Dynamic evaluation of ulcerative colitis model in BALB/c mice induced by dextran sulfate sodium salt

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Research Article

Keywords: animal model, dynamic changes, flow cytometry, sodium dextran sulfate, ulcerative colitis

DOI: https://doi.org/10.21203/rs.3.rs-659387/v1

Abstract

In this experiment, BALB/c mice were induced by dextran sulfate sodium (DSS) to establish ulcerative colitis (UC) model. Treatment by intragastric administration of oxalazine (600 mg/Kg)the disease activity index (DAI), colonic mucosal injury index (CMDI) and histopathological score (HS) of mice in each group were measured at different times. And the changes of T cell subsets in spleen and the content of IL-1 β in serum of UC mice at different time points were detected by flow cytometry and ELISA method. The investigation of the dynamic changes of various indexes in mice during the period of modeling and administration is of great significance for exploring the etiology and pathological changes of the disease, determining the methods of treatment and the research and development of new drugs.

Introduction

Ulcerative colitis is a common chronic non-specific intestinal disease, mainly involving colon, rectal mucosa and submucosa, showing phased and diffuse distribution [1]. Most of the clinical manifestations are abdominal pain, diarrhea, acute post-acute weight and mucous bloody stool, and it has the characteristics of high acute fulminant mortality, high rate of chronic persistent canceration, easy to occur repeatedly, and may be accompanied by serious complications. It has been defined as a precancerous disease and has been listed as a refractory disease by the World Health Organization [2]. With the great changes in people's lifestyle, eating habits and environment, the disease spectrum also changes. The increasing incidence of UC year by year is the epitome of this change. Based on the case statistics of many domestic hospitals, it is inferred that the prevalence rate in China is 11.6/100000 (it may be underestimated), while in western countries, the incidence is 10/100000 ~ 20/100000, the prevalence rate is 100/100000 ~ 200/100000, the age of onset of UC is between 15 and 40 years old, and there may be a second peak between 50 and 80 years old. There is no significant difference in incidence between men and women, and there is family aggregation [3-5]. However, the DSS-induced UC model has the advantages of simplicity, high success rate and good repeatability, and similar to human UC lesions, it is an ideal human UC model, which can be used to study the acute stage of UC, remission stage and canceration of colitis. Therefore, in this study, DSS was used to induce acute ulcerative colitis in BALB/c mice, and oxalazine was given by intragastric administration to treat UC mice. From the general condition of mice, DAI score, organ index, colon CMDI score, spleen T lymphocyte subsets, serum antibody dynamic changes, to explore the etiology and pathological rules of the disease, to determine the treatment and new drug development to provide a reliable and effective animal modeling cycle and administration cycle.

Materials And Methods

Experimental animals

A total of 140 healthy SPF grade BALB/c male mice, aged 6-8 weeks and weighing 18-22 g, were provided by Hunan Shrek Jingda Experimental Animal Co., Ltd., license No.: SCXK (Hunan,China) 2016-0002.

Raised in the barrier system of the Animal experiment Center of Dali University, the temperature is 20-26 °C, the humidity is 40-70 %, and the pressure difference is 25-50 Pa, and the light and shade alternated at 12 h. All experimental studies are in line with the guiding principles of animal research of the China Ethics Committee.

Drugs and reagents

Dextran sulfate sodium (Shenzhen Regent Co., Ltd., batch number: SLBP0889V) oxalazine sodium (Tianjin Lisheng Pharmaceutical Co., Ltd., batch number: 1608005); Red blood cell lysate (Thermo Fisher Co., Ltd., batch number: 4338657); PBS (Solarbio Co., Ltd., batch number: 11310220);Fetal bovine serum (Zhejiang Tianhang Biotechnology Co., Ltd., batch number: 20161013); Rat anti mouse CD3 FITC (batch number: 6053673), Rat anti mouse CD4 PE (batch number: 7023677), Rat anti mouse CD8 APC (batch number: 7081511) and Rat anti mouse CD25 Percp-cy5.5 (batch number: 7032624) are all from BD Co., Ltd.; Occult blood kit (batch number: 20160824) and Mouse IL-1β ELISA kit（batch number: 20170405）are all from Nanjing Jiancheng Bioengineering Research Institute and so on.

Instrument

Electronic analytical balance (model: AL204- IC, Mettler-Toledo Instruments Co., Ltd.); Macro camera (model: DIGITAICAMERA D7100, Nikon); Medical image analysis system (model: BI-2000; Chengdu Tai Meng Technology Co., Ltd.); Flow cytometry (model: FACS Calibur; BD USA); Enzyme labeling instrument (model: SpectraMax M5 position Devices) and so on.

Preparation of main reagents

DSS (3%) configuration: The 120 g DSS was dissolved in 4 L hot-pressed ultra-pure water to obtain 3 % DSS solution, which was then prepared at that time.

The preparation of PBS buffer (PH=7.4): NaCl 8.0 g/KCl 0.2 g/$Na_2HPO_4 \cdot 12H_2O$ 1.44 g/KH_2PO_4 0.24 g. Put the weighing reagent into the flask, add ultra-pure water to 1000 ml, and shake well, then sterilize with high pressure steam (121 °C , 15 min). Storing at 4 °C and set aside.

