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The Comparison of Two Methods of Steroid Therapy via Appendicostomy and Enema in Experimental Acetic Acid Induced Ulcerative Colitis in Dog

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Abstract: Inflammatory bowel disease (IBD) including Crohn's disease (CD) and ulcerative colitis (UC) are chronic and debilitating diseases having unpredictable courses and with a complicated treatment. This study evaluated the effect of corticosteroids in therapy of acetic acid induced UC in dog as an animal model.UC was induced using 6% acetic acid as enema and two methods of treatment of antegrade (via appendicostomy) and retrograde (via enema) steroid therapy were compared histologically. The administration of acetic acid resulted into diarrhea, loose stools, gross bleeding and loss of body weight and histologically, loss of epithelium, crypt damage, depletion of goblet cells and infiltration of inflammatory cells. Comparing the two methods of UC therapy, it was shown that antegrade steroid therapy resolved damages better than the retrograde method. Antegrade steroid therapy may present an opportunity for the treatment of UC and may also broaden the current treatment options.

Key words: Ulcerative Colitis · Acetic Acid · Corticosteroid · Dog · Therapy

INTRODUCTION

Inflammatory bowel disease (IBD) including Crohn's disease (CD) and ulcerative colitis (UC) are chronic and debilitating diseases having unpredictable courses and with a complicated treatment. In children, IBD carries implications extending beyond the gastrointestinal tract health. Identical to other chronic diseases of childhood, patients with IBD are at risk of depression, anxiety, social isolation and altered self-image, negatively affecting the health-related quality of life (HRQOL) of patients [1]. UC is idiopathic, chronic, relapsing and its inflammatory condition is immunologically mediated in the lining of the rectum and colon. In adults, UC is most commonly diagnosed between the third and fourth decades of life, with no difference between males and females [2]. Anti-inflammatory agents such as 5-ASA, topical and systemic corticosteroids and immunomodulators are used in UC therapy [3, 4]. Corticosteroids a re potent inhibitors of T-cell activation and proinflammatory cytokines and are still used in therapy of patients with ulcerative colitis [3, 5]. Systemic corticosteroid therapy was reported to have several side effects limiting its usage but less side effects were observed for the enema route [6, 7].

In patients who had IBDs, especially UC, the repeated cycle of injury and repair of intestinal mucosa has been reported to increase the risk of colon cancer [8-10]. So, a safer and more efficient therapy is required for the treatment and prophylaxis for IBDs and perhaps also for colorectal cancer in humans [11]. To study the etiology of IBD, animal models for experimental colitis were developed and are used to determine the new anti-inflammatory treatments for IBD and various

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pathophysiological aspects of the human disease including mice and rats [12]. Various chemicals were used to induce experimental colitis including dextran sodium sulfate, 2,4,6-trinitrobenzene sulfonic acid, oxazolone, acetic acid and indomethacin [12]. This study compares two methods of antegrade and retrograde steroid therapy in the left colon in acetic acid induced ulcerative colitis in dog as an animal model.

MATERIALS AND METHODS

In summer 2011, 15 out-bred male dogs (1-2 years old; weighs of 15-20 kg) that were provided from Laboratory Animal Center of Shiraz University of Medical Sciences were enrolled. At first for induction of UC and its confirmation, 3 dogs underwent bowel preparation for two days. They did not receive any solid foods for two days but they only had water *ad libitum* for two days. All dogs received 6 ml/kg of acetic acid through a rectal tube. The first dog received 6 ml/kg of 4% acetic acid into the colon for 4 days, the 2nd, 6 ml/kg of 6% acetic acid into the colon for 2 days and the 3rd, 6 ml/kg of 8% acetic acid into the colon for 2 days.

They were followed up with sigmoidoscopy on the 5th and 10th days after injection. Multiple biopsies were provided from mucosa in 10, 20 and 30 cm proximal to the anal verge and transferred into the formalin for histological study to confirm the presence of ulcers in the colon. The distal part of colon of these three dogs was examined macroscopically under sigmoidoscopy according to the criteria described before (Table 1) [13].

