of damage in colitis induced by TNBS. F13A reduces the level of damage, inflammatory cell infiltration, and MPO enzyme activity. APJ may be a therapeutic target in IBD.

Keywords: APJ, F13A, TNBS, ulcerative colitis, rat



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INTRODUCTION

Inflammatory bowel disease (IBD), a chronic global health problem, is a disease that reduces the quality of life of patients and has a medical cost burden due to its acute and chronic complications [1]. Genetic and environmental factors, poor host defense against microorganisms, or inappropriate immune responses may cause IBD [2, 3]. The disease is characterized

by mucosal inflammation in the digestive system, diarrhea, hematochezia, weight loss, and abdominal pain, and has a rather complex etiology [4]. Inflammation, which is very important for the modulation of tissue repair, is a complex process in which various factors and cells play a role. However, uncontrolled inflammation directly contributes to the development of various chronic diseases such as IBD.

IBD mainly includes Crohn's disease (CD) and ulcerative colitis (UC) [2, 5]. CD, which can affect all segments of the gastrointestinal (GI) tract, is characterized by transmural inflammation. UC occurs in the colon and mostly the rectum and is classically a superficial mucosal disease. However, when the disease becomes severe, deep and transmural ulcerations may occur. Although the clinical manifestations of UC and CD are different, dysregulation of the immune response and chronic inflammation in the GI system play a role in the pathogenesis of both subtypes [2, 3]. Treatment strategies for IBD include reducing inflammation and microbial load, suppressing inappropriate immune responses, and neutralizing inflammatory mediators [6].

The apelin receptor (APJ), isolated in 1993, is a G protein-coupled receptor [7]. APJ consists of 380 amino acids and its gene is in the chromosome 11 (q12). The APJ, which has complex effects because it can activate different G proteins and is expressed in all the tissues, has a high sequence homology of approximately 54% with the angiotensin II (Ang II) type 1 receptor (AT1R), the dominant receptor of Ang II [7, 8].

Apelin, the specific ligand of APJ is broken down into bioactive peptides such as apelin-12, -13, -17, and -36 by various enzymes shortly after it is produced as preproapelin with 77 amino acids [9]. Apelin exerts its effects only by activating APJ and has various physiological and pathophysiological roles such as modulation of the immune system, eating and drinking behavior, cardiac contractility, blood pressure, cell proliferation and apoptosis [8]. Recent clinical and experimental studies suggest that the apelin/APJ system modulates inflammation and oxidative processes and thus is involved in the regulation of various diseases [10, 11]. It is known that apelin expression increases in various tissues during the inflammatory process. Although increased colonic apelin production has been demonstrated in patients with IBD

and an animal model of sodium dextran sulfate (DSS)-induced colitis, the role of apelin in this process is not fully understood [12]. The regulatory effects of apelin on intestinal physiology are mostly related to motility, absorption, and enzyme secretion, and the number of studies to elucidate its role in IBD is very limited [13]. Pro-inflammatory cytokines, such as IL-6 and IFN- γ , play critical roles in the pathogenesis of IBD [14, 15]. These cytokines are known to increase apelin expression in the ileum of rats via the Jak/STAT pathway [16]. Findings from studies suggest that the apelin/APJ system plays an important role in IBD. In this study, the role of apelin in the inflammatory process in the colon was investigated in a TNBS-induced colitis model.

MATERIALS AND METHODS

Experimental Animals

In the experiments, 72 male, adult Wistar rats, weighing 200-250 g, were used. The Wistar rats were obtained from the Akdeniz University Experimental Animals Application and Research Center. All animal procedures were following the European Community Council Directive of 24 November 1986. Animals were treated in accordance with the Guide for the Care and Use of Laboratory Animals and were approved by the Akdeniz University Animal Experiments Local Ethics Committee (approval code: 1174/2020.08.001). The rats were allowed to adapt to the laboratory environment for at least 1 week before the experiment. They were housed in a room with a constant temperature ($22 \pm 1^\circ\text{C}$) and 12:12 hour light/dark cycle and fed with standard laboratory chow and tap water.

Drugs and Chemicals

TNBS (Picrylsulfonic acid, cat. no. P2297), hexadecyltrimethylammonium bromide (HTAB, H-5882), and O-dianisidine dihydrochloride (cat. no. D9154) from Sigma (St. Louis, Missouri, USA); phosphate-buffered saline (PBS) tablets (cat. no. 3002) from Invitrogen (Carlsbad, California, USA) and ethanol (cat. no. 100983), formaldehyde (cat. no. 818708), hydrogen peroxide (H_2O_2 , cat. no. 107298), and sodium chloride (cat. no. 106406) were purchased from Merck (Darmstadt, Germany). F13A was synthesized by GenScript (Piscataway, New Jersey, USA).

Experimental Procedure

The animals were randomly divided into groups as control, F13A, TNBS, and TNBS+F13A ($n=18$ in each one). 6 animals in each group were used for histological parameters and 12

Main Points;

- Apelin receptor (APJ) is involved in the development of damage in colitis.
- Trinitrobenzene sulfonic acid (TNBS) application enhanced the level of apelin in colon.
- The APJ blocker F13A reduced the level of damage, inflammatory cell infiltration and MPO enzyme activity in rats with TNBS-induced colitis.

animals were used for other parameters. The weights of the rats were measured both at the beginning and at the end of the experiments. Experimental procedures are summarized in Figure 1. The rats were anesthetized with ether after fasting for 24 hours.

