

Dynamic Monitoring of Cross-Bred Dogs for Translational Research in Ulcerative Colitis

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Abstract

Background: Ulcerative colitis (UC) is a complex, chronic, immune-mediated inflammatory disease. The preclinical studies and assessment of UC drugs remain a major challenge. Dogs share similar environmental, genomic, anatomical, and intestinal physiologic features with humans. However, the model of UC in dogs is not understood and lacks systematic assessment.

Methods: We developed a model for UC in the cross-bred dogs by different dose of acetic acid retention enema. Simultaneously, physiological pathologic indicators, including blood routine examination, inflammatory factor and colonoscopy, were monitored continuously for 14 days. Furthermore, olsalazine was used to treatment UC to evaluate the feasibility of the model.

Results: The percentage of lymphocytes increases after induction UC by 10% acetic acid; C-reactive protein (CRP) and Cyclooxygenase-2(COX-2) are mainly inflammatory markers after induction UC by 10% or 7.5% acetic acid; acetic acid aggravates mucosal damage in a dose-dependent manner, and OLZ prevents 10% acetic acid-induced UC. The acetic acid concentration is controlled between 7.5 % and 10%, and the continuous damage of this model is 10 days.

Conclusions: We suggest that cross-bred dogs represent a useful clinical model of UC, and it is suitable for translational research. Finally, we provide a model and dynamic endoscope atlas for UC in dogs.

Background

Ulcerative colitis (UC) is a complex, chronic, immune-mediated inflammatory disease, and is clinically characterized by abdominal cramping, pain, and diarrhea[1]. Its incidence is increasing[2]. The potential causes of UC are vague and complicated, including aberrant cytokine secretion[3, 4], dysregulated immune responses[5, 6], and alterations in barrier function[7] and intestinal microbiota[8]. Although therapeutic strategy for UC has exhibited on anti-inflammatory agents, including glucocorticoids, salicylates and corticosteroids. So far, there is no cure for UC.

Animal models in rats or mice which colitis-like symptoms are induced through challenge with toxins or chemicals such as carrageenan[9], 2,4,6-trinitrobenzenesulfonic acid (TNBS), dextran sodium sulfate (DSS), oxazolone[10] or acetic acid[11] have been instrumental in understanding the inflammatory processes of UC. Despite the widespread use of rodent models in biomedical research, there is still controversial in the translational value of mouse studies for human disease [12]. The preclinical studies and assessment of UC drugs remains a major challenge as these models has limitations and drawbacks to compare with clinical diagnosis UC. The internal characteristics of the colon are difficult to analyze dynamically and intuitively through colonoscopy, and the efficacy of drugs in these models cannot be accurately assessed.

Large animal models, such as dogs, are generally more representative than rodents because the anatomical and physiological characteristics of dogs are closer to humans, and they have a relatively

large body size, longer life span. and develop spontaneous, similar diseases, such as inflammatory bowel disease (IBD)[13]. Previous studies have shown that miniature pigs [14] and dogs [15, 16] were induced UC model by acetic acid retention enema and it has been as a new approach for drug-evaluation. In addition, the dog remain considerable to be a superior non-rodent mammalian animal model for preclinical study and is preferred by the FDA for initial safety data of drugs for human application[17], such as colonoscopy. Importantly, it is more conducive to the dynamic study of the mechanism and assessment of treated-UC drugs. However, the model of UC in dogs is not understood and lacks systematic assessment. In an attempt to overcome these problems, we develop a model for UC in the cross-bred dogs by different dose of acetic acid retention enema. Simultaneously, physiological pathologic indicators, including blood routine examination, inflammatory factor and colonoscopy, were monitored continuously for 14 days. Furthermore, olsalazine, a new derivative of 5-aminosalicylic acid, which entered the body as prodrug form, was used to treatment UC to evaluate the feasibility of the model. These results may provide some evidences for evaluation of treated-UC drug.

Methods

Animals

All animals(13dogs) handling—including anesthesia; surgical procedures, post-operative care and sacrifice has been approved by the animal care and use Committee (IACUC) of Dali university, China(ethics permission number: 2018–0627, year: 2018). From October 2017, 1 to 2 years old, -fifteen male (8–12 kg) small-sized cross-bred dogs were obtained from the Laboratory Animal Center affiliated with Dali University, Dali, China. All dogs were housed individually and fed standard food throughout the experiment. The dogs were initially evaluated for any illness by physical examination and laboratory screening. The appropriate amount of dog food is given, and the body temperature, respiratory rate, stool frequency, stool traits, and mental state are recorded every. All sections of this report adhere to the ARRIVE Guidelines for reporting animal research[18, 19]. A completed ARRIVE guidelines checklist is included in Checklist S1.

Induction of colitis

Routine blood examination is a basic screening tool for serious pathology, which has reference value for many diseases. To provide a dynamic parameters of blood routine to clinical diagnosis, we observed continuously blood routine for 14 days(Fig.1A). Compared with the 0 day, WBC(Fig.1B), MO(Fig.1E) and granulocyte(Fig.1F) were sharply elevated at 1 day after 10% ($P<0.0001$), 7.5% ($P<0.01$) or 5% ($P<0.01$) acetic acid, and remained constantly to 14 days. Comparably, RBC(Fig.1C) and HGB(Fig.1F) mildly exhibited downtrend at 1 or 4 days after induction UC by 10% or 7.5% or 5% acetic acid, and not altered much to 14 days. Until 10 days, markedly, there was a dose-dependent manner in LY proliferation after 7.5% or 10% acetic acid-induced UC compare to 0 day (Fig.1D). The routine blood examination of 10%, 7.5% or 5% acetic acid-induced UC have slightly changed after 10day, which have self-healing ability in UC model

caused by acetic acid. These observations indicated that UC is associated with LY proliferation, and LY may use an indicator of diagnosis for UC.