Bowel preparation was done using 30 ml of Senagraph syrup (Sina Daru, Tehran, Iran) through a nasogastric tube. To check for presence of ulcers in the colon, 0.1 mg/kg of acepromazine was administered intramuscular and then a rectal tube was inserted transanally for 30 cm till the tip of tube was inserted into the proximal portion of the colon.

After confirmation of the ulcer formation in the colon, again in the 12 remained dogs, ulcer was induced in the colon identically (6 ml/kg of 6% acetic acid into the colon for 2 days). They were randomly divided into two groups of Group A, that received treatment via appendix (antegrade group) and Group B, that received therapy by enema (retrograde group).

Group A did not receive any solid foods for 12 hours and then underwent appendicostomy. After sedation of the animals, general anesthesia was induced using 10 mg/kg of sodium thiopental (Sina daru, Tehran, Iran) and then they were intubated and were supported with mechanical ventilator. After preparation in supine position, high midline laparotomy was done and the abdominal cavity was explored. Cecum was found and appendix was delivered from the abdominal wall through a small incision in the left upper quadrant of the abdomen and fixed to the fascia using 2-0 vicryl sutures. Then the tip of appendix was cut and its margins were fixed to the subcutaneous tissue with 2-0 vicryl as separated sutures. Then fascia was closed with 1-0 nylon suture and skin was sutured with 2-0 nylon stitches.

Appendicostomy was used for treatment in Group A. After 10 days of acetic acid enema, treatment was started and continued for 5 days by 10 mg/kg of hydrocortisone diluted in 100 ml of saline per day. However, Group A was managed through appendicostomy and Group B via enema. They were followed by sigmoidoscopy on the 5th and 10th day after completing the treatment measures and multiple biopsies were provided from the mucosa in 10, 20 and 30 cm proximal to the anal verge and sent for pathological study in 10% formalin.

On the 5th (3 dogs in each group) and 10th day (3 dogs in each group) after completing treatment, both groups were examined microscopically to compare the two treatment modalities. According to the criteria described before for severity of colitis, all dogs were scored [14]. On the basis of these criteria colonic inflammation ranked from 0 to 3 for eight items. So each dog had two scores, ranged from 0 to 24, one on the 5th day and another on the 10th day after completing treatment. In each group, the results of treatment on the 5th and the 10th day were compared (Table 2).

For histological study, the animals were sacrificed with an overdose of anesthetics. The samples were immediately fixed using 10% buffered formalin overnight at room temperature. A 5 μ thick sections of paraffin-embedded tissues were provided and stained with hematoxylin and eosin and examined at ×10-20 magnification for presence or absence of ulcer, reepithelialization, inflammation, congestion, edema, etc.

Animal selection, all experiments, subsequent care and the sacrifice procedure were all adhered to the same guidelines under supervision of Animal Care Committee of Iran Veterinary Organization. All experiments were carried out under aseptic conditions in Comparative Medicine Research Center of Shiraz University of Medical Sciences. The protocol of anesthesia, surgical procedures, postoperative care and sacrifice were identical for all animals. During the experiments, the animals were housed one per cage, maintained under controlled environmental conditions.

SPSS software (Version 15, Chicago, IL, USA) was used to statistical analysis. The independent sample test was applied to compare the treatment modalities. A *p* value less than 0.05 was statistically considered significant.

RESULTS

In the first dog that received 6 ml/kg of 4% acetic acid for 4 days, in biopsies taken on the 5th and 10th days after administration of acetic acid, no obvious changes were detected and the macroscopic score was 0 (Table 1) and the microscopic one was score was 1 (Table 2). In this dog, minimal lymphocyte infiltration with no ulceration and no obvious macroscopic damage were noticed. The administration of acetic acid resulted into loose stools and diarrhea.