Control group: 500 µl of saline was intrarectally given into 8 cm of (well-formed pellet); grade 1, pasty consistency (not adherent to the anus); grade 2, pasty consistency (adherent to the anus); grade 3, pasty the colon from the anus with the aid of a catheter (diameter 2 mm) attached to a syringe (1 ml).

F13A group: 500 µl of saline was given intrarectally and then apelin receptor antagonist F13A (30 µg/kg/day in saline, i.v.) was administered for three days (saline was given only on day 0, while F13A was given on days 0, 1, and 2).

TNBS group: TNBS (30 mg/kg b.w.) was given intrarectally [17]. TNBS was diluted 1:1 v/v with 50% v/v ethanol solution to give a total volume of 500 µl.

TNBS+F13A group: F13A (30 µg/kg/day in saline, i.v.) was applied for three days following TNBS injection (TNBS was given only on day 0, while F13A was given on days 0, 1, and 2) [18].

Three days after TNBS or saline administration, the stool consistency in the subjects was evaluated at 9 am according to the following criteria (on day 3): grade 0, normal consistency (adherent to the anus, containing blood); grade 4, diarrhea (watery stools sticking to the anus, containing blood) [19]. Then, the subjects under anesthesia (10 mg/kg xylazine and 90 mg/kg ketamine, i.p.) were euthanized by draining blood from the isolated abdominal aorta. The total colons of the subjects were removed, and the lesion areas were evaluated macroscopically. After the colon weight and length were determined, the weight/length ratio, an indirect indicator of inflammation, was calculated [20]. Distal colon tissues were divided into pieces of appropriate size according to the number of parameters and stored at -80°C for biochemical analysis (n=12 in each group) or placed into 10% formalin for histological staining studies (n=6 in each group).

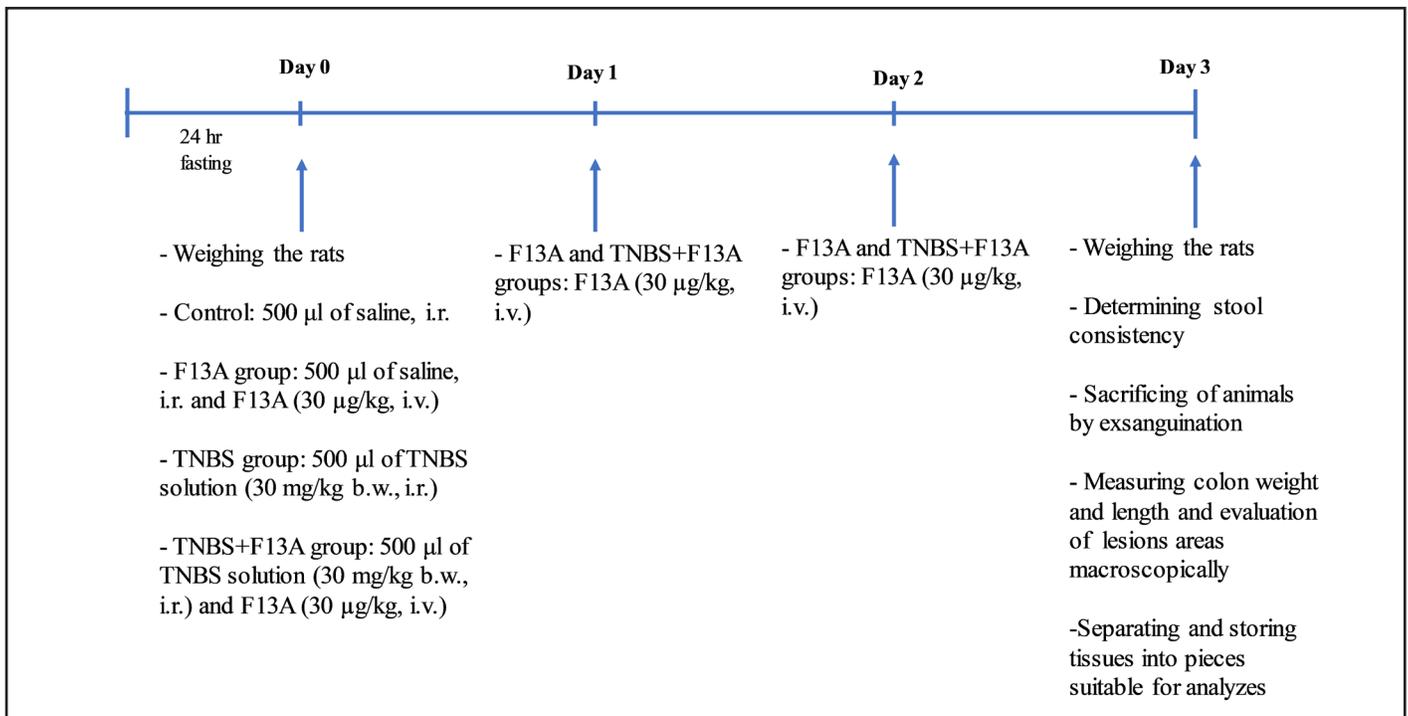


Figure 1. Summary of experimental procedures. On day 0, 500 µl of saline was administered intrarectally (i.r.) to the control group. TNBS (30 mg/kg in 500 µl, 50% ethanol v/v) was administered to TNBS and TNBS+F13A groups. F13A (30 µg/kg/day) was intravenously given to the F13A group after saline administration and to the TNBS+F13A group after TNBS administration. F13A applications were repeated on days 1 and 2. Rats were euthanized on day 3 and colons were removed for various testing (n=18 in the groups).