Monitor of routine blood parameters and inflammatory cytokines

Blood routine parameters, including granulocyte(GR), hemoglobin(HGB), and lymphocyte(LY), monocyte(MO), red blood cell(RBC)and white blood cell(WBC) , inflammatory cytokines were dynamically monitored. In the procedure for the UC model, blood was collected according to the timeline shown in Fig.1A, and then centrifuged at 3500 rpm for 10 min at 4 °C. The plasma or serum was stored at -80 °C to detect the content of C-reactive protein(CRP), cyclooxygenase-2(COX-2), Interleukin-1beta (IL-1 β), Interleukin10(IL-10), inducible nitric oxide synthase(iNOS) and tumor necrosis factor alpha (TNF- α). Timeline of routine blood parameters and inflammatory cytokines assessment are illustrated schematically(Fig.1A). Canine COX-2 ELISA Kit (Number: 20171107), Canine IL-1 β ELISA Kit (Number: 20171107), Canine iNOS ELISA Kit (Number: 20171109), Canine CRP ELISA Kit (Number: 20171107), Canine TNF- α ELISA Kit (Number: 20171107), Canine IL-10ELISA Kit (Number: 20171109)were purchased from Nanjing Jiancheng Bioengineering Research Institute (Nanjing,China).

Colonoscopy

Timeline of colonoscopy are also illustrated schematically(Fig.1A). Bowel preparation was performed on the day of induction. In detail, before colonoscopy all dogs were orally given 10 % MgSO₄ (5 mL/kg, in distilled water) in order to clean the intestines. All animals were anaesthetized with xylazine hydrochloride injection (0.02 mL/kg), and then normal saline is used to flush the end of the colon. The electronic endoscope was slowly inserted into the colon about 20 cm, and then the camera was moved to transverse colon, descending colon or sigmoid colon to observe colonic mucosa dynamically. Colonic mucosa was assessed using published protocols[20, 21] with some modifications (Table 1).

Variables	Severity of changes			
	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
Inflammatory cell	No Inflammatory cell	Increased mildly	Increased moderately	Increased Severely
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)	Dense inflammatory cell infiltration

Table 1
The variables used for microscopic scoring

H&E staining

The model we explored decreased the appetite and emotion of animal and resulted in severe and persistent pain. In addition, the colonic mucosa biopsy could not accurately judge the pathological

characteristics of tissue. Thus, all animals were euthanized with xylazine hydrochloride injection (0.2 mL/kg) and dissected to further investigated the pathological changes via H&E staining. In detail, Two weeks following post-induction of UC, the dogs were euthanized with xylazine hydrochloride injection (0.2mL/kg), samples were provided from mucosa in 10 cm proximal to the anal verge and moved to 4% paraformaldehyde(4%PFA) (Tianjin Fuchen Chemical Reagents Factory, China, Number: 20150408) for histological studies. Briefly, colons were collected, post-fixed(4%PFA,12–24h), and then dehydrated by Automatic dehydrator (Leica ASP–300S, Germany), embedded in paraffin (Biological tissue embedding machine, Xiaogan Hongye Medical Instrument Co., Ltd., model: BM-VIII). Subsequently, the coronal sections of colon (5µm) were prepared by a rotatory microtome (Leica RM2245, Germany). The sections were subsequently stained with hematoxylin and eosin (H&E). Macroscopic damage of colon was evaluated as described by Mehrabani et al reported[22](Table 2).

Score	Macroscopic morphology
0	The mucosa is pale with normal vascular pattern and without mucosa hyperemia, edema
,1	The mucosa is still smooth but mucosa hyperemia, edema and the refractive index enhanced
2	The mucosa is hyperemia, edema, and granularity. The mucosa is friability with contact bleeding
3	The mucosa becomes congested and rough mucus membrane with Mucosal edema, spontaneous bleeding or contact bleeding. Erosions, ulcers are observed
4	The mucosa becomes extensively congested and rough mucus membrane with mucosal edema, marked and spontaneous bleeding or contact bleeding, erosions, ulcers are obviously observed

Table 2
Criteria for scoring of macroscopic damage

Drug treatment

Olsalazine(OLZ), a new derivative of 5-aminosalicylic acid(Tianjin Lisheng Pharmaceutical Co., Ltd. China, Number: 1608005), is an anti- inflammatory agent was used as positive control to evaluate model. 6 male crossbred dogs were used for induction of UC by 10% acetic acid. The experiment was carried out by self- comparison, OLZ(35.6mg/kg, in distilled saline)were continuously given by intragastric administration (i.g.) for 10 day at the beginning of UC. All indicators are evaluated as described Fig.1A.

Statistical analysis

The statistical analysis was performed with Graph Pad Prism 7 software. Homogeneity of variance was tested using Levene’s, and if not statistically significant ($P > 0.05$), one-way ANOVA was used for

statistical analysis. Kruskal-Wallis tests were performed if the variance was not consistent ($P < 0.05$). If the Kruskal-Wallis test was statistically significant ($P < 0.05$), the Mann-Whitney method was further used to make any pairwise comparison between the means. Data were expressed as mean \pm S. E. M. (standard error of mean) or SD (standard deviation).

Result

The percentage of lymphocytes increases after induction UC by 10% acetic acid

Routine blood examination is a basic screening tool for serious pathology, which has reference value for many diseases. To provide a dynamic parameters of blood routine to clinical diagnosis, we observed continuously blood routine for 14 days (Fig.1A). Compared with the 0 day, WBC (Fig.1B), MO (Fig.1E) and granulocyte (Fig.1F) were sharply elevated at 1 day after 10% ($P < 0.0001$), 7.5% ($P < 0.01$) or 5% ($P < 0.01$) acetic acid, and remained constantly to 14 days. Comparably, RBC (Fig.1C) and HGB (Fig.1F) mildly exhibited downtrend at 1 or 4 days after induction UC by 10% or 7.5% or 5% acetic acid, and not altered much to 14 days. Until 10 days, markedly, there was a dose-dependent manner in LY proliferation after 7.5% or 10% acetic acid-induced UC compare to 0 day (Fig.1D). The routine blood examination of 10%, 7.5% or 5% acetic acid-induced UC have slightly changed after 10 day, which have self-healing ability in UC model caused by acetic acid. These observations indicated that UC is associated with LY proliferation, and LY may use an indicator of diagnosis for UC.

