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The effect of L-glutamine on mucosal healing in experimental colitis is superior to short-chain fatty acids

Deneysel kolitte L-glutamin'in mukozal iyileşmeye etkisi kısa-zincirli yağ asitlerinden üstündür

Ekrem KAYA[1], Alper CEYLAN[2], Nurten KARA[3], Hakan GÜVEN[2], Levent YILDIZ[4]

Department of [1]Surgery, Uludağ University, School of Medicine, Bursa

Departments of [2]Surgery, [3]Molecular Biology and [4]Pathology, Ondokuz Mayıs University, School of Medicine, Samsun

Background/aims: *The effects of short-chain fatty acids and glutamine on diseased colonic mucosa, such as in inflammatory bowel disease, are not well described. The aim of this study was to investigate the role of L-glutamine and short-chain fatty acids, both via enema and oral administration, on mucosal healing in experimental colitis.* ***Methods:*** *Colitis was induced with trinitrobenzenesulphonic acid in ethanol enema in rats. Saline enema (Colitis group, n: 12), L-glutamine enema (n: 12), short-chain fatty acids enema (n: 12), oral L-glutamine (n:11) and oral short-chain fatty acids (n.11) were applied twice daily for 10 days after induction of colitis. The sham group (n: 12) received only saline enema. Rats were sacrificed at the tenth day. Crypt depth and DNA content were measured in colonic mucosa.* ***Results:*** *Crypt depth was significantly greater in both glutamine groups and short-chain fatty acids enema group than in sham and colitis groups ($p<0.05$). The mucosal DNA contents of the colitis and glutamine enema groups were significantly greater than both short-chain fatty acids groups ($p<0.05$). DNA content in the oral glutamine group was significantly greater than in the short-chain fatty acids enema group ($p<0.05$).* ***Conclusions:*** *L-glutamine enema can accelerate mucosal healing and regeneration in experimentally induced colitis in rats. When compared to glutamine in this study, short-chain fatty acids showed no beneficial effect on colitis.*

Key words: Experimental colitis, glutamine, short-chain fatty acid

Amaç: *İnflamatuvar barsak hastalığı gibi hastalıklı kolon mukozasına kısa zincirli yağ asidi ve glutaminin etkileri tam olarak aydınlatılamamıştır. Bu çalışmanın amacı deneysel kolitte L-glutamin ve kısa zincirli yağ asidi kokteyli lavmanı ve oral uygulamalarının kolon mukozası iyileşmesine etkilerini incelemektir.* ***Gereç ve yöntem:*** *Çalışmada sıçanlarda etanol içinde trinitrobenzensülfonik asid lavmanı ile kolit oluşturuldu. Gruplar; Serum fizyolojik lavmanı (Kolit grubu, n: 12), L-glutamin lavmanı grubu (n: 12), kısa zincirli yağ asidi lavmanı grubu (n: 12), oral l-glutamin grubu (n: 11) ve oral kısa zincirli yağ asidi grubu (n: 11) şeklinde idi. Lavman solusyonu kolit oluşturulduktan sonra on gün süre ile günde iki kere uygulandı. Sham grubunda kolit oluşturulmadan sadece serum fizyolojik lavmanı uygulandı (n: 12). Onuncu gün sakrifiye edilen sıçanlarda kolon mukozasındaki kript derinliği ve mukozal DNA içeriği ölçüldü.* ***Bulgular:*** *Kript derinliği her iki glutamin grubu ve kısa zincirli yağ asidi lavmanı gruplarında sham ve kolit gruplarından fazla bulundu ($p < 0.05$). Mukozal DNA içeriği kolit ve her iki glutamin gruplarında kısa zincirli yağ asidi gruplarına göre daha fazla idi ($p < 0.05$). Oral glutamin grubu mukozal DNA içeriği de kısa zincirli yağ asidi lavmanı grubundan daha fazla idi ($p < 0.05$).* ***Sonuç:*** *Sıçanlarda deneysel kolitte L-glutamin mukozal iyileşme ve regenerasyonunu hızlandırabilmektedir. Kısa zincirli yağ asidi kokteyli ise bu çalışmada glutamin kadar etkili bulunmamıştır.*

