Tropical Journal of Pharmaceutical Research October 2024; 23 (10): 1741-1747
ISSN: 1596-5996 (print); 1596-9827 (electronic)

Available online at http://www.tjpr.org
http://dx.doi.org/10.4314/tjpr.v23i10.20

Original Research Article

Efficacy of intraperitoneal ghrelin in the treatment of acetic acid-induced colitis

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Sent for review: 14 July 2024 *Revised accepted: 6 October 2024*

Abstract

Purpose: *To investigate the effectiveness of intraperitoneal administration of ghrelin in the treatment of acetic acid-induced colitis.*
Method: *A total of 30 Wistar-Albino rats were randomly and equally assigned to control, colitis-induced and study groups. The control group received normal saline (2 mL, 0.9 % w/v rectally). Colitis was induced with acetic acid (2 mL rectally). The study group administered ghrelin (3 - 7 nmol) intraperitoneally for 5 days after induction with acetic acid. Ghrelin was administered 1 h after acetic acid induction.*
Results: *Macroscopic damage scores were significantly higher in colitis-induced group compared to control group ($p < 0.001$). However, macroscopic scores were significantly lower in study group (treated with ghrelin) compared to other groups ($p < 0.001$). Furthermore, MDA levels were significantly higher in colitis-induced group and study groups ($p < 0.01$) compared to control group. There was no significant difference in total antioxidant capacity (TAC) in all groups.*
Conclusion: *Ghrelin significantly lowers macroscopic damage and MDA levels in acetic-acid colitis. Further long-term studies on intraperitoneal ghrelin treatment in chronic colitis models may be able to clearly show the effects of treatment on oxidative markers.*

Keywords: *Colitis, Ghrelin, Malondialdehyde, Total antioxidant capacity*

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INTRODUCTION

Inflammatory bowel disease (IBD) is characterized by dysfunction of the intestinal epithelial barrier and recurrent attacks of inflammation. Inflammatory bowel disease (IBD) is classified into Crohn's Disease (CD) and ulcerative colitis (UC) according to the localization and depth of involvement in the intestinal wall [1]. The two subgroups described have both different and similar clinical and pathological features. Ulcerative colitis usually affects the large intestine, and it may present as proctitis, proctosigmoiditis, left-sided colitis, and pancolitis. Crohn's disease affects any part of the small intestine, large intestine or rectum. It more commonly affects the perianal region and terminal ileum [2].

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There is evidence that inflammatory mediators (such as chemokines, and cytokines) are a major factor in the etiology of IBD [3]. This inflammatory process causes severe diarrhea, bleeding and abdominal pain. Colitis models are one of the methods frequently used to demonstrate the effect of a drug on inflammatory bowel disease. Many experimental animal models are used to mimic IBD. Examples include chemical (trinitrobenzene sulfonic acid dextran sodium sulfate, oxazolone, acetic acid, etc.), adaptive transfer, and spontaneous colitis models. Acetic acid-induced colitis model is a widely used model that is very similar to IBD in terms of secreted inflammatory mediators and histopathological features (neutrophil infiltration, edema, mucosal and submucosal necrosis, ulceration, vascular dilatation) [4].

Ghrelin is a polypeptide hormone produced in many tissues such as entero-endocrine and inflammatory cells [5]. Ghrelin prevents the release of cytokines such as tumor necrosis factor (TNF)-α, interleukins (IL)-6, IL - 1 from human monocytes and T lymphocytes [6]. Previous studies have reported increased serum concentrations of ghrelin in chronic inflammation, such as UC and CD [7]. Various defense mechanisms, known as antioxidant defense systems or simply antioxidants, have developed in organisms to prevent the harmful effects of free radicals. Previous studies have shown increased free radicals in blood and tissue samples of IBD cases, and that anti-oxidant treatments have positive effects on disease course. Several studies in the literature have investigated the efficacy of ghrelin treatment in experimentally induced colitis models [8,9].