COX-2 and CPR were mainly inflammatory markers after induction UC by 10% or 7.5% acetic acid

To explore the effect of acetic acid with different concentrations on the inflammatory response in different periods of ulcerative colitis, the expression profiles of proinflammatory and anti-inflammatory factors were measured. At 0 day, the release of the proinflammatory factors were no statistical difference. At 1 day after UC, the release of the proinflammatory factors in addition to the CRP, including IL-1 β , TNF- α , iNOS and COX-2 were not elevated in the 10%, 7% or 5% acetic acid group.

At 4 or 7 days after UC, the secretion level of IL-1 β was significantly increased in 10% acetic acid group ($P < 0.01$, $P < 0.05$, respectively, versus BV or 7% or 5% acetic acid group) (Fig.2A). At 7 days after UC, the secretion of TNF- α reached a peak in 10% acetic acid group ($P < 0.05$) (Fig.2B). Compared with the 0 days, there was no significance in acetic acid with different dosage (Fig.2C) at 1, 4, 7, 10 or 14 days. In contrast, remarkable increased COX-2 were observed in 10% or 7% acetic acid group at 4, 7, 10 or 14 days (versus 0 day, respectively, 10% acetic acid: $P < 0.01$, $P < 0.0001$, $P < 0.01$, $P < 0.01$; 7% acetic acid: $P < 0.05$, $P < 0.01$, $P < 0.05$, $P > 0.05$) (Fig.2D). Moreover, besides 5% acetic acid group, CRP was continuously elevated for 14 days (versus 0 day, all $P < 0.0001$) as shown in Fig.2E. However, acetic acid with different dosage did not

affect the secretion of anti-inflammatory mediators, such as IL-10. The present observation indicated that COX-2 and CPR were mainly inflammatory markers after induction UC by 10% or 7.5% acetic acid.

Acetic acid aggravates mucosal damage in a dose-dependent manner

The invasion site UC starts in the rectum and generally extends proximally in a continuous manner through part of, or the entire, colon. Therefore, transverse colon, descending colon or sigmoid were selected to endoscopy as shown in Fig.3A,C. Before inducing UC with acetic acid(at 0 day, all dogs), normal colonic mucosa appears smooth and red with no injury. In contrast, following induction of the UC model, especially at 1,4,7 or 10 days, colonic mucosa became widely congested and ulcers and edema were observed along with the development of rough mucus membrane with mucosal erosion(Fig.3C), which was markedly in 10% acetic acid group. Besides sigmoid and descending colon, 5% acetic acid group had a less obvious manifestation than 7% or 10% acetic acid group(Fig.3C). Although different doses of acetic acid extensively caused colonic mucosal damage, especially descending colon and sigmoid colon, but recovered at 14 days in 5% or 7% acetic acid group (Fig.3C).

Concurrently, colon mucosa damage index(CMDI) of each group was detected at 0, 1, 4, 7, 10 or 14 days. There was a similar result to CMDI as endoscopy (Fig.3B). Briefly, in comparison with not inducing UC with acetic acid(at 0 day), increased CMDI scores was radically observed in different doses of acetic acid at 1 day($P<0.0001$). Moreover, CMDI scores were gradually to reduce over time in 5% acetic acid group(at 4,7,10 or 14 days, respectively, $P<0.0001$, $P<0.0001$, $P<0.05$, $P>0.05$) and 7% acetic acid group(at 4,7,10 or 14 days, respectively, $P<0.0001$, $P<0.0001$, $P<0.0001$, $P<0.05$) versus 0 day. Significantly increased CMDI scores were observed in 10% acetic acid group at 4,7,10 or 14 days (all $P<0.0001$, versus BV) (Fig.3B). In addition, compared with 5% acetic acid group, increased CMDI scores were maintained at 4,7,10 or 14 days($P<0.01$, $P<0.001$, $P<0.01$, $P<0.05$) after 10% acetic acid-induced UC.

Endoscopy and histopathology are crucially for determination disease activity in UC[23, 24]. As is shown in Fig.4B, the structure of the submucosa, muscularis, and adventitia of the colonic mucosa is clear, the mucosa epithelium is integrated, and the goblet cells are clearly visible, there is no inflammatory cell infiltration in control group. But, at 14 days after UC, HE staining revealed infiltration of inflammatory cells in mucosa and around the crypts that were polymorphonuclear leukocytes and lymphocytes. Multiple ulcerations were also observed in 10% acetic acid group, and indicated that there was the presence of a crypt abscess. Multifocal areas of ulceration and inflammation were present in the submucosa and it was widely edematous. Group 5%, 7.5% or 10% acetic acid had different degrees of injury. Among them, 10% acetic acid group was the most severe($P<0.05$)(Fig.4A). The above results indicated that acetic acid aggravates mucosal damage in a dose-dependent manner.

OLZ prevents 10% acetic acid-induced UC

In addition to its own comparison, it is also compared with the model group. Same changes of WBC, MO and GR were seen between group model and group OLZ-treatment (Fig.5A-a, A-d, A-e). As previously mentioned(Fig.1C), the percentage of lymphocytes increases after induction UC by 10% acetic acid, which was reduced by OLZ at 7 days($P<0.0001$). Remarkable decreased RBC was also observed in the OLZ group at 1, 4 or 7 days after UC($P<0.0001$, versus 10% acetic acid). However, there is no practical significance, because base value of the RBC of OLZ group is relatively low(Fig.5A-b). At 7 or 10 days HGB was lower than 10% acetic acid group(Fig.5A-f).