Preparation of spleen single cell suspension: The fresh spleen of mice was placed on a 200-mesh cell sieve and ground into a single cell suspension with the core of a 5 ml syringe. The cell suspension was collected and centrifuged, and the supernatant was discarded. Adding 1 ml red blood cell lysate, after the red blood cell is fully lysed, add 3 % FCS-PBS of 4 ml to wash twice, then discard the supernatant, and add RPMI-1640 complete culture medium to re-suspend spleen single cells. The cell suspension was stained with 0.4 % trypan blue staining, and the cell concentration was adjusted to 10^7/mL.

Experimental methods

Establishment of experimental model

One hundred and forty healthy male BALB/c mice were fed adaptively for one week. Except for the normal group, the mice were given 3 % (w/v) DSS aqueous solution to drink freely for 7 days. After the establishment of the model, the mice were fed with hot pressure sterilized ultra-pure water for 10 days, and the whole process takes a total of 17 days [6].

Grouping and administration

Eighteen mice were randomly selected as the normal group, and the rest of the model mice were evaluated by disease activity index (DAI) at the 7^{th} day. According to the DAI score, the model mice with very mild inflammation were excluded (Very mild inflammation at 0-3, mild inflammation at 4-6, moderate inflammation at 7-9, and severe inflammation at 10-12). The rest of the mice were randomly divided into model group and oxalazine group (600 $mg \cdot Kg^{-1}$) according to the severity of inflammation, with 24 mice in each group (The number of mice killed during modeling was not included in the model group). At the end of the model, each group was given corresponding drugs according to 0.1 $ml \cdot kg^{-1} \cdot d^{-1}$, while the model group and normal group were given 0.1 $ml \cdot kg^{-1} \cdot d^{-1}$ saline once a day for 10 day. The mice in the normal group and the model group were scored by DAI at 0, 1, 4, 7, 8, 10, 14 and 17 day respectively, and the drug group was scored by DAI at 8, 11, 14 and 17 day after the experiment. Except for the normal group, 6 mice were randomly killed on the day of DAI score in other groups [7] (In the normal group, the mice were killed at 0,7 and 17 day to observe the effect of environment on the experiment).

General status observation

The changes of body mass, drinking water, diet and fecal characteristics of mice in each group were observed and recorded during the experiment.

Measurement of colonic length and calculation of organ index in mice

The mice were killed after taking blood from their eyeballs. The liver, spleen, lung, thymus and colon were taken, and the organ mass was weighed. The organ index (organ index = organ mass (mg)/mouse body weight (g)) was calculated. The intestines were dissected longitudinally along the mesenteric edge of mice, the intestinal contents were cleared, the colon was weighed and the length of colon was measured.

Disease activity Index (DAI) score

According to the standard of Hamamoto et al. [8], the weight of mice was weighed, the characteristics of feces and occult blood in feces were observed. According to the score of Table 1, the scores of weight loss, fecal traits and fecal occult blood were summed up, and the disease activity index of each mouse was calculated to evaluate the disease activity.

CMDI score

The removed colon was laid flat on white paper, and the colonic mucosal injury was observed and scored according to Ekstr ö m GM standard [9] and Luk standard [10] (Table 2).

Observation of colonic pathomorphology and HS score

"Swiss rolls" is a routine method for histological and immunohistochemical staining of intestinal tissue: the longitudinally sectioned colon is cut in half, wrapped, fixed and preserved in 10% neutral formalin solution for HE staining. The pathological sections were observed under light microscope according to the standard of Ekstr ö m GM et al. [11] (Table 3), and the colon HS score was performed.

Determination of lymphocyte subsets

100 μL spleen cell suspension (10^6 spleen cells) was added to the flow tube, and the corresponding mixed fluorescent antibody was added for FACS Calibur detection.The ratio of T lymphocytes ($CD3^+$) to single spleen cells and the proportion of Th cells ($CD3^+CD4^+CD8^-$), Tc cells ($CD3^+CD4^-CD8^+$) and Treg cells ($CD3^+CD4^+CD25^+$) to T lymphocytes in spleen were analyzed.

Detection of 1 β content

The blood extracted from eyeballs of mice was placed at 4 ℃ for 4 h, and the supernatant was obtained by centrifugation at 3000 rpm (4 ℃) for 10 min. The other half of the longitudinally dissected colon was made into 10% homogenate in an ice bath, and the supernatant was obtained by centrifugation at 3000 rpm (4 ℃) for 10 min. According to the instructions, the contents of related biochemical factors in serum and colonic mucosa were determined by ELISA method, and the data were calculated by ELISACalc.

Statistical method

SPSS 23.0 and GraphPad Prism 5.0 were used to describe and analyze the data. Rank sum test is used to compare the rank data, and the concentrated trend and discrete trend are expressed by median (M) and quartile (QR) spacing, respectively. The measurement data are expressed as , the data with normal distribution and uniform variance are analyzed by t-test and one-way ANOVA, and the data that are not consistent with normal distribution are tested by rank sum test. The continuous data were analyzed by repeated analysis of variance and univariate analysis of variance. LSD test was used for pairwise comparison between groups. $P<0.05$ was used as the standard of statistically significant difference.

