Original Article / Özgün Araştırma

Investigation of the Effects of Apelin 13 on Experimental Ulcerative Colitis

Apelin 13'ün Deneysel Ülseratif Kolit Üzerindeki Etkilerinin Araştırılması

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Abstract

Objective: The aim of this study was to investigate the effects of apelin (APL)-13 in a trinitrobenzenesulphonic acid (TNBS) induced experimental colitis model. It is also to test whether APL-13 has an antioxidant effect in addition to its proliferative and anti-inflammatory effect in colon inflammation by looking at antioxidant parameters.

Materials and Methods: Forty-five Wistar albino rats were divided into six groups as control, sham control, APL control, colitis (TNBS), colitis + ethanol, and colitis + APL. The control group was fed a standard diet without any treatment. The sham group was treated in the same colitis group, but saline was given instead of TNBS. The APL control group was given APL, but no colitis was induced. Colitis + ethanol and colitis + APL group was given ethanol and 100 µg/kg APL intraperitoneally (i.p.) for 3 days after colitis. For the biochemical analysis of the tissues, levels of myeloperoxidase (MPO), malonyldialdehyde (MDA), superoxide dismutase, catalase and glutathione were measured. Histopathological observations occurred.

Results: There were significantly increased MDA and MPO levels the animals that were grouped as TNBS and the antioxidant parameters were shown significantly decreased. Administration of APL-13 did make a significant decrease in colon MDA and MPO levels and antioxidant parameters were shown significantly increased compared to TNBS groups. The histological image of the TNBS + APL group was observed to have reduced mucosal damage, necrosis and edema compared with the TNBS group.

Conclusion: In conclusion, our study showed that APL-13 has therapeutic effects on TNBS induced ulcerative colitis. APL-13 may be an effective substance that can be used to eliminate the negative effects of ulcerative colitis.

Keywords
Apelin 13, inflammation, oxidative damage, TNBS, ulcerative colitis

Anahtar Kelimeler
Apelin 13, enflamasyon, oksidatif hasar, TNBS, ülseratif kolit

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Öz

Amaç: Bu çalışmanın amacı, trinitrobenzen sülfonik asit (TNBS) ile indüklenen deneysel kolit modelinde apelin (APL) 13'ün etkilerini araştırmaktır. APL-13'ün kolon enflamasyonunda proliferatif ve antiinflamatuvar etkilerinin yanında antioksidan etkilerini biyokimyasal ve histopatolojik analizler ile test etmektir.

Gereç ve Yöntemler: Kırk beş adet Wistar albino sıçan, kontrol, sham kontrol, APL kontrol, kolit (TNBS), kolit + Etanol ve kolit + APL olmak üzere altı gruba ayrıldı. Kontrol grubuna tedavi uygulanmadan standart yem verildi. Sham grubu ve kolit grubuna intrarektal salin ve TNBS verildi. APL kontrol grubuna APL uygulandı, ancak kolit yapılmadı. Kolit + Etanol ve Kolit + APL gruplarına kolit sonrası 3 gün boyunca 100 µg/kg APL intraperitoneal olarak (i.p.) verildi. Dokuların biyokimyasal analizi için miyeloperoksidaz (MPO), malonildialdehit (MDA), süperoksit dismutaz, katalaz ve glutatyon seviyeleri ölçüldü. Histopatolojik değerlendirmeler gerçekleştirildi.

Bulgular: TNBS grubu hayvanların MDA ve MPO düzeylerinde anlamlı düzeyde

artış olduğu ve antioksidan parametrelerinin önemli ölçüde azaldığı görüldü. APL-13 uygulamasının TNBS grubuna göre kolon MDA ve MPO seviyelerinde önemli bir düşüş sağladığı ve antioksidan parametrelerin ise TNBS grubuna göre önemli ölçüde arttığı gösterildi. TNBS + APL grubunun histopatolojik değerlendirmesinde TNBS grubuna göre daha az mukozal hasar, nekroz ve ödem olduğu gözlendi.

Sonuç: Sonuç olarak, çalışmamız APL-13'ün TNBS'ye bağlı ülseratif kolit üzerinde terapötik etkileri olduğunu göstermiştir. APL-13, ülseratif kolitin olumsuz etkilerini ortadan kaldırmak için kullanılabilecek etkili bir madde olabilir.

Introduction

Ulcerative colitis is a recurrent, inflammatory disease that effects the digestive system, limited to the colon. It is characterized by superficial and continuous ulcers from the rectum to the proximal colon (1,2). One of the most commonly used animal models is the rat model induced by 2, 4, 6 trinitrobenzene sulfonic acid (TNBS) (2).