As predicted by our model, we found that OLZ co-treatment with 10% acetic acid for 10 days substantially preserved activity of TNF- α , iNOS, IL-10(Fig.5Ab-d). We have found that COX-2 and CPR were mainly inflammatory markers after induction UC by 10% acetic acid(Fig.1). However, OLZ can only reduce the secretion of CRP and IL-1 β at 7 or 10 days after UC(CRP: $P<0.01$, $P<0.001$; IL-1 β : $P<0.05$, $P<0.01$ versus 10% acetic acid), not COX-2.

As shown in Fig.5C, following OLZ-treatment after UC, mucosal injury, including ulcers, edema and mucosa hyperemia or bleeding were gradually ameliorated. However, there is no cure for this model. Compared with model group, CMDI was reduced in OLZ-treated group at 10 days after UC($P<0.05$). Similarly, decreased HS was seen in OLZ-treated group($P<0.01$) (Fig.5E). Ulcer, mucosa atrophy and inflammatory cell infiltration significantly reduced in OLZ-treated group (Fig.5F).

Discussion

Acetic acid-induced UC is one of the acknowledged experimental model to study UC[25]. The induction of this animal model is associated with prolonged neutrophil infiltration. The mucosal barrier structure is destroyed by acetic acid administration, and then the inflammation is started, which are similar to the inflammatory properties of arachidonic acid metabolism abnormality in human colitis. Structural weakening of the colonic mucus barrier is an early event in ulcerative colitis pathogenesis[26]. It has been widely used to study the effects of drugs for UC[25, 27]. Many reports showed that mice or rats as experimental models of UC[28] [11, 29]. But, dogs share similar environmental, genomic, anatomical, and intestinal physiologic features with humans[30]. In this paper, we suggest that cross-bred dogs represents a useful clinical model of UC.

Here described investigations, the first of this study was to the dynamic variation of blood routine and inflammatory response in dogs. Inflammation in inflammatory bowel disease (IBD) is sustained by an exaggerated response of lymphocytes[31]. Studies have shown that the intestinal lymphocytes and peripheral blood lymphocytes is increased, which were early activation markers in UC patients[32]. All peripheral immune cells migrated[33] and recirculated from the intestine to the blood, which was an important factor in UC pathogenesis. This is supported by our research, which showed that the percentage of lymphocytes gradually increases in dogs after UC by 10% acetic acid. Lymphocytes, including effector memory T cells are endlessly recirculating by blood, tissues and lymphatics, and surveying for antigens. Subsequently, engagement with their homologous antigens trigger the

proliferation and activation of T cells, which quickly transform into cytokine-producing effectors, which sustain recruitment and activation of other immune cells, thereby initiating, spreading, and amplifying inflammation [34–36]. Blocking of T cell trafficking is a highly attractive therapeutic strategy in IBD. We detected therapeutic effect of OLZ has found that the percentage of lymphocytes increases after induction UC by 10% acetic acid, which was reduced by at 7 days. Besides at 1 day parameters of blood routine, including WBC, RBC, MO, GR, HGB, were increased sharply after acetic acid-UC, changes were slight at 4,7,10 or 14 days. Previous research has exhibited the presence of a correlation of both peripheral blood mononuclear and neutrophils cells with disease activity. Undoubtedly, neutrophils and macrophages are interestingly associated with IBD pathophysiology[37]. And monocytes also be detected early in IBD as biomarkers of inflammation[38, 39]. For this model, we also discovered MO were rapidly elevated at 1 day after different doses of acetic acid retention enema. Taken together, the increased Ly ratio is continuous, but increased MO is only in the early stages of UC.

Cytokine-driven immune networks crucially have a crucial role in the pathogenesis of UC, where they control multiple aspects of the inflammatory response[40]. Intrinsic layer dendritic cells (DCs) and macrophages are the main antigen presenting cells (APCs) found in the inflamed mucosa of IBD. Following activation, which occurs in response to combination of Toll-like receptor (TLR) signaling and the commensal microbiota, these cells produce excessive pro-inflammatory factors, such as IL-1 β , IL-6, IL-18 and TNF[41]. In this paper, the release of TNF- α and IL-1 β were continuously measured for 14 days, but only increase their secretion in the middle stage of disease activity for 10% acetic acid-induced UC. In addition, loss of IL-10 signal transduction in severe infant-onset IBD was involved in production of IL-1 by macrophages that resulted in activation of CD4+ T cells[42]. In our present study, there was no changes of IL-10 after UC by different acetic acid doses-induced. Expression of inflammatory proteins which include COX-2 and iNOS are believed to play a key role in regulation inflammation[43, 44]. Our findings agree with reported showing that throughout remarkable elevated COX-2 was found in UC using 7% or 10 acetic acid-induced. However, poor activation of iNOS produces in this model. CRP is measure that is used to determine the severity of UC[45–47]. Additionally, patients exhibiting with acute severe ulcerative colitis (ASUC) usually have fatal systemic inflammation as evidenced by an elevated CRP and conspicuous hypoalbuminemia. Evidence from dynamically measurement of CRP content in our system, significant CRP raised was continuously observed in acetic acid UC for 14 days. Activation of CRP and COX-2 produces abundant inflammatory mediators which may conduce to the development of intestinal damage and additionally, COX-2 acts in synergy with CPR to accelerate the inflammatory response. Our data revealed that COX-2 and CPR were mainly inflammatory markers after induction UC by 10% or 7.5% acetic acid. OLZ, as a positive drug, can only reduce the secretion of CRP and IL-1 β at 7 or 10 days after UC. Mucosal evaluation is independently essential in UC. In clinical trials of UC, endoscopic evaluation of mucosal disease activity is often used to determine eligibility and response to treatment. However, preclinical trials don't do enteroscopy in rodents result in exiting systems for new drug appaisement. Perhaps this is why the drug for UC is effective in preclianical, not clinically. Endoscopic evaluation is the current gold standard to assess mucosal lesions[48]. In this paper, we provide a model and dynamic endoscope atlas for UC in dogs. We performed dynamic monitoring of enteroscopy on the transverse