This study investigated the efficacy of ghrelin treatment in acute experimental colitis models using tissue oxidative markers (total antioxidant capacity (TAC)), malondialdehyde (MDA) and histopathological parameters.

EXPERIMENTAL

Animals

A total of 30 male Wistar albino rats weighing between 150 - 200 g and aged 8 - 12 weeks were randomly assigned to control, colitis-induced and study groups. During the study, animals were kept under a 12 h light-dark cycle, standard humidity and temperature (23 °C) and fed with standard rat food. Solid food was withdrawn at 24 h and drinking water at 2 h before experimental procedures. To prevent coprophagia, wire mats were placed inside the cages.

Ethical approval

The study was conducted in the Experimental Research Centre Laboratory of Ankara Training and Research Hospital. Ethical approval was obtained from the Local Ethics Committee of Ankara Training and Research Hospital (approval no. 29.01.2011-22) and conducted according to the guidelines on the care and use of laboratory animals [10].

Treatment

The animals were randomly and equally assigned into control, colitis-induced group and study groups. Control group received 2 mL 0.9 $\%^{w}/_{v}$ saline infusion, colitis-induced group received 4 % acetic acid rectally to induce colitis. Study group received ghrelin (3 - 7 nmol intraperitoneally for 5 days) after inducing colitis with 4 % acetic acid. Ghrelin was administered 1 h after acetic acid induction. After an overnight fast, all rats were anesthetized and a polyethylene tube was advanced 4 - 5 cm from the anal canal into the colon lumen.

Thereafter, 0.9 $\%^{w}/_{v}$ saline was administered rectally to control group, 2 mL acetic acid was administered to induce colitis (colitis-induced and study groups) through the lumen and the rats were kept in an inverted position (head-down) for 2 min. Daily intraperitoneal (3 - 7 nmol) ghrelin was administered for 5 days. The rats were fed normally for 5 days and weighed daily.

Defecation characteristics and stool shape were observed daily. At the end of the experiment, rats were sacrificed after general anesthesia, the abdomens were opened with a longitudinal incision and the colon was exposed. The colon was removed from the closest point to the anus up to transverse colon. Then, the colon was cut longitudinally along the mesenteric edge and opened. Macroscopic scoring was performed after the mucosa was washed with physiological serum [4].

Evaluation of parameters/indices

Macroscopic features

The colon was removed from each rat in all the groups, evaluated macroscopically, and then photographed. Hyperemia, inflammation, erosion, and ulcers were common macroscopic features. Lesions in the colon were graded using the macroscopic damage scoring criteria of Morris *et al* [11] (Table 1).

Table 1: Macroscopic damage score

Score	Macroscopic appearance
0	No damage
1	Localized hyperemia, no ulcer or erosion
2	The presence of ulcer or erosion not accompanied by significant inflammation
3	The presence of ulcer or erosion is accompanied by inflammation in one area.
4	Ulceration and/or inflammation in two or more areas
5	Inflammation and ulceration in two or more large areas, or ulceration and inflammation wider than 1 cm along a large area of the colon

Microscopic evaluation

Samples of the colon were preserved in 10 % formaldehyde, fixed in cassettes, and paraffin for microscopy. Sections of tissue samples were evaluated and photographed with an optical microscope by a pathologist blinded to the characteristics of the study groups. Ulcers, inflammation, granuloma, depth, fibrosis, intraepithelial lymphocytes, and loss of goblet cells were evaluated in the microscopic examination using microscopic damage scoring criteria [12] (Table 2).

Table 2: Microscopic damage score

Histological lesion	Score
Ulcer	
None	0
Ulcer < 3mm	1
Ulcer > 3mm	2
Inflammation	
None	0
Mild	1
Severe	2
Granuloma	
None	0
Present	1
Depth layer	
None	0
Submucosal Layer	1
Muscular layer	2
Serosal layer	3
Fibrosis	
None	0
Mild	1
Severe	2

Antioxidant parameters

A segment (1 cm) was separated from the proximal part of the colon segment and used for TAC and MDA measurement using high-performance liquid chromatography method (HPLC). These samples were stored in – 80 ^{o}C deep freezer until measurements were made.