Results

Changes in general physical signs

In the first three days of the experiment, the body weight of the model mice changed little, and the third day, mice began to develop bloody stools, loose stools, severe weight loss and reduced water consumption. The 7^{th} day, mice showed mental malaise, hematochezia or diarrhea, curled up in piles and dull color. The intestinal wall of the mice was swollen and congested, but no obvious ulcer was found in the naked eye. At the 10^{th} day, the body weight of the mice slowly recovered, the drinking water returned to normal, the symptoms of diarrhea were mild, the hematochezia decreased or even disappeared, but the

activity did not increase. The activity and body weight of the 14th day mice increased, and the coat color returned to a lighter luster. At the end of the model, after administration of oxalazine (600 mg·Kg^{-1}), the weight loss of mice slowed down, followed by weight gain, coat color gradually returned to luster, and the amount of exercise increased.

Impact of DAI score

The feces of mice in the normal group were basically formed, and the feces of some mice were loose or weakly positive for occult blood. However, with the increase of days of drinking DSS, stool thinning and bloody stool appeared, weight loss; weight gain, stool score, occult blood score decreased after administration. The scores of DSS-induced acute UC mice increased gradually at the 1st, 4th, 7th, 8th and 10th day DAI. The 10th day DAI score reached the peak in the experiment. Compared with the normal group, the DAI score of each model group was significantly higher than that of the normal group at different time points (P < 0.01).The DAI score of the mice in the drug group was significantly lower than that in the model group at different time periods of treatment (P < 0.01). The results are shown in figure 1.

Changes of colonic length and CMDI

The colon length of mice in the normal group changed little, but the colon length in the model group decreased gradually with the increase of days of drinking DSS, and the length of colon recovered from the 8th day (figure 2a). At 0 day, the general morphology of mouse colon was normal, the mucosa was smooth and smooth, and no mucosal hyperemia, edema, erosion and ulcer were found. With the increase of days of drinking DSS, colonic inflammation, colonic mucosal congestion and edema, intestinal wall thickening and erosion, ulcer, oval ulcer, marginal hyperemia and edema, CMDI score also showed an upward trend. After stopping drinking DSS, it gradually recovered and reached the peak at the 10th day CMDI (figure 2C).Compared with the model group, when oxalazine was given for 1, 4 and 7 day, the CMDI of mice decreased significantly (P < 0 01). At the 17th day, there was no significant difference in CMDI between the model group and each treatment group (P > 0.05), and there was no significant change in colon length from the beginning to the end (figure 2b and 2d).

Score of colonic histopathology in mice

As shown in figure 3, a small number of goblet cells were lost and a small amount of inflammatory cells infiltrated in normal mice. In the model group, with the increase of modeling days, there was a large area loss of goblet cells, different degrees of crypt defect, and a large area of inflammatory cells infiltrated into the mucous membrane and submucosa. After discontinuation of DSS, the defects of goblet cells and crypt cells gradually recovered, and the degree of inflammatory cell infiltration decreased, but it was still significantly different from that of the normal group.The HS score showed that compared with 0 day, the colonic HS score of mice at each time point increased in varying degrees, and the HS score of 8th, 10th, 14th, 17th day model mice was significantly higher than that of 0 day (P < 0.01), the 10th day HS score reached the peak, and then showed a downward trend, as shown in Table 4.

Compared with the model group, the mice in the treatment group showed crypt blood stool, goblet cell repair and less inflammatory cell infiltration, which showed that the scores of HS decreased with the increase of administration days. The HS score decreased significantly on the 14^{th} day, but there was no significant difference on the 17^{th} day, as shown in Table 5 and figure 4.

Changes of T cell subsets in model mice at different time points

$CD3^+$ T lymphocytes were selected and analyzed by CD4, CD8 and CD25 staining. As shown in figure 5, with the increase of days of drinking DSS, the proportion of $CD3^+$ decreased gradually ($P <$ 0.01). Compared with the mice on the 0th day, the proportion of CD4+ gradually increased, the proportion of $CD3^+$, $CD8^+$ cells did not change significantly, and the proportion of $CD3^+CD4^+/CD3^+CD8^+$ gradually increased, and peaked at the 10^{th} day.However, when drinking ultra-pure water, the proportion gradually recovered. The proportion of $CD3^+CD4^+CD25^+/CD3^+CD4^+$ decreased gradually before the 10^{th} day, reached the lowest value at the 10^{th} day, and then gradually recovered.

Effect of organ index of model mice at different time points

As shown in figure 6, compared with 0 day, the liver index increased at all time points, and the liver index of 10^{th} day, 14^{th} day and 17^{th} day mice increased significantly (P<0 01 or P<0 05). The spleen index at each time point was higher than that of 0 day, and the spleen index of the first day, 8 day and 10 day mice was significantly higher than that of 0 day mice ($P <$ 0 01 or $P <$ 0 05). Compared with 0 day, the lung index increased at all time points, and the lung index of 8 day, 10 day and 14 day mice increased significantly ($P <$ 0 01). Compared with 0 day, the thymus index decreased at each time point, and the 10th day decreased significantly ($P <$ 0.05). The colon index of mice increased at all time points, except for the 17th day, the colon index increased significantly at all time points ($P <$ 0.01). And all the indexes reached the extreme value at the 10th day, and recovered gradually to 0 day after stopping drinking DSS.

Changes of serum IL-1 β content in UC mice at different time points

As shown in figure 7, the content of IL-1 β in serum of mice changed with time. Compared with the 0th day, the serum IL-1 β increased at all time points after the establishment of the model, and the contents of the 7^{th}, 8^{th} and 10^{th} day IL-1 β increased significantly (P < 0 01 or P < 0 05), indicating the success of the model.