colon, descending colon or sigmoid colon. Edema, erythema, loss of vascularity, mucosal granularity and friability, ulcers and erosions were observed by endoscopic findings. At 1 day, these findings typically begin sigmoid colon, and at 4,7,10 or 14 days, these manifestations extend proximally to transverse colon in a continuous manner with a gradual transition to normal-appearing mucosa. However, there was more seriously pathological phenomena in the 10% acetic acid-induced UC. Additionally, HE staining revealed infiltration of inflammatory cells in mucosa and around the crypts that were polymorphonuclear leukocytes and lymphocytes. Multiple ulcerations were also observed in 10% acetic acid group, and indicated that there was the presence of a crypt abscess. Multifocal areas of ulceration and inflammation were present in the submucosa and it was widely edematous.

The data above demonstrate that 10% acetic acid-induced UC in cross-bred dogs is more suitable for preclinical drug evaluation. First-line therapy in mild to moderate disease is the 5-ASA drugs[49], which can be administered as suppositories, enemas, or oral formulations, such as OLZ. We tested OLZ as a positive drug in our model system to verify the success of the acetic acid-induced UC. Our data confirm that OLZ prevents 10% acetic acid-induced UC.

Finally, the present study has certain limitations. Firstly, we found that The proportion of lymphocytes increased was not classified for T cell subtypes. Secondly, only sigmoid colon was stained by pathology. Thirdly, evidence that acetic acid-induced UC may occur self-healing came from colonoscopy. We speculate that the 10% acetic acid-induced model is ASUC.

Conclusion

In summary, we suggest that cross-bred dogs represent a useful clinical model of UC. The acetic acid concentration is controlled between 7.5 % and 10%, and the continuous damage of this model is 10 days. The standard for the success of the model is marked by steadily increased lymphocytes and CRP or COX-2. Finally, we provide a model and dynamic endoscope atlas for UC in dogs. These findings support drug-evaluation for UC.

Abbreviations

APC: Antigen presenting cell; ASUC: Acute severe ulcerative colitis ; CMDI: Colon mucosa damage index; CRP: C-reactive protein; DC: Dendritic cell; DSS: Dextran sodium sulfate; FDA: Food and Drug Administration; GR: Granulocyte; HGB: Hemoglobin; IACUC: the Institutional Animal Care and Use Committee; IBD: Inflammatory bowel disease; iNOS: Inducible nitric oxide synthase;IL-1 β : Interleukin-1beta; IL-10: IL-1 β ; LY: Lymphocyte; MO: Monocyte; OLZ: Olsalazine; PFA: Paraformaldehyde; RBC: Red blood cell; TLR: Toll-like receptor; TNBS:2,4,6-trinitrobenzenesulfonic acid; TNF- α : Inducible nitric oxide synthase; UC: Ulcerative colitis; WBC: White blood cell.

Declaration

The authors declare that they have no conflict of interests.

Authors' contributions

Conceptualization, YZ,CGZ and HL; Data curation, HRZ; Formal analysis; Funding acquisition, YZ,CGZ,HRZ, and HL; Investigation, HL; Methodology,YJW, SSL, QL, ZYY,YSX and HRZ; Project administration, YZ and HZ; Resources, HL and ZPX; Validation, HRZ and YJW; Roles/Writing - original draft, YJW and HRZ; Writing - review & editing, HRZ, YuZ and CGZ. All authors critically read and revised the

Ethics approval and consent to participate

All animal research procedures were performed as approved by the Institutional Animal Care and Use Committee (IACUC) of Dali University. This article does not contain any studies with human participants performed by any of the authors.

Consent for publication

Not applicable.

Availability of data and materials

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

Competing interests

We declare that there is no financial conflict of interest in this research work.