Total antioxidant capacity

Total antioxidant capacity (TAC) was examined with commercial ImAnOx (TAS/TAC) kit (Immun Diagnostic, Bensheim, Germany). Absorbances of enzyme-containing samples and enzyme-free samples were taken at 450 nm in the ELISA reader. Total antioxidant activity (TA) was obtained using Eq 1.

TA (µmol/L) = 392-(392-CC)x(ΔA sample/ΔA calibrator) …….. (1)

Where: CC is the calibrator concentration; ΔA is changes in absorbance

Malondialdehyde

Tissue samples were cut into thin pieces with scissors, centrifuged at 5000 rpm for 2 min and then homogenized (Ultra Turrax IKA T18 Basic) for 10 times the volume with cold potassium chloride (KCl). The homogenate was centrifuged for 15 min (5000 rpm), and the supernatant obtained after centrifugation was subjected to high-performance liquid chromatography (HPLC), and a commercial MDA kit (Immundiagnostik AG, Germany) was used for MDA measurement. Reaction changes in the samples with MDA were examined under fluorescence and then phase changes were examined (at appropriate pH, 30 °C with a chromatographic C18 column, flow rate of 0.8 mL/min). Protein concentration in the supernatant was evaluated using the method of Lowry *et al*, [13].

Statistical analysis

Data was analyzed using Statistical Packages for Social Sciences (SPSS version 22.0; Inc., Chicago, USA). Measurement data was presented in mean ± standard deviation (SD). Count data was presented in frequency and percentages. Kruskal - Wallis test and Mann - Whitney U-test were used for analysis of quantitative independent data. Kaplan Meier method was used in survival analysis. $P < 0.05$ was considered statistically significant.

RESULTS

Survival rate

At the end of the experimental study, 7 rats in the colitis-induced group, and 2 rats in the study group died. There was no death reported in control group. Survival rates were significantly higher in study group compared to colitis-induced group ($p < 0.05$). There was a significant increase in weight in control group after

treatment compared to other groups ($p < 0.05$; Table 3).

Table 3: Body weight of the rats before and after the study

Group	Pre-study weight (g)	Post-study weight (g)	*P*-value
Control	157.57±8.81	174.574±16.78	0.005
Colitis-induced	173.75±17.11	165.62±19.95	0.1
Study	161.25±47.83	175.50±57.19	0.4

Macroscopic scores

The macroscopic score was significantly lower in study group compared to colitis-induced group ($p < 0.05$; Table 4). There was a significant decrease in macroscopic scores in study group compared to colitis-induced group ($p < 0.05$). There was no macroscopic change in control group (Figure 1).

Table 4: Macroscopic scores

Group	Macroscopic score
Control	$0.0\pm0.0^{*\#}$
Colitis-induced	$3.50\pm0.57^{*\#a}$
Study	$1.12 \pm 0.83^{\#a}$

Note: $^{*}P < 0.05$ vs control, $^{\#}p < 0.05$ vs control and colitis-induced group, $^{a}p < 0.05$ vs colitis-induced group

Microscopic indices

There was a significant decrease in microscopic scores in study group compared to colitis-induced group ($p < 0.05$; Table 5). The presence and depth of ulcers, severity of inflammation, granuloma, and fibrosis were evaluated microscopically in all the groups at the end of the study (Figure 2).

Table 5: Evaluation of microscopic scores

Group	Microscopic score
Control	$0.0\pm0.0^{*\#}$
Colitis-induced	$4.50\pm1.82^{*\#a}$
Study	$0.46\pm0.25^{\#a}$

Note: $^{*}P < 0.05$ vs control, $^{\#}p < 0.05$ vs control and colitis-induced group, $^{a}p < 0.05$ vs colitis-induced group

Malondialdehyde (MDA) and total antioxidant capacity (TAC)

Levels of MDA and TAC were significantly higher in colitis-induced and study groups compared to control group ($p < 0.05$; Table 6).