As shown in figure 8, the content of IL-1 β in serum of mice changed with the time of administration. Compared with the normal group, the content of IL-1 β in serum of mice at the 1^{st}, 4^{th}, 7^{th} and 10^{th} day groups increased significantly ($P <$ 0.01). Compared with the model group, the content of IL-1 β in serum of PB-1 group increased significantly in the 1th day ($P <$ 0.01). At the 4^{th} day, the content of IL-1 β in serum of oxalazine group increased significantly ($P <$ 0.01). The content of IL-1 β in serum of the 7th day oxalazine group was significantly higher than that of model group ($P <$ 0 01 or $P <$ 0 05), but the content of IL-1 β in serum of the 10^{th} day mice was not significantly different from that of model group ($P >$ 0 05).

Discussion

The results of this experiment showed that the DAI score, CMDI score and colon HS score of mice increased at first and then decreased at each time point after DSS modeling. Compared with the 0^{th} day, the DAI score, CMDI score and HS score increased significantly at the 4th day, while the colon length decreased significantly ($P < 0.01$). Each index reached the maximum at the 10th day and showed a trend of recovery. The 7^{th} day mice were still in the acute stage, but all the indexes decreased slightly at the beginning of the 10^{th} day. Slight hyperemia, edema and no obvious ulcer were observed in the colon of the 14^{th} day mice. CMDI score and colon HS score were still significantly higher than those at 0 day (P < 0 01), indicating that the model was successful and stable. There was no significant difference in colon length, CMDI and HS between the 17^{th} day and the normal group (normal group), suggesting that the mice had strong self-healing ability and basically returned to the normal level at the 17^{th} day.

IL-1 β is a kind of cytokine secreted by monocytes, mast cells, smooth muscle cells and endothelial cells. It plays a key role in the initiation and development of host response in response to pathogen invasion. It is an important regulator of immunity and inflammation. At each time point, the content of IL-1 β in serum increased at first and then decreased. The content of IL-1 β in serum at the 7^{th}, 8^{th}, 10^{th} and 14^{th} day was significantly higher than that at the 0^{th} day, and reached the peak at 10 day, suggesting that IL-1 β was involved in inflammatory reaction.

Thymus and spleen are important immune organs, the weight of spleen and thymus can reflect the function of non-specific immunity, and colonic index can also indirectly reflect the changes of intestinal mucosal barrier. After drinking DSS, the liver index increased significantly, while the spleen index and thymus index decreased significantly. After the occurrence of UC, the weight of the animals lost, and the colon index showed an upward trend due to colonic mucosal barrier injury, such as congestion, shortening, narrowing of intestinal cavity, thickening of intestinal wall and so on.After stopping the modeling, the organ index recovered gradually. It is suggested that the occurrence of UC is related to the possible regulation of immune system.

The acute UC model was successfully induced when mice drank 3% DSS for 7 days freely, and the model was stable. After administration of the 10^{th} day after the establishment of the model and observation of the colonic lesion cycle, all the indexes of the mice basically recovered at the 17^{th} day. Through the observation of the appearance of mice and the naked eye of colon, it was found that the mental state and fecal properties of rats also returned to the normal level. Therefore, the experimental period of this model is 14 days, in which the modeling time is 7 days, and the administration time is 7 days.

Declarations

Acknowledgements

The authors thank this experiment was carried out under the guidance and help of the teacher of Hunan Agricultural University and colleagues for their contributions and hard working.

Funding information

Yunnan Young and Middle-aged academic and technological leaders Reserve Talent Project

Grant No.2014HB027

Chinese Academy of Sciences "Western Light Young Scholars" Project

Grant No.Kefa Renxin 2016 No. 84

Pu'er Science and Technology Bureau Science and Technology Plan Project

Grant No.2014kjxm01

Pu'er College Innovation team Project

Grant No.CXTD005

Pu'er College key Scientific Research Project

Grant No.2020XJGH07

Conflict of InterestsConflict of Interest disclosure

The authors declare no conflict of interest in this study.

The animal welfare and ethics statement

Healthy SPF BALB/c mice were raised in the barrier system of the Animal Experimental Center of Dali University, and the mouse feed and corncob cushion were purchased from Chengdu Dashuo Experimental Animal Co., Ltd. (batch number: 20170601). All experimental studies are in line with the guiding principles of the China Ethics Committee on animal welfare and animal research.

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Tables

Table 1 Evaluation of disease activity index (DIA).

DIA score	Stool consistency	Occult blood test	Weight lossa (%)
0	Normal	Negative□-□	<1
1	Normal`Sparse stool	Weak positive(+)	1-5
2	Sparse stool	positive(++)	5-10
3	Sparse stool`Diarrhoea	Strong positive(+++)	10-15
4	Diarrhoea	Bloody stool	≧15

Note: normal feces = formable faeces; sparse stools = sparse faeces that do not stick to the anus; diarrhea = liquefied feces that stick to the anus. Weight loss ratio range, excluding the first number, including the latter number. Weight loss (%) = (body weight at a certain point after modeling-pre-modeling weight) / pre-modeling weight × 100 %. DAI score = weight loss score + stool trait score + occult blood score.

Table 2 CMDI scoring criteria

Score	The mucous membrane of the colon
0	No damage
1	Mild hyperemia and edema, smooth surface, no erosion or ulcer
2	Hyperemia and edema, rough mucous membrane, granular sensation, erosion or intestinal adhesion.
3	Severe hyperemia and edema, necrosis and ulcer formation on the surface, thickening of intestinal wall or necrosis and inflammatory polyps on the surface.
4	Severe hyperemia and edema, mucosal necrosis and ulcer formation, whole intestinal wall necrosis, death caused by toxic megacolon.