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Figures

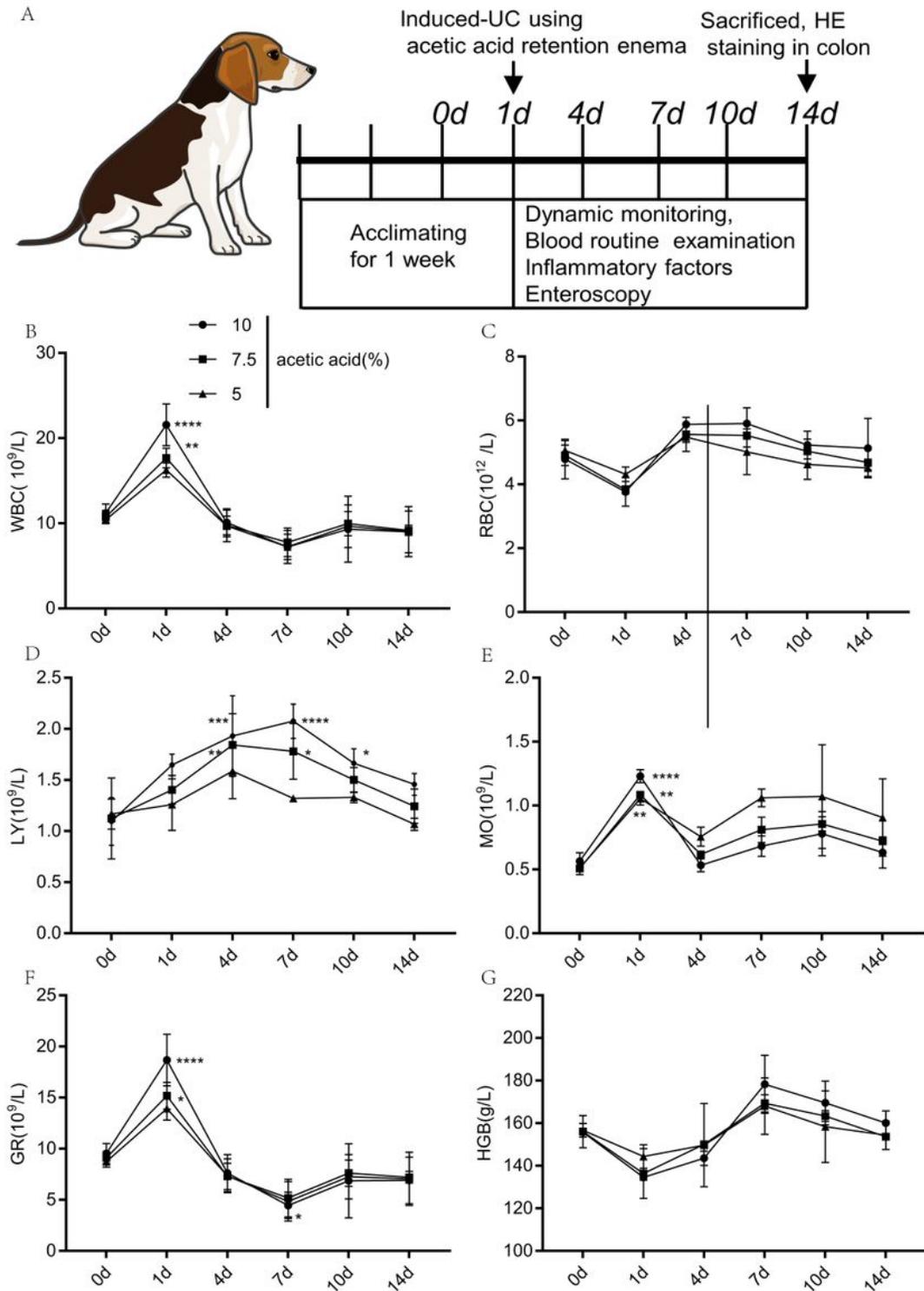


Figure 1

Monitor of routine blood parameters in UC model. (A) The timeline of routine blood examination, inflammatory factors and intestinal mucosal injury are schematically illustrated. Excepted for the lymphocyte (LY) (D), routine blood parameters of acetic acid-induced UC mildly improved as evaluated by white blood cell (WBC) (B), red blood cell (RBC) (C), monocyte (MO), granulocyte (GR), and hemoglobin (HGB)

at 1, 4, 7, 10 and 14 days. n=3-4, results are expressed as means \pm SEM. * P < 0.05, ** P < 0.01, ***P<0.001, ****P<0.0001 versus 0 day. All comparisons were self-comparisons.