Anahtar kelimeler: Deneysel kolit, glutamine, kısa zincirli yağ asidi

INTRODUCTION

Efforts to describe the etiopathogenesis of the inflammatory bowel disease (IBD) have increased considerably in recent years. The etiology of this disease remains unknown. Current treatment modalities are not satisfactory for this clinical entity.

Various theories have been proposed about the etiology of IBD, but the most popular are oxidant injury of the colonic mucosa and energy deficiency of the colonocytes (1, 2). It is known that colonocytes use the short-chain fatty acids (SCFAs: butyrate, propionate and acetate), glutamine and glucose as a metabolic fuel (3). Furthermore, previous studies demonstrated that these agents (SCFA and glutamine) have mucosatrophic effect in normal

Address for correspondence: Ekrem KAYA
Department of Surgery, Uludag University, School of Medicine
16059 Görükle, Bursa, Turkey
Fax: +90 224 442 83 98
E-mail: ekremkaya@uludag.edu.tr

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mucosa. Especially SCFAs enhance colonic anastomotic healing, increase mucosal blood flow and increase colonic mucosal DNA content (4-6). Butyrate, most avidly taken up and metabolized by the colonocytes, is an SCFA (7, 8). In contrast, the effects of SCFAs on diseased mucosa such as in IBD are not well described and no consensus exists among the investigators (9-13).

Glutamine, although mainly a metabolic fuel for the small-bowel cells (3), is also used increasingly as a fuel by the colonocytes in colitis. It has been demonstrated that glutamine increases mitotic activity in colon of parenterally fed rats (14). Glutamine-enriched elemental diets have been shown to reduce portal endotoxin level in experimental colitis (15). It has also been shown that prophylactic glutamine supplementation in enteral diets modulates the inflammatory activities of cytokines in trinitrobenzenesulphonic acid in ethanol (TNBS-E) induced colitis (16).

In our previous study, we suggested that glutamine enema, probably mediated by increasing the antioxidant capacity of the tissue, reduces colonic mucosal injury in TNBS-induced colitis in rats. But we did not observe a similar beneficial effect with SCFA enema (17). The aim of this study was to determine the effect of SCFAs (relative increase of butyrate molar ratio) and glutamine on the mucosal healing process and any difference observed with oral administration of these agents.

MATERIALS AND METHODS

Seventy specific pathogen-free Wistar-albino rats weighing 112-232 g were used with institutional approval of the Ondokuz Mayıs University Animal Care and Use Committee. The rats were housed in individual rack-mounted cages in a room with controlled temperature (24°C) and 12-hour light/dark cycle. Standard rat chow and tap water were provided *ad libitum*. All rats were fasted for 16 hours before TNBS-E enema and before the laparotomy procedure. Cleansing enema was also applied to the rats to facilitate contact of colitis agent to the whole colonic mucosa before colitis induction with the standard enema solution (Enema set, Bıçakçılar Tıbbi Cihazlar Sanayi ve Tic. AS, Istanbul, Turkey) using the 8F lubricated polypropylene catheter via the rectum.

Induction of Colitis

All rats were put in a special cage where they could fit comfortably. An 8F polypropylene catheter (Unoplast ® AS/DK- 300, Handested, Denmark) was lubricated with jelly and inserted 8-10 cm via the anal canal into the colon and tied at, or just proximal to, the splenic flexure. A mixture of 25 mg 2, 4, 6 trinitrobenzenesulphonic acid (SIGMA Chemical Co., St Louis, MO) and 0.25 ml 50% ethanol (TNBS-E) was instilled through the catheter at this position. The catheter was flushed with 2 ml air after instillation of colitis agent. After these procedures, the rats were kept in Trendelenburg position for 20 seconds to prevent immediate discharge of instillate.