Table 3 scoring criteria of histopathology

Score	Epithelial cell	Degree of inflammatory cell infiltration
0	Normal form	No infiltration.
1	Loss of a small number of goblet cells	Infiltrate into the basal layer of the crypt
2	Massive loss of goblet cells	Infiltrate into the muscular layer of the mucosa
3	Loss of a small number of crypt cells	The infiltration penetrated into the muscular layer of the mucosa, accompanied by mucosal thickening and obvious edema.
4	Massive loss of crypt cells	Infiltrate into the submucosa

Note: Colonic HS score = "epithelial cell" score + "inflammatory cell infiltration" score.

Table 4 changes of colonic mucosal injury index in model mice at different time points

Number of days	Quantity	HS score									P value (rank sum test)
		0	1	2	3	4	5	6	7	8	Compared with 0 day
0	6	0	0	6	0	0	0	0	0	0	-
1	6	0	0	3	3	0	0	0	0	0	0.180
4	6	0	0	2	2	0	1	0	0	1	0.065
7	6	0	0	1	0	0	0	5	0	0	0.015
8	6	0	0	0	0	0	0	1	5	0	0.002
10	6	0	0	0	0	0	0	1	1	4	0.002
14	6	0	0	0	0	0	0	5	0	1	0.002
17	6	0	0	0	3	1	0	2	0	0	0.002

Note: α = 0.05 and the α'of Benferroni correction method is 0.007.

Table 5 effects of Ento-PB on HS of UC mice at different time points

Colonic HS score			
Group	Epithelial cell score	Inflammatory cell infiltration score	Total score
	8th day		
Normal group	1.0±0.0	1.0±0.0	2.0±0.0
Model group	3.2±0.2**	3.6±0.2**	6.8±0.2**
Oxalazine group	3.2±0.2**	3.2±0.2**	6.4±0.4**
	11th day		
Normal group	1.0±0.0	1.0±0.0	2.0±0.0
Model group	3.6±0.2	3.8±0.2	7.4±0.4
Oxalazine group	2.2±0.5△	2.2±0.5△△	4.4±1.0△△
	14th day		
Normal group	1.0±0.0	1.0±0.0	2.0±0.0
Model group	3.2±0.2	3.2±0.2	6.4±0.4
Oxalazine group	1.6±0.4△△	1.6±0.4△△	3.2±0.8△△
	17th day		
Normal group	1.0±0.0	1.0±0.0	2.0±0.0
Model group	2.2±0.4**	2.2±0.4**	4.4±0.7**
Oxalazine group	1.5±0.3	1.5±0.3	3.0±0.7

Figures

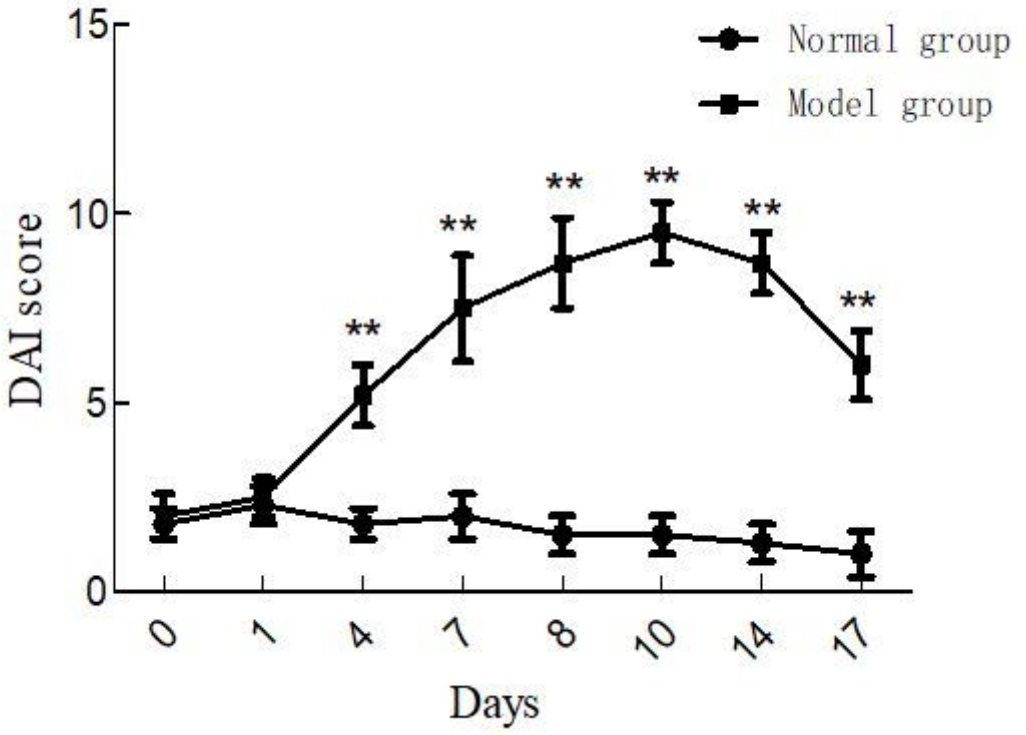

Figure 1

Cchanges of DAI score in mice at different time Note: Compared with the normal group, *P <0.05, **P<0.01. Compared with the model group, △P <0.05, △△P<0.01 (The following is the same).