In the second dog that received 6 ml/kg of 6% acetic acid for two days, typical changes of ulcerative colitis such as diffuse inflammation and multiple ulcers were detected and the macroscopic score was 5 (Table 1) and the microscopic sum of scores was 24 as each dog had microscopic sum of scores, ranged from 0 to 24 (Table 2). In this dog, pattern of ulcerative colitis was seen. In biopsies taken on the 5th day, inflammatory cells (polymorphonuclear leukocytes and lymphocytes) were observed in the mucosa and around the crypts. On the 10th day multiple ulcerations were detected in the mucosa and inflammatory cells were present in the crypts (crypt abscess). The submucosa was diffusely edematous and contained multifocal areas of ulceration and inflammation. Extensive infiltration by polymorphonuclear leukocytes, eosinophils and lymphocytes was apparent (Figures 1-3). The administration of acetic acid resulted into loose stools, diarrhea, bleeding and loss of body weight.

In the 3rd dog that received 6 ml/kg of 8% acetic acid for two days, diffuse necrosis was detected. In this dog, in biopsies taken on the 5th and 10th days, extensive mucosal necrosis was seen throughout the colon while the macroscopic score was 5 (Table 1) and the microscopic sum of scores was 24 (Table 2, Figure 4). The administration of acetic acid lead to dysentry, gross bleeding and loss of body weight.

In the treatment groups on the basis of the scoring criteria, each dog had two sums of scores, ranged from 0 to 24, one on the 5th day and another on the 10th day after completing treatment. Table 3 demonstrates the results of antegrade treatment in group A and Table 4, the findings of retrograde treatment in group B. In antegrade method,

Table 1: Criteria for scoring of macroscopic damage

Score	Macroscopic morphology
0	No damage
1	Localized hyperemia, but no ulcer
2	Linear ulcers with no significant inflammation
3	Linear ulcer with inflammation at one site
4	Two or more sites of ulceration and/or inflammation
5	Two or more major sites of inflammation and ulceration or one major site inflammation and ulceration extending 41 cm along the length of the colon

An Inflammation was defined as regions of hyperemia and bowel wall thickening.

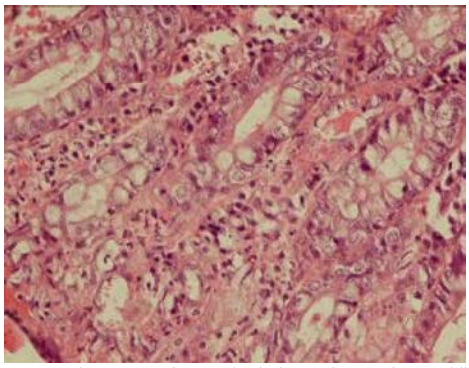

Fig. 1: Crypt abscess after administration of 6 ml/kg of 6% acetic acid for two days (H&E, x400).

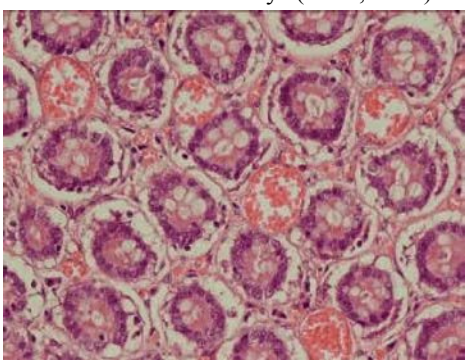

Fig. 2: Vascular congestion after administration of 6 ml/kg of 6% acetic acid for two days (H&E, x400).

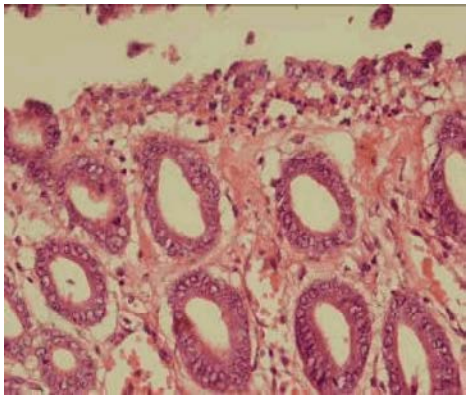

Fig. 3: Surface ulceration & goblet cell depletion after administration of 6 ml/kg of 6% acetic acid for two days (H&E, x400).