Macroscopic Evaluation of Damage

After the subjects' colons were opened longitudinally from the mesenteric margin and the mucosa was exposed, it was washed with physiological saline to clear the luminal content. Then damaged areas on the mucosa were examined macroscopically using a scoring system [21]. Morphological damage scoring criteria are as follows grade 0, no ulcer, no inflammation; grade 1, local hyperemia without ulcers; grade 2, hyperemia and ulcers without inflammation; grade 3, hyperemia and ulcers with inflammation at one side; grade 4, ulcers and/or inflammation in two or more areas; grade 5, ulcers and inflammation extending more than 1 cm in 2 or more areas; grade 6-10 if the ulcer size was larger than 2 cm, the score was increased by "1" for each additional cm.

Histopathology

For the microscopic evaluations, samples taken from the colon were fixed in 10% formalin fixative. Then the tissues were embedded in paraffin and 5 µm thick sections were taken from the blocks. After hematoxylin-eosin (H-E) staining of the sections, changes in the colon tissue were examined by light microscopy. In addition, based on previous studies, inflammatory cell infiltration, submucosal edema, mucosal necrosis, and mucosal ulceration were semi-quantified based on a scoring system [22]. The following criteria were used for scoring: Based on cell infiltration, submucosal edema, and mucosal necrosis as grade 0, no damage; grade 1, limited; and grade 2, diffuse; for mucosal ulceration grade 0, no ulceration; and grade 1, ulceration is present.

Enzyme Immunoassays

The homogenates of the colons obtained from the experimental groups were tested for apelin and the proinflammatory cytokines (TNF- α , IL-1 β , and IL-6) using enzyme immunoassay kits (Bioassay Technology Laboratory, cat. numbers respectively: E1026Ra E0764Ra and E0119Ra, E0135Ra).

Briefly, the colon tissues were rinsed in ice-cold PBS (pH 7.4) and weighed before homogenization. Then tissues were homogenized in PBS (tissue (g)/PBS (mL)=1:9) with an ultrasonic cell disrupter on ice. The homogenates were centrifuged for 15 minutes at 12 000 rpm at 4°C. The analysis of the parameters in supernatants was carried out according to the instructions in the kit. After measurement of the protein concentrations with the Bradford method, supernatants were assayed in well plates and the absorbance of the samples was determined at 450 nm.

The cytokine and apelin levels were expressed as nanograms or picograms per milligram of protein.

MPO Enzyme Activity

MPO enzyme activity was measured as an indicator of neutrophil infiltration into the colon tissues [23]. Briefly, the colon tissues were homogenized in 50 mM PBS, pH 6.0, containing 0.5% HTAB (1 ml solution per 50 mg wet tissue). Then, homogenates were centrifuged at 12 000 g for 15 min (+4°C). One unit of enzyme activity in the clear supernatants was expressed as the quantity of product formed in 3 minutes. MPO enzyme oxidizes O-dianisidine dihydrochloride in the presence of H₂O₂ and absorbance of the produced compound is measured at 460 nm. After the measurement of protein concentration in the samples, MPO activity was determined by mixing 100 µl of the sample with 2.9 ml of reaction buffer containing 0.167 mg/mL O-dianisidine dihydrochloride and 0.0005% H₂O₂ in 50 mM PBS, pH 6.0. The change in absorbance for 3 min was measured at 37°C and enzyme activity was expressed as U/mg protein.

Statistical Analysis

The number of subjects was calculated according to the power analysis performed in the light of the information in the literature, assuming 95% confidence (1- α) interval, 95% test power (1- β) and effect size $d = 0.5$. The data were analyzed with SPSS program version 23.0 software (Chicago, Illinois, USA). The suitability of the data to the normal distribution was evaluated with the Kolmogorov-Smirnov and Shapiro-Wilk tests. One-way ANOVA followed by Tukey's multiple comparisons test was used to compare the percent change in body weight, stool consistency score, colon weight/length ratio (g/cm), macroscopic injury score, and histopathological changes in the groups. Kruskal-Wallis and Mann-Whitney U tests were used to compare apelin, proinflammatory cytokines, and MPO activity in the colon of the groups. Values were expressed as the mean \pm standard error of the mean (SEM). A value of $p < 0.05$ was considered statistically significant.