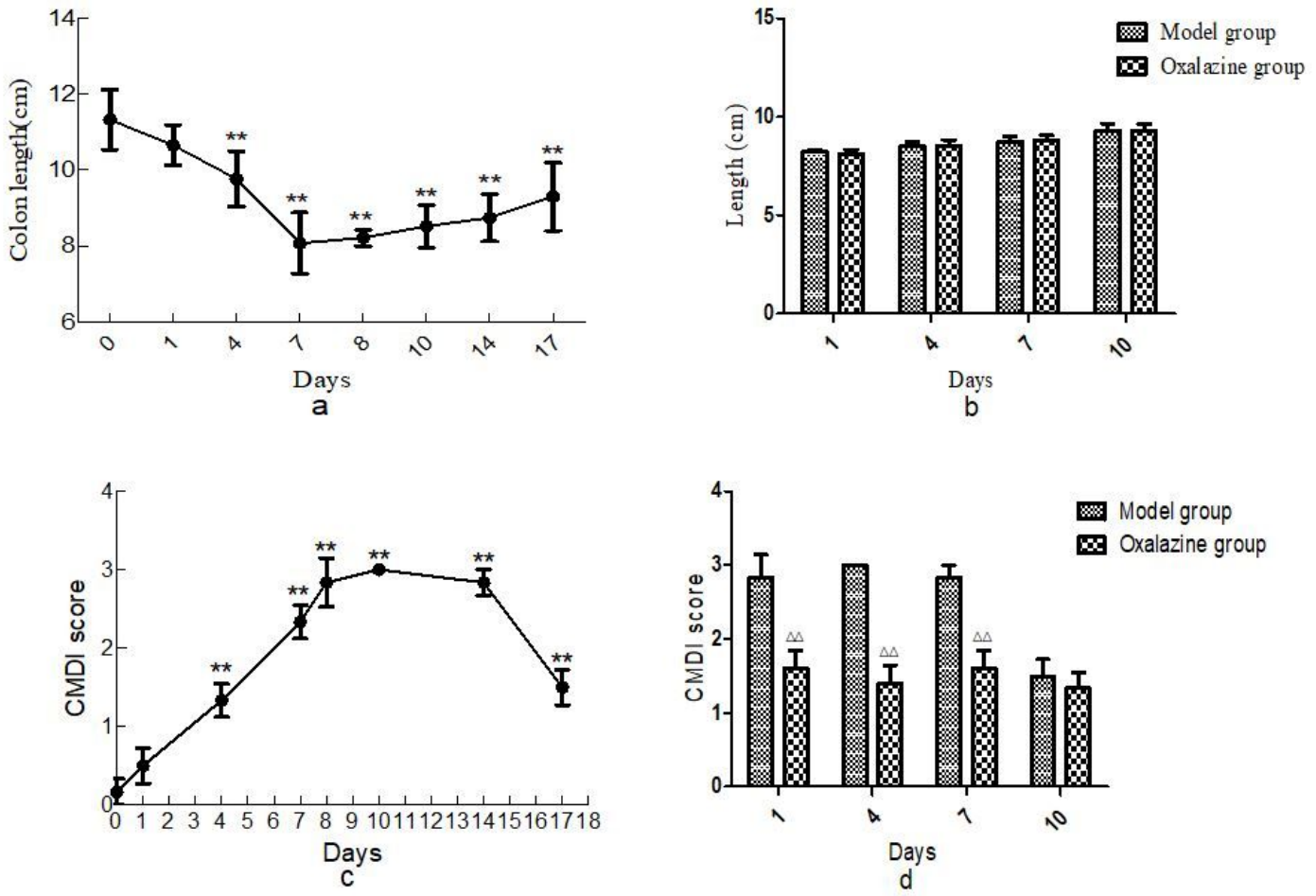

Figure 2

Changes of colonic length and CMDI in UC mice at different time points Note: a: The colonic length of mice at different time points during the modeling period. b: Colonic length of mice at different time points during administration.c: Changes of CMDI score of mice at different time points during modeling. d: Changes of CMDI score in mice at different time points during administration.