Table 2: The variables used for microscopic scoring

	Severity of changes			
Variable	0	1	2	3
Ulceration	No ulcer	Erosion or single ulceration not exceeding lamina muscularis mucosa	Multifocal ulcerations not exceeding the submucosa	Ulcerations exceeding the submucosa
Mucus cell depletion	Preserved mucus cell	Mild depletion in a few cells	Moderate depletion (<50% of cells)	Severe depletion or complete disappearance of mucosa
Crypt abscesses	No abscess	1-3 abscesses/ slide	4-9 abscesses/slide	10 or more abscesses/slide
Inflammatory cysts	No cysts	1-3 cysts/slide	4-9 cysts/slide	10 or more cysts/slide
Mucosal atrophy	Normal thickness	Mild atrophy (<10%)	Moderate atrophy (10-50%)	Severe atrophy (<50%)
Edema (submucosa)	Normal thickness	Mild edema (submucosal Expansion<10%)	Moderate edema (submucosal expansion,10-100%)	Severe edema (submucosal Expansion>100%)
Inflammatory cell infiltration	No inflammatory cell infiltration	Mild inflammatory cell infiltration	Moderate (distributed but not dense) inflammatory cell	Dense inflammatory cell infiltration
Vascular dilatation	Normal blood vessels	Mild dilatation of single blood vessel	Moderate dilatation of several blood vessels	Severe dilatation of several blood vessels

Table 3: Results of antegrade treatment in group A

	5th day after treatment completion					10th day after treatment completion				
Variable	1	2	3	4	5	1	2	3	4	5
Ulceration	0	1	1	0	0	0	0	0	0	0
Mucous cell depletion	0	1	1	2	2	1	1	0	0	0
Crypt abscess	1	1	1	1	0	0	1	0	0	1
Inflammatory cyst	0	0	0	0	0	0	0	0	0	0
Mucosal atrophy	1	1	1	1	1	0	0	0	0	0
Submucosal edema	1	1	1	1	1	0	0	0	0	1
Inflammatory cell	2	2	3	2	2	1	1	1	1	1
Vascular dilatation	2	2	2	2	2	1	2	2	2	1
Total	7	9	10	9	8	3	5	3	3	4

Table 4: Results of retrograde treatment in group B

	5th day after treatment					10th day after treatment				
Variable	1	2	3	4	5	1	2	3	4	5
Ulceration	3	1	3	2	2	1	1	1	1	1
Mucous cell depletion	2	2	2	2	2	2	1	2	1	2
Crypt abscess	2	1	2	2	2	1	1	1	1	1
Inflammatory cyst	1	0	1	1	1	0	0	0	0	1
Mucosal atrophy	3	2	3	2	2	1	1	1	1	0
Submucosal edema	2	2	2	2	2	1	1	1	1	2
Inflammatory cell	2	2	2	2	2	2	2	3	2	2
Vascular dilatation	2	2	2	3	2	2	1	2	3	2
Total	17	12	17	16	15	10	8	11	10	11

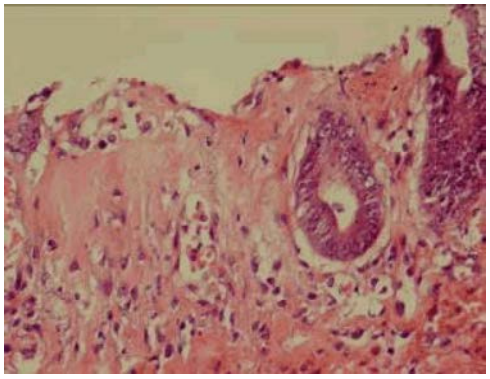

Fig. 4: Surface ulceration with necrotic material after administration of 6 ml/kg of 8% acetic acid for two days (H&E, x400).

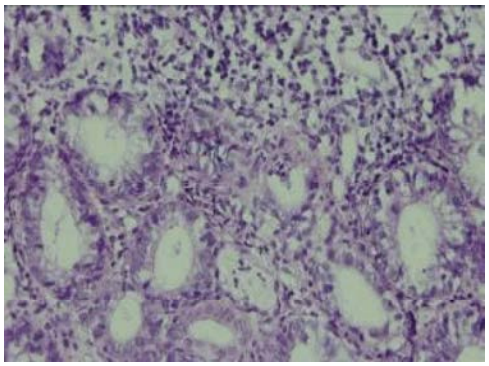

Fig. 5: Healing with some PMN infiltration in the colonic gland via appendicostomy after 5 days (H&E, x400).