In previous studies, it is reported that many factors (for example neutrophil infiltration and excessive production of proinflammatory mediators such as cytokines, arachidonic acid metabolites) play a role in the pathogenesis of inflammatory bowel disease (IBD), especially in the TNBS model. In addition, reactive oxygen metabolites cause tissue damage in many inflammatory diseases, especially colitis. The degree of tissue is damage caused by free oxygen radicals depends on the effectiveness of the intracellular defense systems. Defense systems consist of many enzymes and free radical scavengers [catalase (CAT), superoxide dismutase (SOD), reduced glutathione (GSH), peroxidases]. These antioxidant defense mechanisms are responsible to prevent the initiation of lipid peroxidation and the formation of free radicals (3-5).

Apelin is an endogenous peptide which found in many tissues such as brain, kidney, heart, lung, adipose tissue, gastrointestinal tract and breast tissue and binds to apelin receptors. Due to its presence in many tissues, apelin is effective on many functions such as heart contraction, blood pressure, appetite and drinking behavior, hypothalamo-pituitary-adrenal axis, stomach, insulin and cholecystokinin secretion (6).

The apelin gene encodes a 77 aa pre-proprotein with the signal peptide at its end of N-terminal. After translocation and signal to the endoplasmic reticulum, the remaining 55 aa proprotein is split into several active fragments such as apelin (APL)-13, APL-17 and APL-36. Each of these are peptides containing the number of amino acids specified in their names. The presence, concentration and effects of these peptides in human plasma are being investigated. In IBD, the inflammation in the colon affects the production of many molecules synthesis, absorption and secretion (7). APL-13 is found in many tissues, organs and systems in humans and rodents such as the central nervous system, liver, kidneys, lungs, adipose tissue and cardiovascular system. In various scientific studies have conducted in recent years, APL-13 has been shown to have effects against oxidative damage (8,9). In addition, apelin has been determined to be a useful adipokine with anti-diabetic and anti-obesity properties. Because of these properties, apelin seems to be a powerful and promising therapeutic target in metabolic disorders (10).

The aim of this study is to investigate the effects of APL-13 in the TNBS-induced experimental colitis model. It is also to test whether APL-13 has an antioxidant effect in addition to its proliferative and anti-inflammatory effect in colon inflammation by looking at antioxidant parameters.

Material and Methods

Experimental Animal Material

The approval of Adnan Menderes University Animal Experiments Local Ethics Committee (ADU-HADYEK), numbered 64583101/2014/171 (date: 10.09.2014). Forty-five Wistar albino rats were used in the ADU Experimental Animals unit. Analyzes were carried out in ADU Central Research Laboratory and Faculty of Medicine Laboratories.

Experimental Groups

Rats were divided into six groups as control, sham control, APL control, colitis (TNBS), colitis + ethanol, and colitis + APL. The numbers of the rats from control groups were 7 and the others were 8. Control group animals were fed with normal standard diet without any treatment. Sham group animals were treated in the same colitis group, but saline was given instead of TNBS. Apelin was given to animals in the APL control group, but colitis was not performed. Colitis + ethanol

and colitis + APL group animals were given ethanol and 100 µg/kg APL intraperitoneally (i.p.) every day for 3 days after colitis, each in their own group.

Induction of Colitis

Animals to be treated with colitis were fasted approximately 24 hours before the administration of colitis and their intestines were emptied on the operation day, under the dose of 75 mg/kg ketamine and 8 mg/kg xylazine anesthesia, 25 mg TNBS diluted in 0.8 mL saline. The colitis agent, prepared by dissolving in 37% ethanol, was injected 8 cm inside the anal orifice with the help of a polyethylene cannula. Sham control group animals were treated in the same colitis group, but saline was given instead of TNBS (2,11,12).

Sacrification

After all animals were subjected to manipulations of in their groups during the experiment, all animals were sacrificed 3 days later and their colons removed. After the columns were taken, biochemical and histopathological analyzes were performed.

Biochemical Analyses

Standard biochemical procedures were applied and supernatants were used for analyzes. The levels of malonyldialdehyde (MDA) (Cat. No: K739, BioVision®, Milpitas, CA, United States), myeloperoxidase (MPO) (Cat. No: K744, BioVision®, Milpitas, CA, United States), GSH (Cat. No: K264, BioVision®, Milpitas, CA, United States), SOD (Cat. No: K335, BioVision®, Milpitas, CA, United States) and CAT (Cat. No: K773, BioVision®, Milpitas, CA, United States) were measured. Biochemical parameters were measured using ELX800TM (Biotek Instruments Inc. Winooski, USA).