Groups

2 ml saline (n: 12), 2% L-glutamine (n: 12) and SCFA cocktail (n: 12) enemas were applied twice daily to the rats for 10 days after induction of colitis in colitis, rectal glutamine (Rectal-Gln), and rectal SCFA groups, respectively. Oral glutamine group (Oral-Gln, n: 11) and oral SCFA group (n: 11) were fed 2% glutamine and SCFA cocktail, respectively, 2 ml, twice daily via an oro-gastric tube for 10 days. The sham group (n: 12) received only saline enema (Table 1).

Table 1. Procedures performed according to study group

	Study Groups	
Groups	**n**	**Procedures Performed**
Sham	12	only saline enemas
Colitis	12	colitis + saline enema
Rectal Gln	12	colitis + 2% Gln enema
Oral Gln	11	colitis + 2% Gln oral
Rectal SCFA	12	colitis + SCFA enema
Oral SCFA	11	colitis + SCFA oral

Gln: Glutamine, SCFA: Short-chain fatty acid

Solutions

The SCFA cocktail (150 mM, pH: 6) contained acetate, propionate, and butyrate as sodium salts (SIGMA Chemical Co.) in a ratio of 55: 25: 20. Glutamine solution was a 2% solution of L-glutamine (SIGMA Chemical Co.).

All rats were weighed and sacrificed at the tenth day under ether (Diethyl ether, CARLO ERBA Co.) anesthesia and laparotomy was performed.

Histopathologic Examination

After midline laparotomy, the colon was excised totally, opened longitudinally and cleaned of fecal material with gentle jet of saline solution (0.9% Na Cl). The distal 2 cm segment of the colon was

excised and examined histopathologically. Histopathologic examination was made under light microscope and crypt depth was measured as micrometer (μm). In addition, the colonic mucosa was investigated for inflammation, edema, hemorrhage and ischemia by two independent pathologists who were not informed about the specific groups.

Measurement of DNA Content

The remaining proximal segment of the colonic mucosa (brush-border) was stripped off and homogenized in 1.15% cold KCl (Fisher Sonic Dismembrator, Model 300). DNA content was measured in this homogenate by the method described by Hogan et al. (18).

Statistical Analysis

The results of each of the treatment groups were compared with those of the colitis and sham groups. Assessment was done using Kruskall-Wallis variance test. If there was any significance between the groups, the Mann-Whitney U (MWU) test was used for comparison between two groups. $P<0.05$ was considered significant.

RESULTS

During the first three to four days, all the rats receiving TNBS-E colitis showed a reduced level of activity, some piloerection and diarrhea. In the colitis and both SCFA groups, diarrhea was observed over the 10 days. Initial mean body weights and changes did not differ significantly in any group throughout the study.

Histopathologic Findings

Inflammation in the colonic mucosa was seen in only one rat in the sham group, but it was seen in various degrees in the other groups (In Sham 8%, in Colitis 70%, in Rectal-Gln 60%, in Oral-Gln 60%, in Rectal SCFA 95%, and in Oral SCFA 95%). Degree of inflammation did not differ significantly among study groups except in the sham group. In the colitis group, loss of mucosal crypt was significantly greater than in the other groups (Crypt loss ratios: 0% in Sham, 40% in Colitis, 7% in Rectal-Gln, 15% in Oral–Gln, 7% in Rectal SCFA and 7% in Oral SCFA groups).

Crypt depth was greater in Rectal-Gln, Oral-Gln, and Rectal SCFA groups than in sham and colitis groups ($p<0.05$, MWU test) (Figure 1). There were no significant differences between sham and colitis groups.