DISCUSSION

Various genetic and environmental factors stimulate microbial intestinal flora and this results in the activation of intestinal immune systems (T lymphocytes, B lymphocytes, monocytes, eosinophils, neutrophils, etc.) and non-immune systems (epithelial and mesenchymal cells, etc.), which induce inflammation and tissue damage [14]. Various experimental models have been used in clarification of the disease etiopathogenesis and examination of new treatment options. Validity of experimental colitis models created with acetic acid has been shown in previous studies [15]. Acetic acid-induced colitis develops acutely with a tendency for rapid healing, hence, this current study employed an acute colitis model induced with acetic acid.

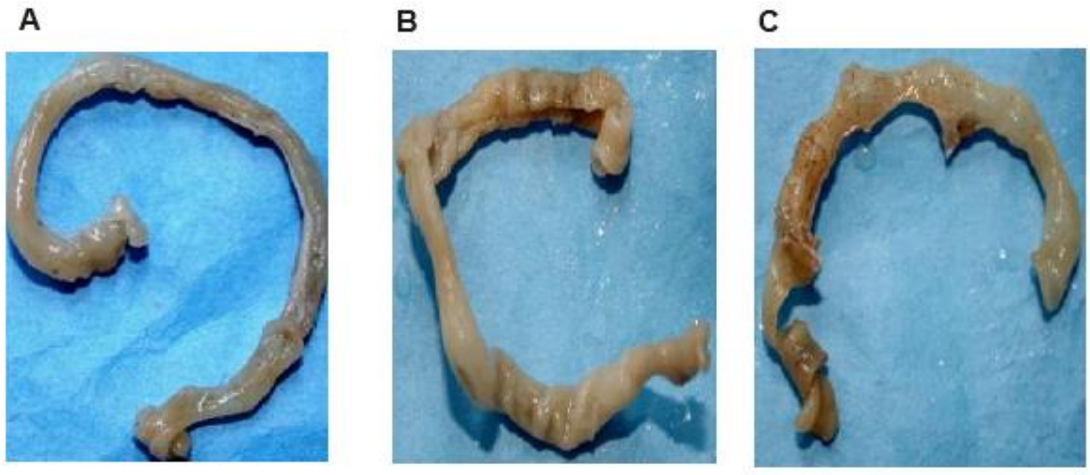

Figure 1: Macroscopy of the excised colon of rat. **A:** Control group administered 0.9 % saline infusion (no macroscopic change was observed). **B**: Colitis-induced group was administered 4 % acetic acid (fibrosis and areas of necrosis were observed). **C**: Study group administered intraperitoneal ghrelin daily after colitis induction with 4 % acetic acid (mild hyperemic areas were observed)