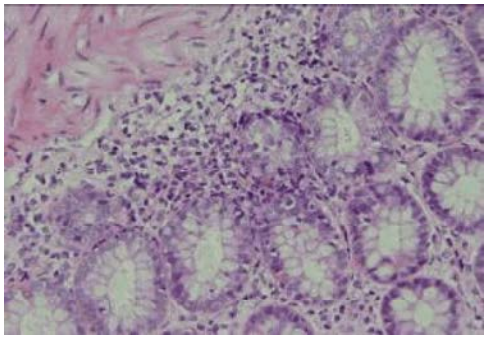

Fig. 6: Healing with mild inflammation in lamina propria after treatment via appendicostomy after 10 days (H&E, x400).

the histological scoring was from 7 to 10 on day 5 and 3 to 5 on day 10 (Figures 5 and 6). In retrograde group treated via enema, the scoring was 12-17 on 5th day and 8-11 on 10th day (Figures 7 and 8).

A statistically significant mucosal healing was seen on the 10th day compared to the 5th day in both groups (p =0.009 in group A, p =0.007 in group B). Treatment on

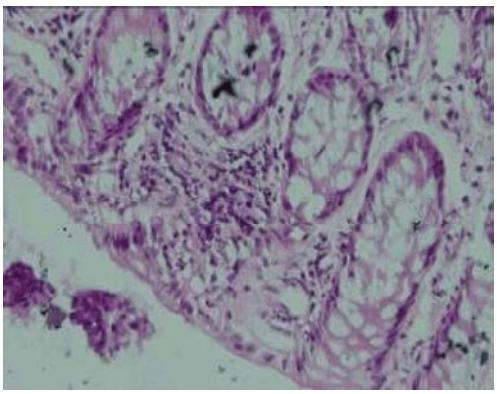

Fig. 7: Inflammation is still visible after treatment via enema after 5 days (H&E, x400).

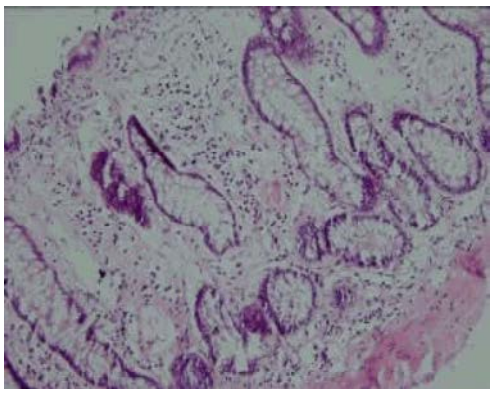

Fig. 8: Inflammation and atrophy are seen after treatment via enema after 10 days (H&E, x200).

the 5th day showed that Group A had a significantly better improvement (p =0.039) and on the 10th day, Group A had a significant improvement when compared to Group B (p =0.041). Two dogs died during the experiment and were excluded from the results.

DISCUSSION

Several studies reported mice, rats or hamsters as experimental models of UC [7, 15-18], but there was no available report on dog as an animal model for UC [19, 20]. Intracolonic administration of 6% acetic acid resulted in a rapid development of severe ulceration and inflammation of the colon associated with weight loss, diarrhea and hematochezia. These features are similar to what happens in human UC where the major symptoms include diarrhea, rectal bleeding and weight loss. In our study, macroscopic findings revealed marked ulceration and hemorrhage. Histological findings were increased infiltration of polymorphonuclear leukocytes, lymphocyte and existence of cryptic abscesses, which are identical to human UC.

In relation to induction of UC, there are also many reports on various chemicals used to induce experimental colitis including dextran sodium sulfate, 2,4,6-trinitrobenzene sulfonic acid, oxazolone, acetic acid and indomethacin [12]. We used 6% acetic acid for induction of UC as it is easily available and inexpensive chemical. Our results confirmed induction of UC by sigmoidoscopy and histologically.