Histopathological Analyses

Standard histological preparation methods were used and the sections were stained with hematoxylin and eosin. Olympus BX51 light microscope was used for examination. The blind histology expert, examined tissue damage with light microscopy as a blind observation of tissue edema formation, mucosal damage, necrosis, hemorrhage and inflammation, graded the results between 0 and 3; Grade 0: normal histopathology; Grade 1: mild histopathological deterioration; Grade 2: moderate histopathological deterioration; Grade 3: severe histopathological deterioration (13).

Statistical Analysis

GraphPad 7 statistical program (GraphPad Software, Inc., CA, USA) was used for statistical analysis (Means and standard deviations were given). One Way ANOVA test and Mann-Whitney U test were used for statistical evaluation. $P<0.05$ was considered statistically significant. The asterisk indicates that there was a statistically significant difference compared to the TNBS group, while the ns sign indicated that there was no statistical significance.

Results

Biochemical Results: SOD and CAT activities as well as GSH levels were measured to investigate the antioxidant properties of APL against TNBS administration. In addition, MDA and MPO levels were measured to determine the oxidative damage and leukocyte infiltration levels. Biochemical results are presented in Table 1.

MDA Levels: As shown in Figure 1a. here we determined that MDA levels were significantly increased in the TNBS group compared to the control group. Colonic MDA measurements showed a statistically significant decrease in the TNBS + APL group compared to the TNBS group ($p<0.05$).

MPO Activity: We also determined the MPO activity levels and showed that MPO of the rats in the TNBS group were significantly higher than that of the control group. APL administration resulted in a statistically significant decrease in colon MPO levels between the TNBS + APL and TNBS groups ($p<0.05$). The results are shown in Figure 1b.

GSH Levels: Statistically significant decreases were determined in colonic GSH levels of rats in the TNBS group compared to the control groups. GSH concentration in colon was significantly increased in the TNBS + APL group that treated with APL compared to TNBS group ($p<0.05$). The results are shown in Figure 1c.

SOD Activity: Colonic SOD levels were decreased in the TNBS group compared to the control group ($p<0.05$). In contrast, SOD activities were increased significantly in the TNBS + APL group that applied APL compared to the TNBS group ($p<0.05$). These results show that APL administration supports antioxidant activity by supporting SOD activity. The results are shown in Figure 2a.

Table 1. Biochemical and histopathological results

	Biochemical results				Histopathological results					
	MPO (U/g tissue)	MDA (nmol/g tissue)	GSH (nmol/g tissue)	CAT (mU/mL)	SOD (U/mL)	Mucosal damage	Necrosis	Inflammation	Edema	Hemorrhage
Control	15.69±0.85*	280.97±20.22*	20.46±2.88*	6.14±1.02*	4.51±0.65*	0	0	0	0	0
Sham control	16.26±1.66*	290.19±28.06*	21.86±2.45*	5.89±0.75*	5.14±1.25*	1	0	0	1	0
APL control	14.36±1.64*	278.53±19.47*	19,79±1.70*	5.64±1.02*	5.19±0.47*	0	0	0	0	0
TNBS	19.42±2.13	340.68±20.24	14.18±1.87	3.15±0.59	2.26±0.45	3	3	2	3	2
TNBS + ethanol	20.15±2.34ns	341.45±31.65 ns	13.25±2.26 ns	3.89±0.59 ns	2.89±0.66 ns	3	2	2	3	2
TNBS + APL	16.36±2.07*	303.05±17.37*	17.51±1.59*	4.16±0.47*	3.45±0.38*	2	1	1	1	1

*If p<0.05 comparing result with TNBS group, nsComparison with TNBS group if p>0.05, MPO: Myeloperoxidase, MDA: Malonyldialdehyde, GSH: Glutathione, SOD: Superoxide dismutase, TNBS: Trinitrobenzene sulfonic acid, APL: Apelin