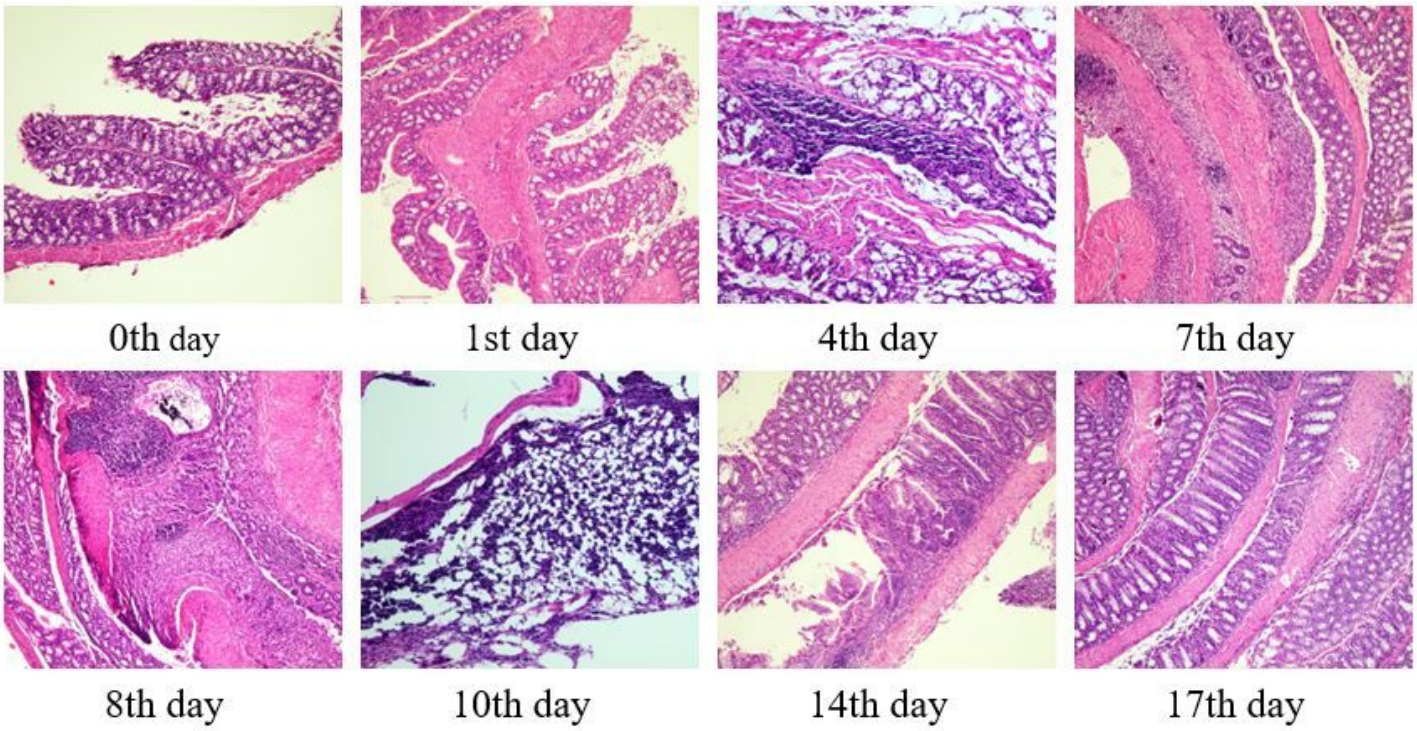

Figure 3

HS picture of colon of model mice at different time points after modeling (HE, 10 × 10)