Several therapeutic methods were used in experimental animal models of UC used rats as model of UC with 3% acetic acid [18]. They showed that administration of oral licorice extract could significantly reduce the colonic inflammatory response and edema. In dog as animal model of UC, it was demonstrated that UC was induced by 6% acetic acid, transrectally. In the presence of *T. polium* extract, the colonic architecture was restored with an increased number of healthy cells and a reduction in inflammatory cells [20, 21]. In another study in dog, ulcerative colitis was identically induced with 6% acetic acid as enema while *C. officinalis* could successfully resolve the damages of UC [19].

Current therapies for IBD are predominantly anti-inflammatory and immune-modulating agents such as salazosulfapyridine, mesalazine, corticosteroids, azathioprine, 6-mercaptopurine, methotrexate and cyclosporin [22]. Most of the available treatments are targeted to amelioration symptoms of the ulcerative colitis. However, in terms of clinical outcome, a treatment to prevent relapse could be as important as a treatment to ameliorate symptoms [23].

Lin *et al.* (2007) showed that fibratide reduced the severity of experimental dextran sulfate sodium (DSS)-induced ulcerative colitis in mice and showed that it may be potentially useful in the treatment of IBD [23]. Topical application of GM-CSF on mucositis shortened healing time by suppressing inflammatory reaction and proliferating epithelia [15]. Tozaki *et al.* reported that chitosan capsules may be useful carriers for the colon-specific delivery of anti-inflammatory drugs including 5-ASA and the healing of TNBS-induced colitis in rats [24]. It was shown that treatment with ECP antibody, improved DSS-induced colitis in rats, possibly due to an increase in the regenerative activity of the colonic epithelium and downregulation of the immune response and suggested that anti-ECP may promote intestinal wound healing in patients with UC [17].

In this study, we compared two methods of steroid therapy for UC via appendicostomy (antegrade) and enema (retrograde). In antegrade method, the histological scoring was from 7 to 10 on day 20 and 3 to 5 on day 25. In retrograde group treated via enema, the scoring was 12-17 on 20th day and 8-11 on 25th day. Therefore, better results were visible in antegrade method in comparison to retrograde rout. The difference may be due to peristaltic movement of colon pushing materials from proximal site to the distal part of the colon. In antegrade method, steroids also stay more in the area and prolong the contact of medication with the colon mucosa but in retrograde method, steroids do not cover the proximal part of the colon. Reber *et al.* [16] showed the importance of glucocorticoids in treatment of IBD.

The colonic mucus layer was demonstarted as a potential therapeutic target for IBD. This is based on the histologic findings that IBD patients often show thinner mucus layer and depletion of goblet cells in the colonic epithelium [25]. The mucus layer, which is composed of mainly mucins, acts as a physical barrier to protect the epithelium from agents disturbing epithelium integrity. It may also prevent the intestinal microflora from triggering abnormal immune [25]. A recent study by Van der Sluis *et al.* in which mice with disrupted mucus synthesis suffered from more serious colitis, emphasized the importance of mucus in inflammation. Gastrointestinal tissue injury is usually followed by healing that needs granulation tissue production, i.e. proliferations of fibroblasts, deposition of connective tissue matrix and most important angiogenesis for reconstruction of mucosal microvessel critical for delivery of oxygen and nutrients to the healing site [25]. In the final stage of healing, re-epithelialization and reconstruction of epithelial structures happens [26].

After the development of mucosal injuries, the intestinal epithelium rapidly tends to reestablish its integrity. To reestablish mucosal integrity, epithelial cells migrate into the wounded area (epithelial restitution) and they then proliferate to replace the decreased cell pool. Studies over the past several years have shown that a variety of soluble peptides, prostaglandins, growth factors and cytokines are secreted in a coordinated fashion in the injured area to restore mucosal integrity [27]. Comparing our two methods of UC therapy, it was shown that antegrade steroid therapy resolved damages better than the retrograde method. So antegrade steroid therapy may present an opportunity for the treatment of UC and may also broaden the current treatment options.

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Conflict of Interests: None declared.

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