RESULTS

Body Weight, Stool Consistency Score, Colon Weight/Length Ratio, and Macroscopic Colonic Injury

As seen in Figure 2A, the percentage change in body weight was found to be 0.4 in the control, 2.1 in the F13A group, 17.7 in the TNBS group, and 10.2 in the TNBS+F13A group. Compared to the control, the body weight loss was significantly increased in the TNBS group ($p < 0.001$). However, administration of F13A

following TNBS significantly prevented this TNBS-related loss in body weight ($p < 0.001$). It was observed that diarrhea developed in the TNBS group and there was a significant increase in the stool consistency score compared to the control ($p < 0.001$, Figure 2B). F13A reduced the enhancing effect of TNBS on stool consistency score.

The ratio of colon weight to length (g/cm) was 0.79 ± 0.04 g/cm in the control group, 0.78 ± 0.02 g/cm in the F13A group, 1.57 ± 0.08 g/cm in the TNBS group, and 1.13 ± 0.07 g/cm in the TNBS+F13A group (Figure 2C and 2D). Compared with the control group, in the TNBS-induced colitis group, colon weight increased about inflammation, and therefore the weight/length ratio significantly increased ($p < 0.001$). Administration of F13A following TNBS caused the weight/length ratio to decrease ($p < 0.001$).

When the degree of damage in the colon wall of the subjects was evaluated macroscopically, no damage was observed in the control group and F13A groups (Figure 2C and 2E). While

significant damage was detected in the colon tissue of rats treated with TNBS ($p < 0.001$), these lesions were significantly reduced by F13A injection following TNBS administration ($p < 0.001$).

Histopathology

In the microscopic examination of the colons of the control group rats, crypts with the normal histological structure were seen (Figure 3A, 3A1, and Table 1). Similarly, it was observed that the normal histological structure was preserved in the F13A group (Figure 3B, 3B1 and Table 1). In the TNBS group (Figure 3C, 3C1, and Table 1), the borders of the histological layers became indistinguishable with severe damage/deformation in the crypts, lesions in the epithelium (mucosal necrosis), intense mucosal/submucosal inflammatory cell infiltration and increased submucosal edema were determined. On the other hand, in the TNBS+F13A group, submucosal edema decreased, and some improvements were observed in the crypts and layers. In the TNBS+F13A group, intense mucosal/submucosal inflammatory cell infiltration and epithelial necrosis were milder than in the TNBS group (Figure 3D, 3D1, and Table 1).