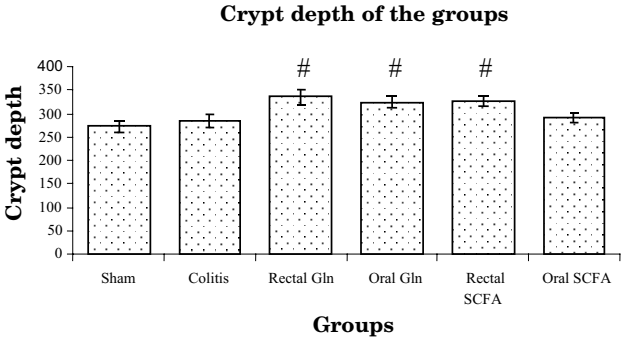

Figure 1. Mean (± SEM) crypt depth of the groups at the tenth day in TNBS-E induced colitis rat model. Crypt depths in both glutamine groups and Rectal SCFA group were significantly greater than in sham and colitis groups (#$p<0.05$). Crypt depths were calculated as micrometer. Gln: Glutamine, SCFA: Short-chain fatty acid

Mucosal DNA Content

The highest mucosal DNA content was measured in the Rectal-Gln group. Mucosal DNA content of both glutamine groups was significantly greater than of both SCFA groups. Mucosal DNA levels of both SCFA groups were less than that of the colitis group ($p<0.05$, MWU test) (Figure 2).

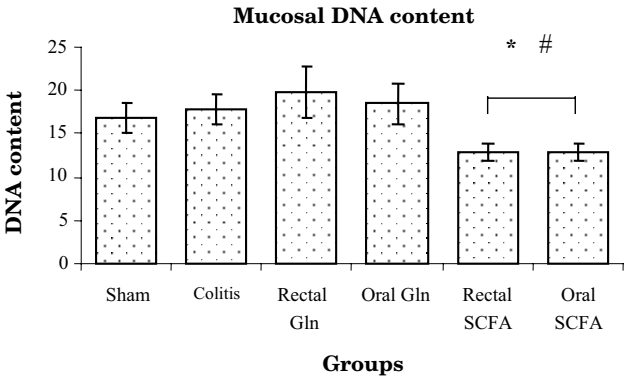

Figure 2. Mean (± SEM) mucosal DNA content of the groups at the tenth day in TNBS-E induced colitis rat model. Mucosal DNA content in both SCFA groups was significantly less than that of both glutamine groups (#$p<0.05$). Mucosal DNA content of both SCFA groups was significantly less than that of the colitis group (*$p<0.05$). DNA contents were calculated as microgram/gram tissue. Gln: Glutamine, SCFA: Short-chain fatty acid

DISCUSSION

Gastrointestinal and systemic manifestations are observed in IBD due to mucosal injury. The main aim of the treatment of IBD is to control or attenuate the mucosal injury. For this purpose, various antioxidant and/or mucosatrophic agents have been used. However, the exact benefits and satisfac-

tory influences of these agents on healing have not been observed in clinical practice. Therefore, clinical and experimental studies have been ongoing to find the optimal and most useful agents. In this study, we investigated the role of glutamine and SCFAs, using both enema and oral administration, on three parameters (histopathologic examination, crypt depth and colonic mucosal DNA content) in an experimentally induced colitis model in rats.

We sacrificed the rats on the tenth day to determine the effect of the above agents because it takes at least a week for these changes to occur in human IBD (19). In an animal study, histological changes were observed in colonic mucosa in TNBS colitis for 15 days (20). We also preferred TNBS-E colitis in this study, because it has been used widely in similar studies and it produces colitis that is morphologically similar to that in human chronic IBD (15, 21, 22). Mucosal inflammation was seen in various percentages in all groups except the sham group in this study. Crypt loss and inflammation were seen mostly in the colitis group. Although these parameters are not clearly objective measurements, these findings demonstrate that TNBS-E produced colitis in this study.