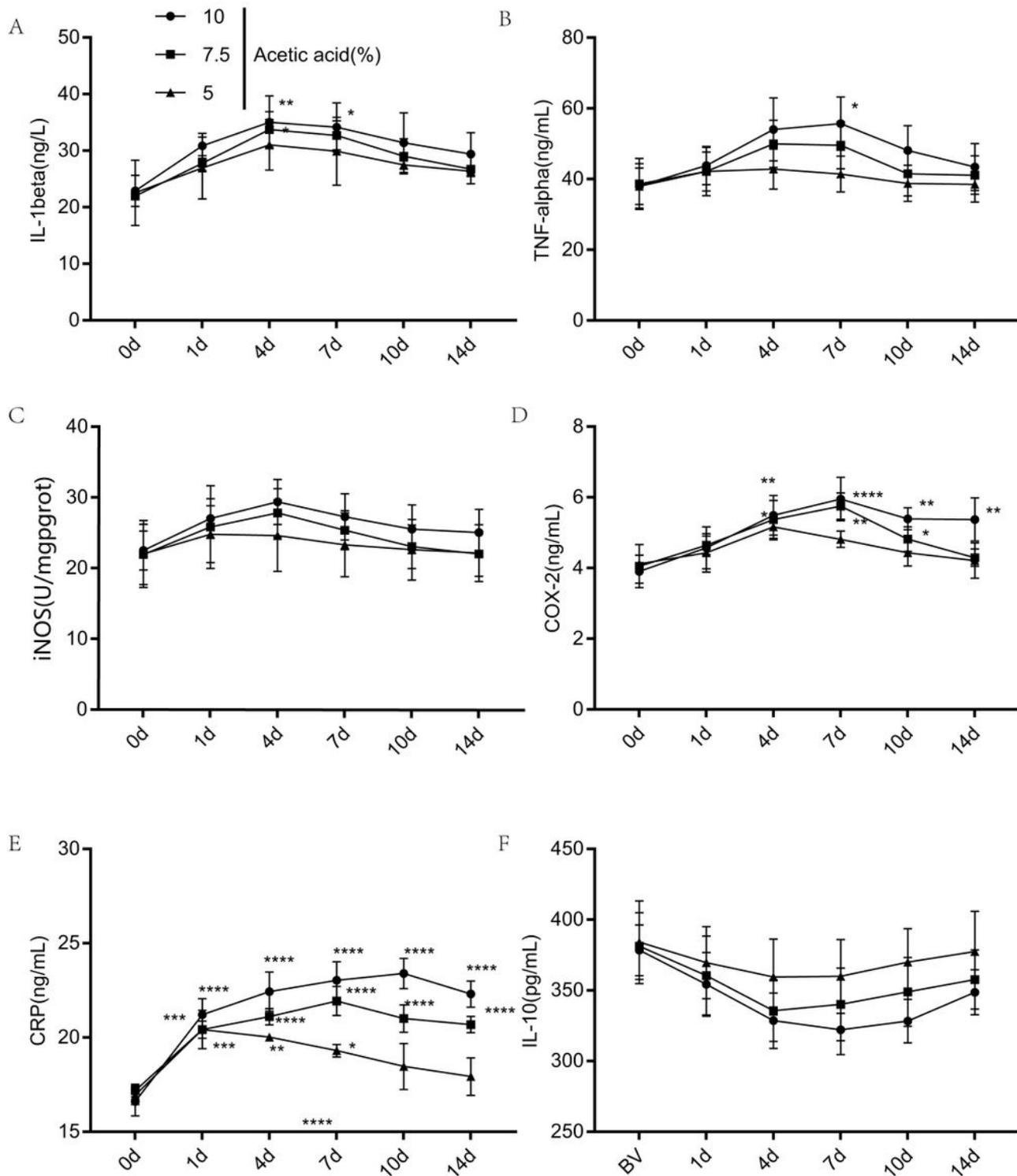


Figure 2

The effect of acetic acid induced-UC on the expression profiles of inflammation mediators at 1, 4, 7, 10 or 14 days. (A-F) Release of proinflammatory mediators or anti-inflammatory cytokine, including IL-1 β (A), TNF- α (B), iNOS(C), COX-2(D), CRP(E) or IL-10(F), were dynamically detected by ELISA Kits. N=3-4,

data are expressed as means \pm SEM. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, **** $P < 0.0001$ versus 0 day (BV). All comparisons were self-comparisons.

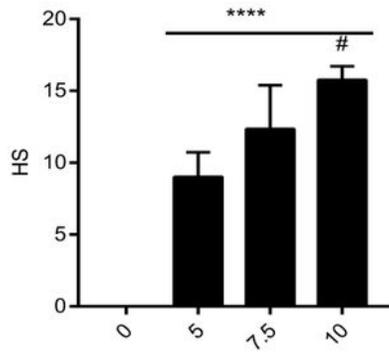


Figure 3

Macroscopic evaluation of the colonic mucosa in UC by acetic acid retention enema. (A) The pattern of the colon was illustrated. (B) Colon mucosa damage index (CMDI) of each group was detected at 0, 1, 4, 7, 10 or 14 days. (C) Dynamic endoscope atlas for UC in dogs. $N=3\sim 4$, data are expressed as means \pm

SEM. * P < 0.05, ** P < 0.01, ***P<0.001, ****P<0.0001 versus 0 day; # P < 0.05, ##P < 0.01,###P<0.001, ####P<0.0001 versus 5% acetic acid group.

A



B

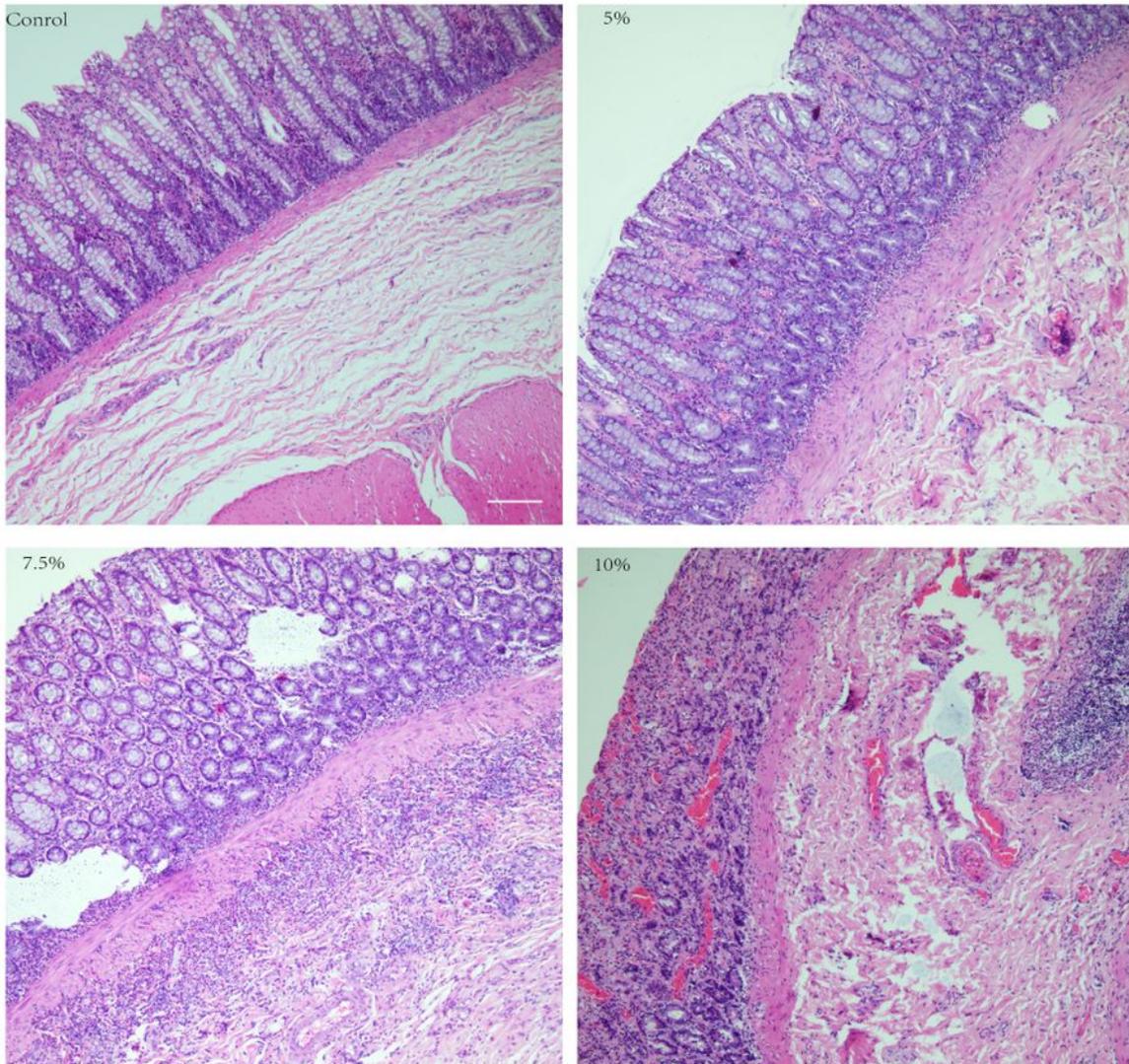


Figure 4

The microscopic appearance of the colonic mucosa. (A) Histopathological scores(HS) of each group were determined. (B) H&E staining photomicrographs on dogs colon sections. Scale bars:50 μ m;

magnification: 400×. Values are expressed as mean± SD, n=3~4 for each group . ****P<0.0001 versus 0 day; #P<0.05 versus 5% acetic acid group.