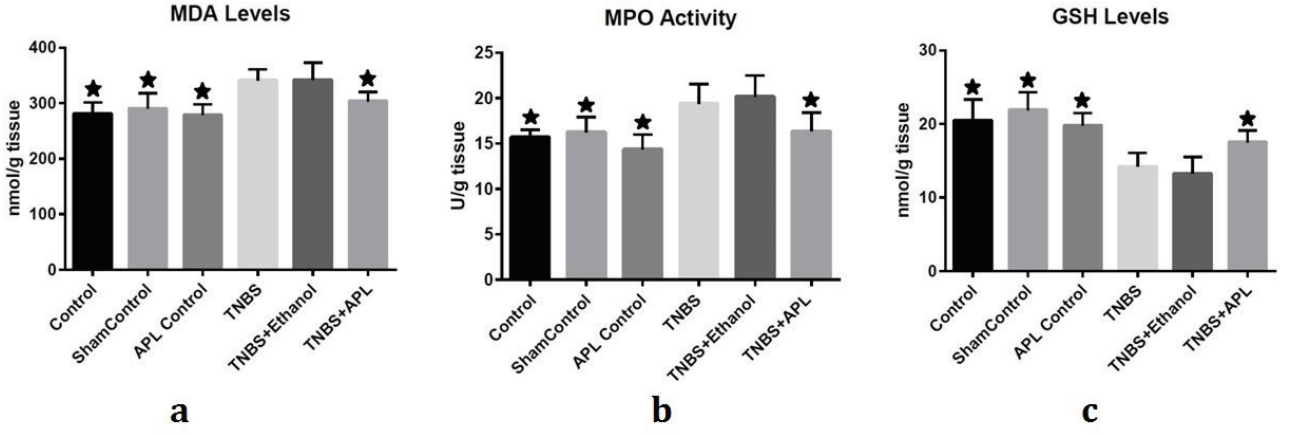

Figure 1. The changes of a) MDA levels, b) MPO activities and c) GSH levels between control and experimental groups
MDA: Malonyldialdehyde, MPO: Myeloperoxidase, GSH: Glutathione

CAT Activity: The results obtained from CAT activity measurements showed that there was a decrease in the TNBS group in comparison to the control group. In contrast, APL supported CAT activity accordingly increased CAT activity was detected in the TNBS + APL group compared to the TNBS only group. The results are shown in Figure 2b.

Histopathological Results: In the microscopic examination of the colon tissues of the rats in the control group (Control, Sham control, APL control), we observed that the mucosal formations were normal (Figure 3). Whereas, in the microscopic examination of the colon tissues of rats in the TNBS group, structural changes consistent with grade 3 which

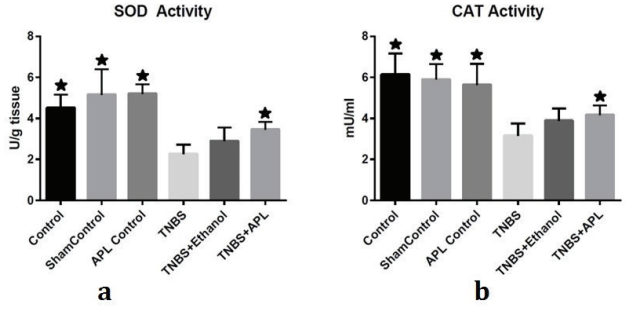

Figure 2. The changes of a) SOD and b) CAT activities between control and experimental groups
SOD: Superoxide dismutase, CAT: Catalase

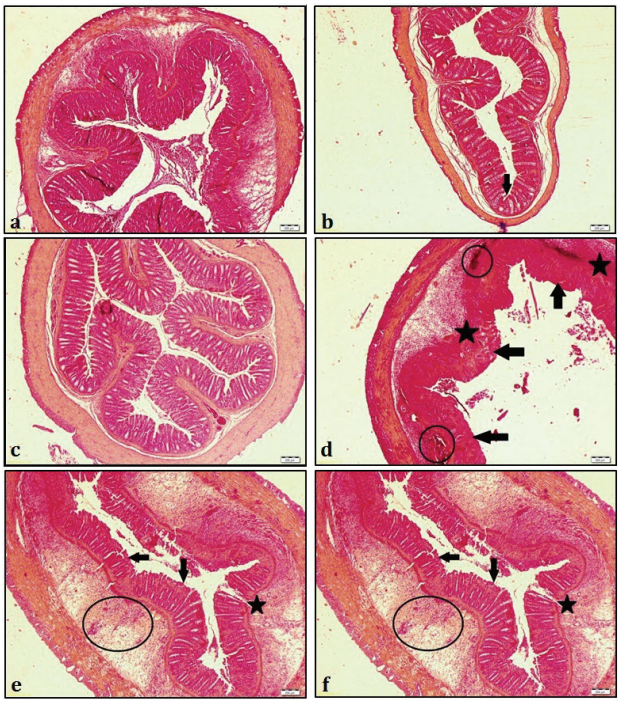

Figure 3. Arrow: Mucosal damage, Circles: Hemorrhagic areas. Asterisks: Inflammation and edema. a) Control group; b) Sham control group; c) APL control group; d) TNBS group; e) TNBS + ethanol group; f) TNBS + APL group
TNBS: Trinitrobenzene sulfonic acid, APL: Apelin

were characterized by mucosal damage and necrosis, hemorrhage, diffuse edema and severe inflammation were detected. The results of the TNBS + Ethanol group treated with ethanol, the solvent of APL-13, were very close to the histopathological results of the TNBS group. The histological image of the TNBS + APL group showed to have reduced mucosal damage, necrosis and edema compared to the TNBS group. Hemorrhagic areas were present but were less than that of the TNBS group.