Crypt depth reflects the inflammation and mucosal cell proliferation (23). Intestinal mucosal regeneration starts from the base of the crypt and cells which proliferate increase the crypt depth. In the present study, colonic mucosal crypt depths were significantly greater in both glutamine groups and in the Rectal SCFA group than in the colitis and sham groups. These findings might be explained by the hypothesis that glutamine and rectal SCFA can stimulate cell turnover. Oral SCFA administration did not have as significant a mucosatrophic effect in this study as SCFA enema. It has been described that small intestinal mucosa has the capacity to absorb SCFA as well as the colon (4, 24, 25), and that the absorption of SCFA in the gut is related to luminal concentration gradient (7). Orally ingested SCFA in this study may not have reached a great enough concentration in the colon due to small bowel absorption. This may explain why oral SCFA has no mucosatrophic effect on the inflamed colon when compared with SCFA enema. On the other hand, the effects of SCFA on colonic mucosa in IBD are controversial (5, 26, 27). Scheppach et al. (28) showed that the SCFA cocktail increases colonocyte proliferation in an in vitro study. In another in vitro study, Finnie et al. (29) showed that Na butyrate, one of the SCFAs, increases colonic mucin synthesis. In some studies, rectal irrigation with SCFAs has produced clinical improvement in ulcerative colitis (10, 26), but this effect was unclear in another study (8). Furthermore, it is shown that butyrate oxidation is impaired in ulcerative colitic terminal ileal mucosa while it is not impaired in normal ileal mucosa (30). Araki and co-workers (31) showed that SCFA and intestinal microflora were altered in a colitis model and these alterations contributed to the progression of colitis. Butyrate is the most important SCFA for colonocytes as a fuel source (4, 30, 32-34). Although the butyrate molar ratio in the SCFA cocktail was increased in this study in contrast to the previous studies (6,17) for a more therapeutic effect; we did not find any beneficial effect. Tarrerias (35) showed that neither butyrate nor the other SCFAs had any beneficial effect on colonic hypersensitivity in TNBS colitis in rats. Their study supports our current results and also our previous study (17).

Colonic mucosal DNA content was correlated with the crypt depth especially in glutamine administrated groups in this study. Increasing DNA content in the intestinal mucosa reflects mucosal proliferation or cell turnover (36). In the present study, mucosal DNA content in both glutamine groups, enema and orally ingested, was higher than in the other groups. Mucosal DNA content of the colitis group was slightly less than of the glutamine groups but more than the other groups. This might be explained by mucosal regeneration due to injury since mucosal healing is very fast in experimentally induced colitis (37).

The effect of L-glutamine on mucosal regeneration was superior to the SCFAs in terms of both mucosal DNA content and crypt depth in this study, as reported in some previous studies (3, 17, 36). Glutamine is a major energy source of the small intestinal cells and colonocytes as proliferating cells and it is taken from both lumen and arterial blood. Glutamine occupies a central role in any metabolic pathway and comprises more than 50% of the body's amino acid pool and synthesis of nucleic acid and glutathione (38-39). Finnie et al. (40) showed in an in vitro study that glutamine metabolism increases in the colon (especially descending colon) in ulcerative colitis patients. Our previous study and other studies have shown that glutamine has an antioxidant effect in the gut mucosa (17, 41, 42). Despite these positive results, the effects of oral glutamine in IBD is controversial in both

clinical and experimental studies (43, 44). There is not enough evidence from the existing study about the effect of glutamine enema in IBD. In this present experimental study, we showed that glutamine enema is more effective than oral glutamine. It is difficult to explain the exact reason for these results; however, the results of our present study support Finnie's results.

In conclusion, the beneficial effects of SCFAs on damaged colonic mucosa in IBD are still controversial. L-glutamine enema appears to accelerate mucosal healing during colitis in rats as indicated by mucosal DNA content and crypth depth. These results also suggest that the beneficial effect of L-glutamine is superior to that of SCFAs in rats with colitis. Similar effects in humans remain to be clarified.

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