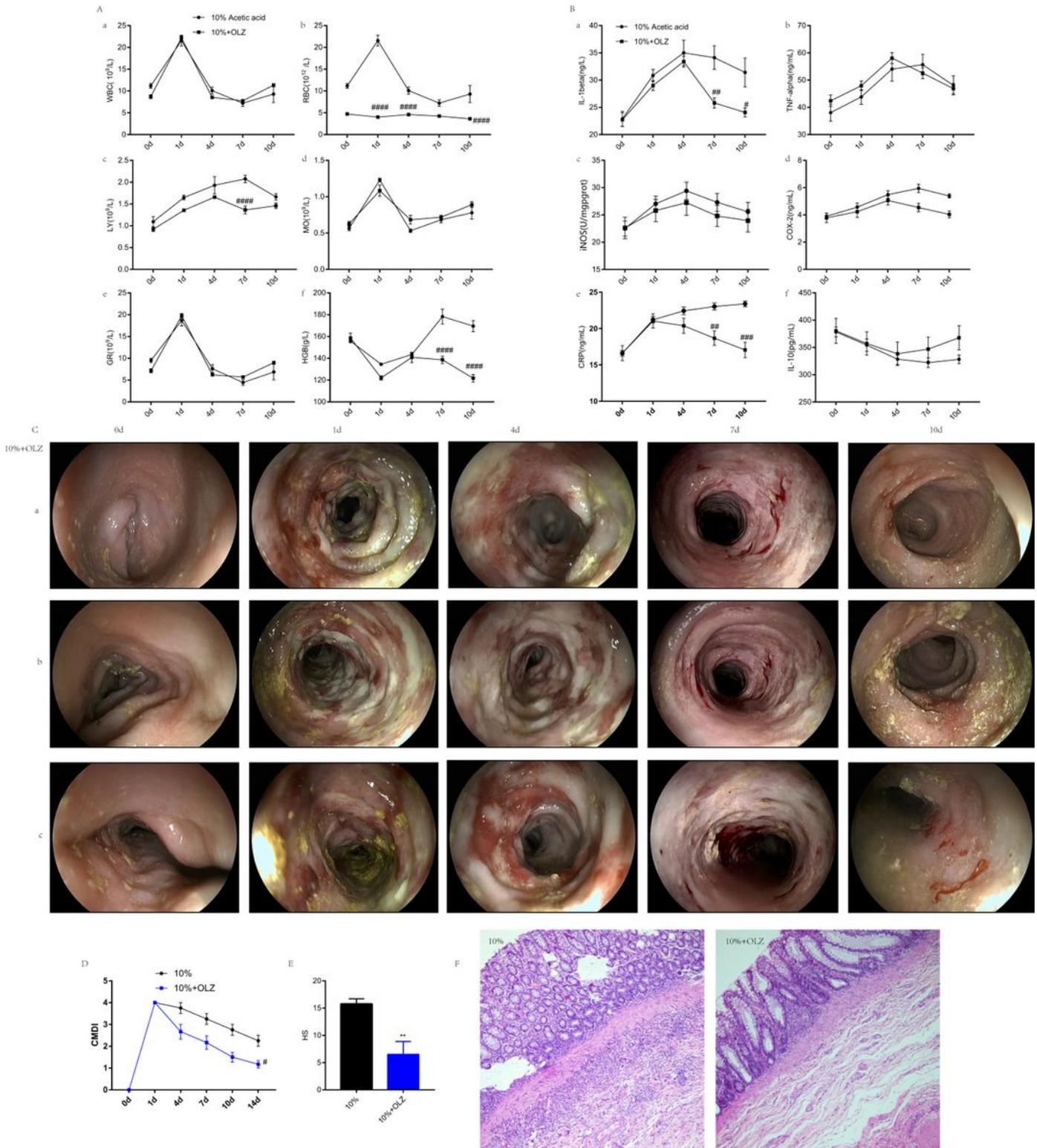


Figure 5

OLZ prevents 10% acetic acid-induced UC. (A) Monitor of routine blood parameters at 0,1,4,7 or 10 days. a-f are successively expressed white blood cell(WBC), red blood cell (RBC), lymphocyte (LY) , monocytes(MO),granulocyte(GR)and hemoglobin(HGB) at 1, 4, 7,10 and 14 days.(B) The effect of OLZ on

the expression profiles of inflammation mediators at 0,1, 4 ,7or 10 days after acetic acid induced-UC. (a-f) Release of proinflammatory mediators or anti-inflammatory cytokine, including IL-1 β (a), TNF- α (b), iNOS(c), COX-2(d), CRP(e) or IL-10(f), were dynamically detected by ELISA Kits. (C)Dynamic endoscope atlas for UC in dogs were exhibited after OLZ-treatment. (D) Colon mucosa damage index(CMDI) of OLZ-treatment was detected at 0,1, 4 ,7or 10 days.(E) Histopathological scores(HS) of each group were determined.(F) H&E staining photomicrographs on dogs colon sections. Scale bars:50 μ m; magnification: 400 \times . Values are expressed as mean \pm SD, n=4~6 for each group . # P < 0.05, ##P<0.01,###P<0.001, ####P<0.0001versus 10% acetic acid group.

Supplementary Files

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