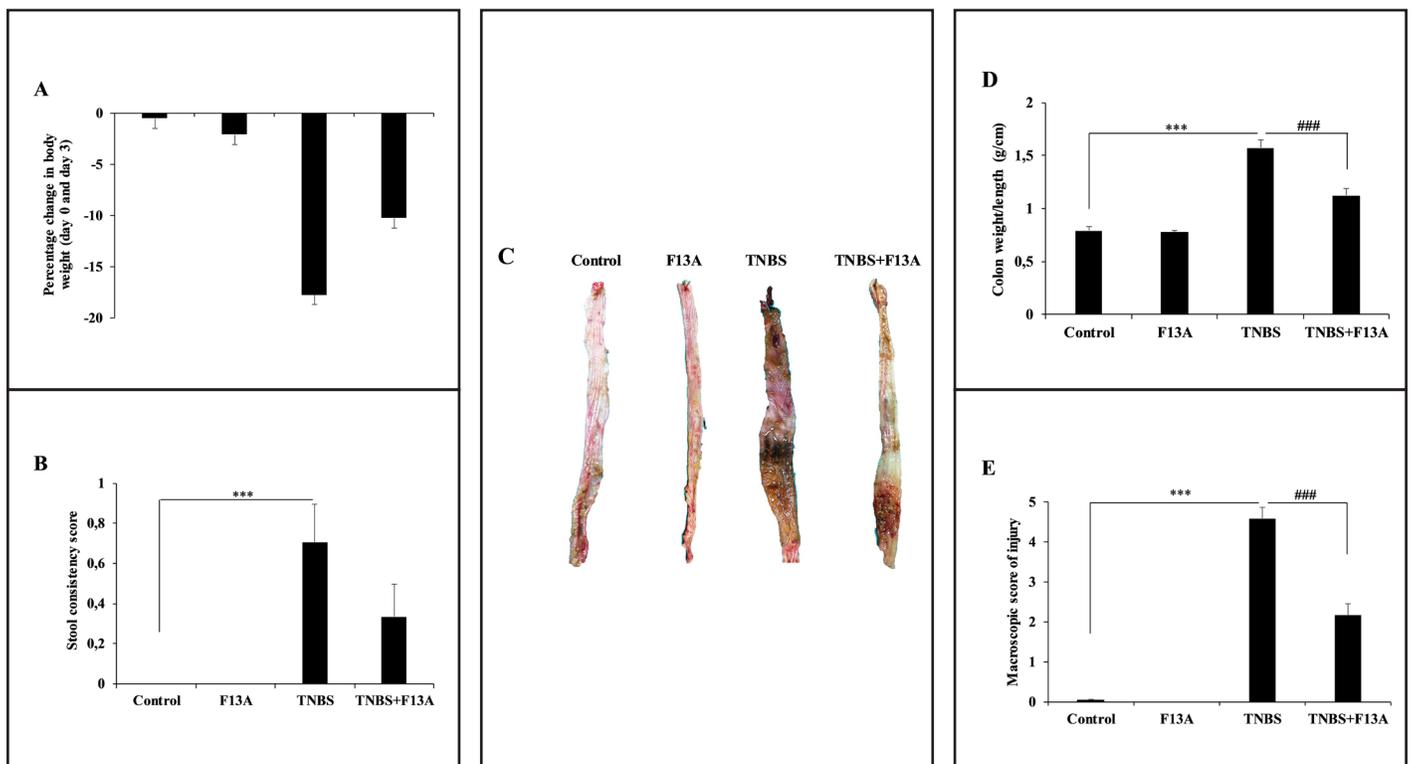


Figure 2. Effect of TNBS and F13A on the percentage change in body weight, stool consistency score, the colon weight/length ratio (g/cm), and macroscopic colonic injury score. The percent change in body weight from day 0 to day 3. B The consistency of the stool was calculated according to a scoring system on day 3. C The representative colon images. D The ratio of the total weight to length of the colons of the groups and E The macroscopic injury calculated according to a scoring system on day 3. Data was presented as mean \pm S.E.M (n=18 in groups). *** $P < 0.001$ vs. control group. ### $P < 0.001$ vs. TNBS-induced colitis group.

Table 1. Semi-quantitative scoring of histopathological changes in the colon.

	Control	F13A	TNBS	TNBS+F13A
Focal mucosal ulceration	0	0	1; 1±0*	0.5; 0,5±1 [#]
Inflammatory cells infiltration in mucosa	0.3; 0±1	0.5; 0.5±1	2; 2±0*	1.2; 1±2 [#]
Submucosal edema	0.2; 0±1	0.5; 0.5±1	2; 2±0*	1; 1±2 [#]
Mucosal necrosis	0	0.2; 0±1	2; 2±0*	1.2; 1±2 [#]

Data are expressed as mean; median ± range and analyzed by One-Way ANOVA followed by Tukey's multiple comparisons test which was used for comparing histological results between different selected groups (* P < 0.05 vs. control group, # P < 0.05 vs. TNBS group, n=6 in each group).

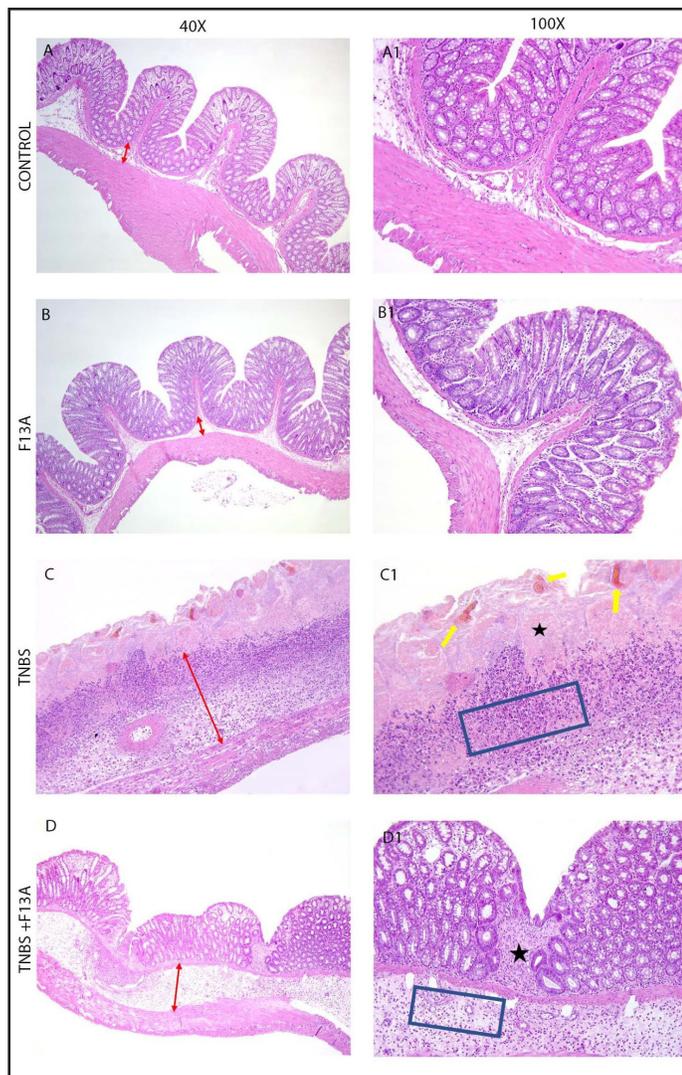


Figure 3. Representative H-E staining images of colon sections (40X and 100X) (n=6). While the tissue sections of the control and F13A groups exhibited a normal tissue appearance (A-A1, B-B1), the tissue sections in the TNBS group had mostly submucosal edema (red arrow), inflammatory cell infiltration (rectangular frame), necrotic areas (asterisk) and ulceration (yellow arrow) was observed (C-C1). Colon tissues of the TNBS+F13A groups were similar to those of the control groups. (D-D1).