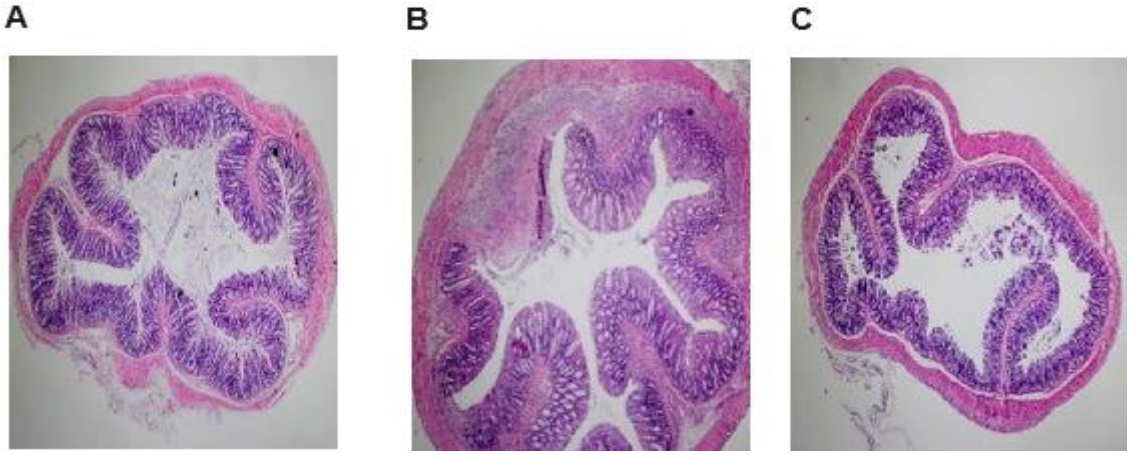

Figure 2: Microscopy showing histoarchitectural section of rat colon from H&E staining. **A:** Control (no pathological changes). B: Colitis-induced (an area of full layer epithelium loss was observed. **C:** Study group (x4)

Table 6: Levels of MDA and TAC

Antioxidant	Control group	Colitis-induced	Study group	*P*-values
MDA	75.57±48.95	285.50±202.94	221.621±43.76	0.01
TAC	539.142±329.72	492.00±339.73	522.00±190.65	0.8

Several studies have shown the positive effects of ghrelin on the gastrointestinal system. The anti-inflammatory effect was first shown by Gonzales-Rey and Delgad [16] in two different colitis models with trinitrobenzene sulfonic acid (TNBS) and dextran sulfate sodium (DSS). Results of clinical and animal experiments have shown that ghrelin has a protective and therapeutic effect on inflammation which develops in many organs and tissues, including the gastrointestinal system [17,18]. Several mechanisms have been responsible for the protective effects of ghrelin on the gastrointestinal tract. These include inducing rapid inflammation response, a reduction in mucosa oxidative stress, improvement of intestinal barrier function, increase in cell vitality and proliferation, and provision of normal micro-circulation [19]. Ghrelin shortens transit time by accelerating gastrointestinal system motility, shortening the potential contact of local inflammatory substances with intestinal mucosa, and reducing inflammation [20].

Another effect is an increase in appetite and food intake by stimulating the energy-regulating center in the hypothalamus. Previous studies have shown that in several clinical conditions such as heart failure [21] and cancer [22], ghrelin treatment decreases inflammatory cytokine levels and improves appetite and weight. Results of the current study demonstrated a greater weight increase in study group (treated with ghrelin) compared to untreated rats induced with colitis. Zhang *et al* [23] showed that in an experimental colitis model formed with dextran sodium sulfate, ghrelin was protective against intestinal epithelial cells apoptosis by modulating the unfolded protein response pathway. Also, ghrelin increases blood flow to the colon mucosa and this may be a potential contributory mechanism to reducing colonic inflammation. An increase in colonic blood flow halts iNOS inhibition and increases NO production with both a direct and indirect effect [24]. Deterioration in the balance between pro-oxidant and anti-oxidant mechanisms is one of the causative factors for IBD. It has been reported that MDA increases in IBD [25]. This study showed significantly higher MDA levels in the colitis-induced and study groups compared to control group. However, tissue MDA values were lower in study group compared to the colitis-induced group.

Total antioxidant capacity (TAC) has been associated with severe intestinal inflammation in IBD [26]. In this current study, there was no significant difference in TAC across groups.

Limitations of the study

Despite histopathological improvement seen after treatment, the fact that there was no significant improvement in oxidative markers may have been due to the short treatment period.

CONCLUSION

Ghrelin significantly improves macroscopic and microscopic inflammation indices, lowers MDA, and increases TAC in experimental acute colitis

models. Further long-term studies on intraperitoneal ghrelin treatment in chronic colitis models may be able to clearly show the effects of treatment on oxidative markers.

DECLARATIONS

Acknowledgment

None.

Funding

None provided.

Ethical approval

Ethical approval was obtained from the Local Ethics Committee of Ankara Training and Research Hospital (approval no. 29.01.2011-22).

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Conflict of Interest

No conflict of interest associated with this work.

Contribution of Authors

The authors declare that this work was done by the authors named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by them.

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