Discussion

IBD has been studied for many years using different experimental animal models, as well as studies on various therapeutic and protective agents. The TNBS induction model is one of the models that best reflect the formation of ulcerative colitis chemically (14,15). In our study, TNBS was used to induce ulcerative colitis; experimental TNBS-administration caused ulcerative colitis, and these results were obtained in biochemical and histopathological scores.

The increases of MPO levels were found in experimental models with ulcerative colitis. The increase of MPO activity, which is an indicator of leukocyte accumulation in tissue, is a marker of tissue damage in addition to other oxidative damage markers (16,17). The results we obtained in our study show that MPO levels increased in the TNBS applied groups. MPO levels reduced in the APL-treated colitis group.

Oxidative stress and associated lipid peroxidation can disrupt the integrity of the intestinal mucosal barrier by exacerbating free radical chain reactions and activate inflammatory mediators. Various studies showed that MDA levels increased after TNBS was administered (11,18). Our results showed that MDA levels decreased in colitis rats treated with APL, and associated histopathological improvements.

The effects of APL-13 on GSH and GSH-Px levels have been demonstrated on different experimental models (19-21). Bircan et al. (20) showed in their renal ischemia study that APL-13 administration increased GSH-Px levels in a dose-dependent manner.

Sagiroglu et al. (21) found in their hepatic ischemia-reperfusion experimental study that the GSH level of the APL-13 group was statistically increased compared to the ischemia group. In our study, GSH levels are increased significantly in the APL-13 applied groups. The increase in GSH level in the APL control group compared to the Sham control group indicates the protective character of APL-13 administration.

The positive effects of ApL-13 administration on the CAT enzyme Foussal et al. (22) demonstrated against oxidative stress in their experimental study. Xu and Li (23) showed that APL-13 administrations increased the CAT level which is decreased in spinal cord ischemia reperfusion injury. In our study, the CAT level was decreased in the TNBS groups, increased with the administration of APL-13. The relationship between the increase in CAT level and the decrease in oxidative stress were shown.

Zhang et al. (24) found that the results of APL-13 administration in rats with oxidative stress were close to the control group without stress. These results were supported by other antioxidant parameters and oxidative stress biomarkers. Pisarenko et al. (25) determined that decreased SOD values in myocardial ischemia-reperfusion injury increased with the administration of APL-13. Hence, our results showing

increased SOD activity in APL-13 group compared to the TNBS administered group, are consistent with the literature.

Experimental studies show that antioxidant parameters are decreased in TNBS applied groups, while oxidative stress parameters are increased (11,12,18). In those studies, antioxidant and oxidative stress levels were supported by histopathological scores. In our study, while the levels of MDA, which is an oxidative stress marker, and the levels of MPO, which is an infiltration marker, are increased. Meanwhile, the antioxidant parameters GSH, CAT and SOD were decreased in the TNBS groups. In contrast, administration of APL-13 with TNBS resulted in improved results close to the control group. These results were compatible with histopathological scores.

Conclusion

In conclusion, our study presented that APL-13 administration may exert therapeutic effects on damage caused by TNBS-induced ulcerative colitis. We determined that APL-13 increased the antioxidant activity and decreased lipid peroxidation and leukocyte infiltration. Here, we show that APL-13 increased the antioxidant power by decreasing the TNBS induced total oxidant content, and brought the histopathological scores closer to the normal levels. This study reveals the antioxidant potential of APL-13. It is likely that APL-13 is a potentially effective substance that can be used in the long term treatments to eliminate the symptoms of ulcerative colitis and the direct or indirect negative effects that may arise due to these processes.

Ethics

Ethics Committee Approval: The approval of Adnan Menderes University Animal Experiments Local Ethics Committee (ADU-HADYEK), numbered 64583101/2014/171 (date: 10.09.2014).

Informed Consent: Experimental study.

Peer-review: Externally and internally peer-reviewed.

Authorship Contributions

Surgical and Medical Practices: F.Ş., Concept: F.Ş., G.C., Design: F.Ş., G.C., Data Collection or Processing: F.Ş., G.C., Analysis or Interpretation: F.Ş., G.C., Literature Search: F.Ş., Writing: F.Ş.

Conflict of Interest: No conflict of interest was declared by the authors.

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