Apelin

Compared to the control group (2.27 ± 0.72 ng/mg protein), blocking the APJ receptor with F13A did not significantly affect the apelin level in colon tissue (1.54 ± 0.12 ng/mg protein) (Figure 4). The rats treated with TNBS exhibited a significant increase in apelin level (16.43 ± 2.02 ng/mg protein, $p < 0.001$). But F13A administered following the application of TNBS prevented the increase in colonic apelin level due to TNBS (2.51 ± 0.25 ng/mg protein, $p < 0.001$).

Proinflammatory Cytokines

As observed in Figure 6. in the control group, the TNF- α (Figure 5A), IL-1 β (Figure 5B), and IL-6 (Figure 5C), the important proinflammatory cytokines, were 30.12 ± 2.85 ng/mg protein, 313.85 ± 23.40 pg/mg protein and 1.36 ± 0.18 ng/mg protein, respectively. F13A treatment did not cause any change in these parameters (42.70 ± 5.64 ng/mg, 224.73 ± 12.12 pg/mg, and 2.24 ± 0.23 ng/mg, respectively). In rats treated with TNBS, a significant increase in TNF- α levels (329.15 ± 32.59 ng/mg protein, $p < 0.001$), IL-1 β (2222.11 ± 225.12 pg/mg protein, $p < 0.001$) and IL-6 (14.08 ± 1.21 ng/mg protein, $p < 0.001$). However, F13A administration immediately after TNBS treatment decreased the changes due to TNBS in these parameters (35.93 ± 4.61 ng/mg protein, $p < 0.001$; 289.04 ± 16.59 pg/mg protein, $p < 0.001$; 1.98 ± 0.28 ng/mg protein, $p < 0.001$, respectively).

MPO Enzyme Activity

As seen in Figure 6, MPO activity, which is an indicator of neutrophil infiltration into the tissue, was found to be 80.62 ± 9.08 U/mg protein in the colon tissue in the control group, while it was 68.61 ± 6.97 U/mg protein in the F13A group. The rats treated with TNBS exhibited a significant increase in MPO activity (148 ± 16.88 U/mg protein, $p < 0.05$). However, blocking the APJ receptor immediately after TNBS treatment decreased the changes due to TNBS (87.92 ± 8.74 U/mg protein, $p < 0.05$).

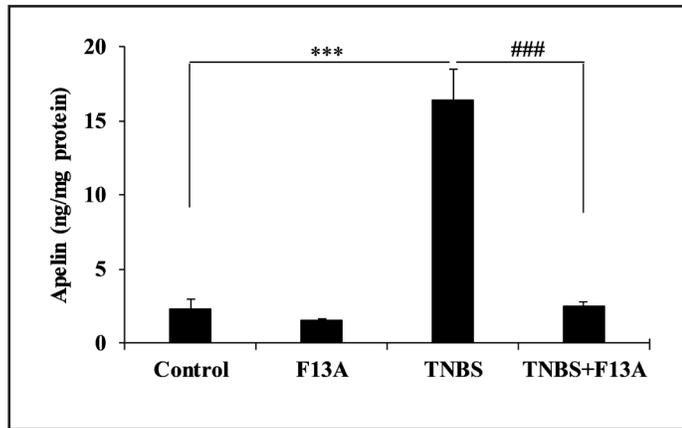


Figure 4. Effect of TNBS-induced colitis on the apelin level in the colon. Data was presented as mean ± S.E.M (n=12 in groups). ***P<0.001 vs. control group. ###P<0.001 vs. TNBS-induced colitis group.

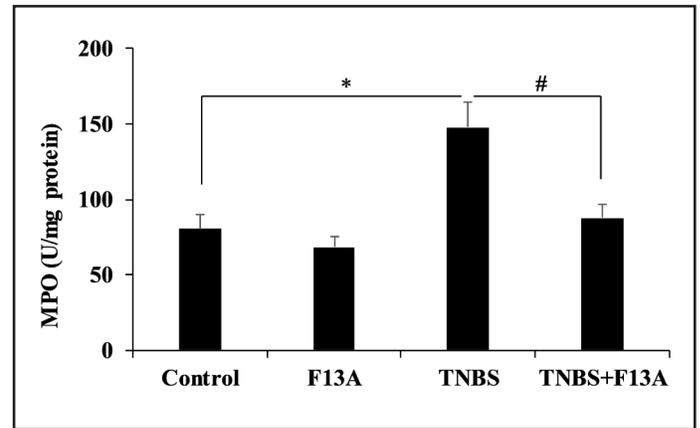


Figure 6. Effect of TNBS and F13A on the MPO enzyme activity in colon tissue. Data was presented as mean ± S.E.M (n=12 in groups). *P<0.05 vs. control group. #P<0.05 vs. TNBS-induced colitis group.

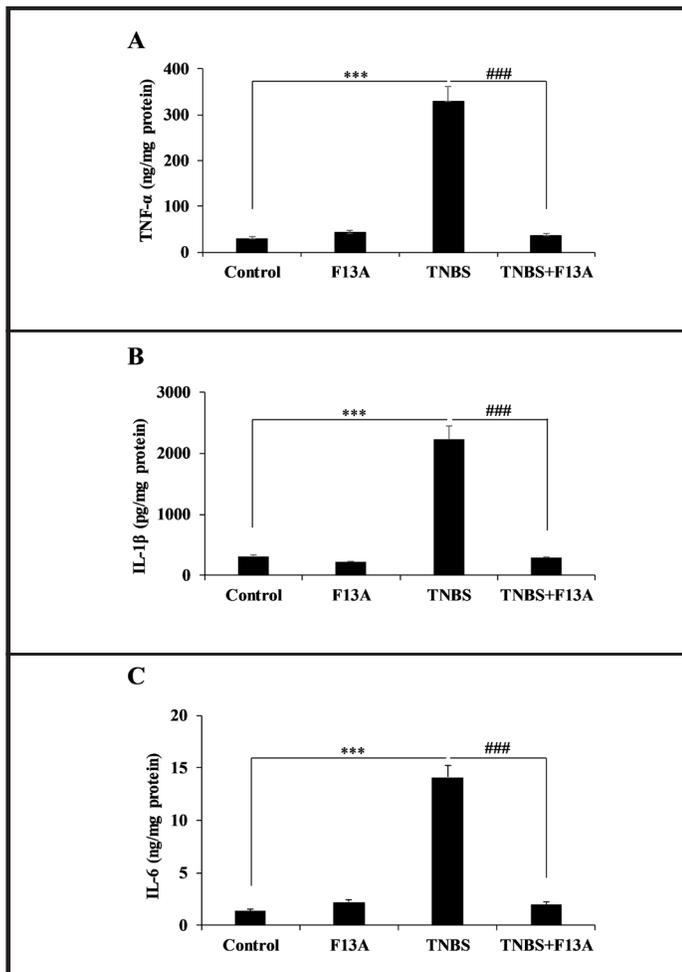


Figure 5. Effect of TNBS and F13A on the proinflammatory cytokines in colon tissue. A TNF-α level; B IL1β level; and C IL-6 level. Data was presented as mean ± S.E.M (n=12 in groups). ***P<0.001 vs. control group. ###P<0.001 vs. TNBS-induced colitis group.

DISCUSSION

The role of the apelinergic system, which is known to modulate inflammatory processes and oxidative stress, in the pathophysiology of IBD has not been clarified to date [10, 11]. In our study, by blocking APJ with F13A, the effects of apelin, whose expression is increased during TNBS-induced colitis, were prevented. Thus, the role of endogenous apelin in the pathophysiology of IBD was shown for the first time. Our major findings were as follows: (i) Blocking the APJ prevented macroscopic and microscopic injury induced by TNBS in the colon. (ii) Blocking the APJ suppressed inflammation by decreasing MPO activity and the proinflammatory cytokines. (iii) Blocking the APJ ameliorated TNBS-induced changes in body weight and colon weight/length ratio.

Immunological, genetic, and environmental factors play a role in the pathogenesis of IBD. It has been thought that it may occur because of excessive activation of the immune system and abnormal interactions. In this study, colitis mimicking UC was induced by intrarectal administration of TNBS dissolved in diluted ethanol [24]. Ethanol acts as a barrier breaker and increases intestinal permeability. TNBS passes into the subepithelial space, causing a rapid immune response [24]. Immune cells that migrate to the site of inflammation release cytokines [25]. Overproduction of cytokines damages tight junctions and causes tissue injury. Therefore, bacterial penetration into the submucosa is increased [26]. In this study, we focused on stool consistency, weight loss, the ratio of colon weight to length, macroscopic and microscopic damage levels in the colon, TNF-α, IL-6, and IL-1β levels, and MPO activity as

indicators of inflammation. Weight loss accompanying diarrhea was observed in the TNBS group. In addition, it was observed that TNBS caused an increase in colonic apelin production, apart from mucosal damage, proinflammatory cytokines, and MPO activity. Similar findings related to inflammation have been reported in previous studies in which colitis was induced by TNBS [27, 28]. Inflammation-related findings approached control values with the administration of F13A, thus our data suggest that F13A, an APJ antagonist, may be a therapeutic approach for IBD.

The apelin/APJ system has important functions for physiological and pathophysiological mechanisms in humans and animals, and these roles vary according to the bioactive peptides of apelin, localization of APJ expression, and activated different signaling pathways [13, 29]. In the GI tract of young rats, apelin and APJ mRNA levels are highest at birth and progressively decline into adulthood. The APJ is expressed on epithelial cells, goblet cells, and smooth muscle cells, as well as neurons of the enteric nervous system [30, 31]. It is also known that apelin mRNA expression decreases in the GI tract from the stomach to the colon [32]. In the present study, it was observed that the production of apelin, whose expression is low in the colon under normal conditions, was high in TNBS-induced colitis. While proinflammatory factors can affect the expression of apelin, conversely, apelin can affect the level of proinflammatory factors [10]. It is known that apelin expression is significantly increased in various pathological processes such as liver damage, fibrosis, cirrhosis, non-alcoholic fatty liver disease, pancreatitis, and gastroesophageal and hepatocellular cancers [13]. In general, apelin, which has its receptor on immune cells, reduces inflammation by suppressing immune cell migration and secretion of proinflammatory cytokines and inhibits apoptosis by modulating caspases [10, 11, 29, 33]. In our previous studies, we observed that apelin-13 had a protective effect against ischemia reperfusion injury in the gastric mucosa by activating vago-vagal reflex mechanisms [34, 35]. Also, it was observed that blocking apelin receptors after ischemia delays the healing process in stomach [36]. Similarly in the colon, the application of synthetic pyro-apelin-13 for 3 days in the recovery phase after colitis was induced with DSS in mice, increased cell proliferation [12]. In another study, it was determined that chronic (4-week) application of apelin-13 diminished the disease activity index, and inflammatory score, and induced lymph angiogenesis in mice that spontaneously developed colitis [37]. In these last two studies, the effects of exogenously applied apelin after the

development of colitis were examined. However, in our present study, the effect of endogenous apelin during the inflammatory process was abolished by blocking the APJ. Thus, it was shown for the first time that the pathology caused by TNBS in the colon tissue was reduced when the effects of APJ were inhibited by F13A.

The apelinergic system can regulate inflammation in tissues by different mechanisms. Administration of Fc-apelin, which has a long half-life, reduces lipopolysaccharide-mediated liver damage by suppressing macrophage infiltration, oxidative stress, and apoptosis [38]. Although the activation of APJ is thought to reduce oxidative stress inflammation and apoptosis, some studies say the opposite [10, 39]. Apelin stimulates the expression of NADPH oxidase through the APJ and contributes to atherosclerosis by inducing proliferation dependent on oxidative stress in vascular smooth muscle cells [40]. Jo2-induced damage and apoptotic changes in the liver were less observed in APJ^{-/-} mice [41]. Plasma apelin levels and hepatic APJ expression were increased in cirrhotic rats [42]. It was observed that hepatic fibrosis decreased with F13A application. These studies suggest that the apelinergic system exerts regulatory effects through different mechanisms in various tissues in different situations.

Intestinal permeability plays a critical role in the development of digestive system diseases such as IBS and IBD [43, 44]. Disturbances in the intestinal barrier cause bacterial translocation, impaired absorption of nutrients and motility, and visceral hypersensitivity [26, 45]. It was reported that accelerated colonic transit caused by acute stress is reduced by intraperitoneally administered APJ antagonists [46]. It is also thought that APJ contributes to corticotrophin-releasing factor (CRF)-mediated changes in colonic motility. Furthermore, the APJ agonist activates the CRF-TLR4-proinflammatory cytokine signal, causing visceral hypersensitivity and increased epithelial permeability in the colon [47]. In the GI tract, CRF signaling regulates not only motility, but also permeability, inflammation, and immunity [48]. CRF signaling which may also play a role in the regulatory effect of the apelinergic system in IBD, was not evaluated in the present study. Moreover, it was reported that LPS or CRF activates the AT1 system to cause colonic hyperpermeability and injury [47]. AT1 receptor expression increases in the colon with colitis [49]. AT1 signaling activates NF- κ B and induces inflammation [50]. There has been a report suggesting the existence of signal interaction between apelin-APJ and the AT1 system. Non-activated APJ via apelin may suppress AT1 receptor activation [51]. In this regard, the effects

due to the blockade of APJ may also be due to the suppression of AT1 signaling. The relationship between CRF and AT1R signals during APJ blockade was not evaluated in this study. It would be useful to reveal these mechanisms in elucidating the role of APJ during colitis.

CONCLUSIONS

In conclusion, in this study, it was shown that blocking the APJ in a TNBS-induced colitis model alleviated inflammation, edema, and infiltration of inflammatory cells, and decreased cytokine and damage levels (Figure 7). Our findings have shown that the apelin/APJ system contributes to the pathophysiology of IBD, further studies are needed to explain how the apelinergic system acts. Clarifying the signaling mechanisms between the apelinergic system and CRF or AT1 will also be beneficial in terms of the approach to ulcerative colitis treatment.

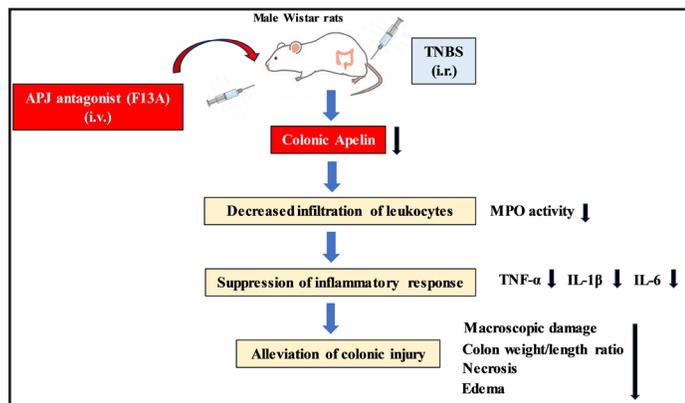


Figure 7. TNBS increased apelin production in colon tissue and also caused leukocyte infiltration into the tissue and pro-inflammatory cytokine response. APJ antagonist F13A administered after TNBS reduced these effects of TNBS.

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Conflict of interest statement: The authors declare that they have no conflict of interest.

Ethics Statement: All animal procedures were following the European Community Council Directive of 24 November 1986. Animals were treated in accordance with the Guide for the Care and Use of Laboratory Animals and were approved by the Akdeniz University Animal Experiments Local Ethics Committee (approval code: 1174/2020.08.001).

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