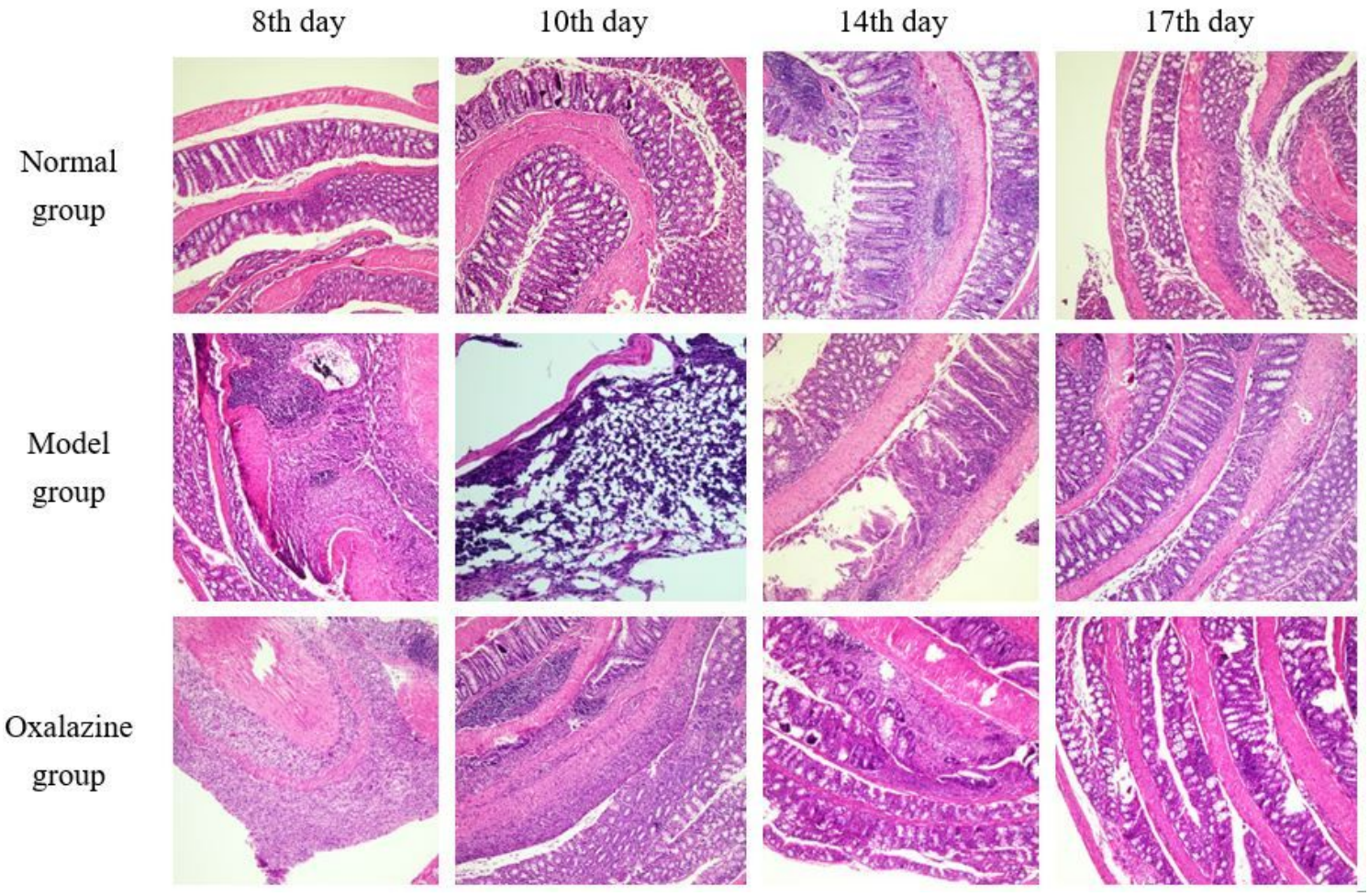

Figure 4

changes of HS score of UC mice at different time points in each administration group (HE, 10 × 10)

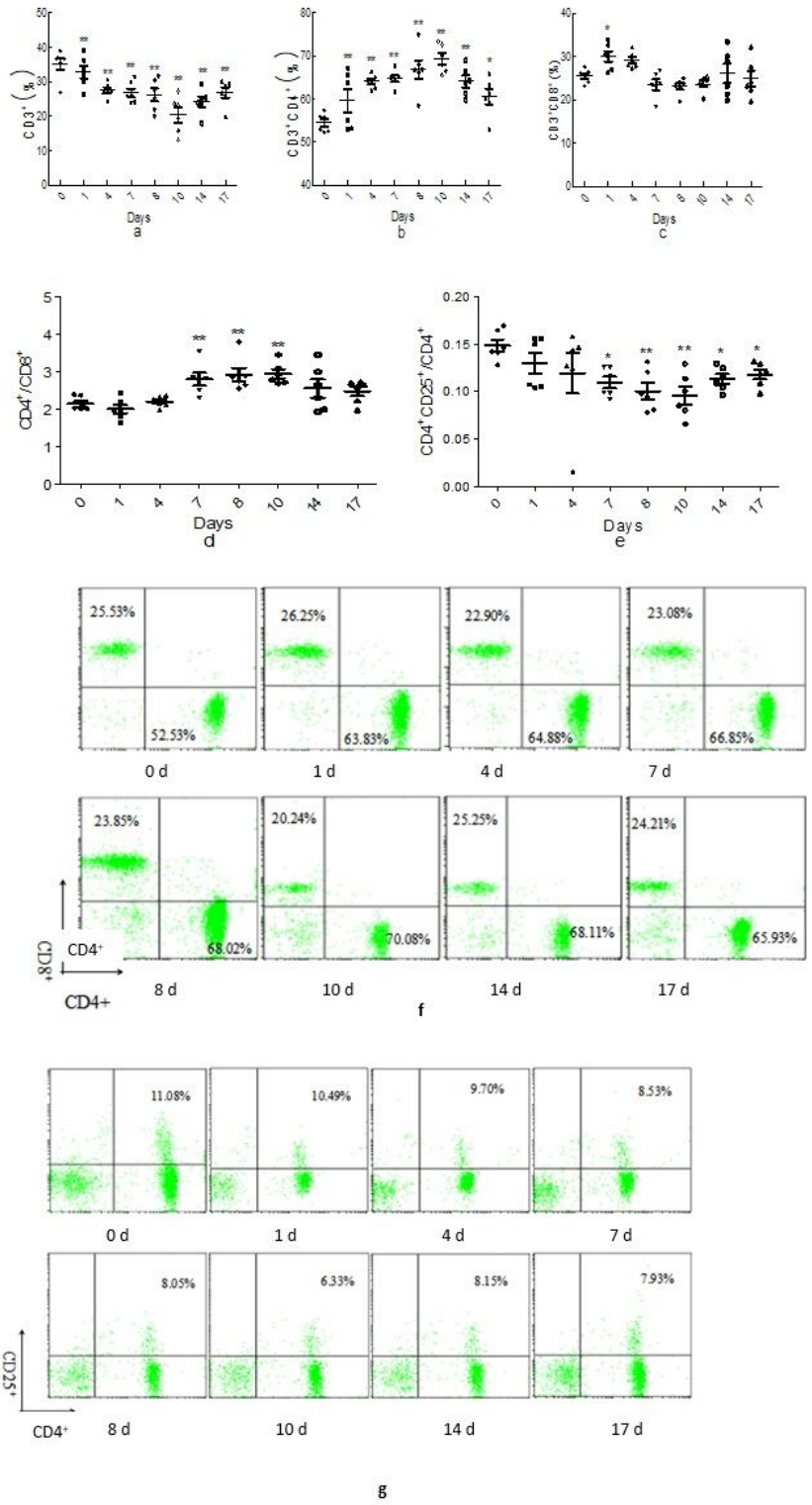

Figure 5

Changes of T lymphocyte subsets in splenocytes of model mice at different time points Note: a: CD3+ occupies cellular level. b: CD3+CD4+ occupies cellular level. c: CD3+CD8+ occupies cellular level. c: CD3+CD4+/CD3+CD8+ occupies cellular level. d: CD3+CD4+CD25+/CD3+CD4+ occupies cellular level. f: Flow analysis of CD4+ and CD8+T cells of model mice at different time points. g: Flow analysis of CD4+CD25+T cells in spleen of model mice at different time points

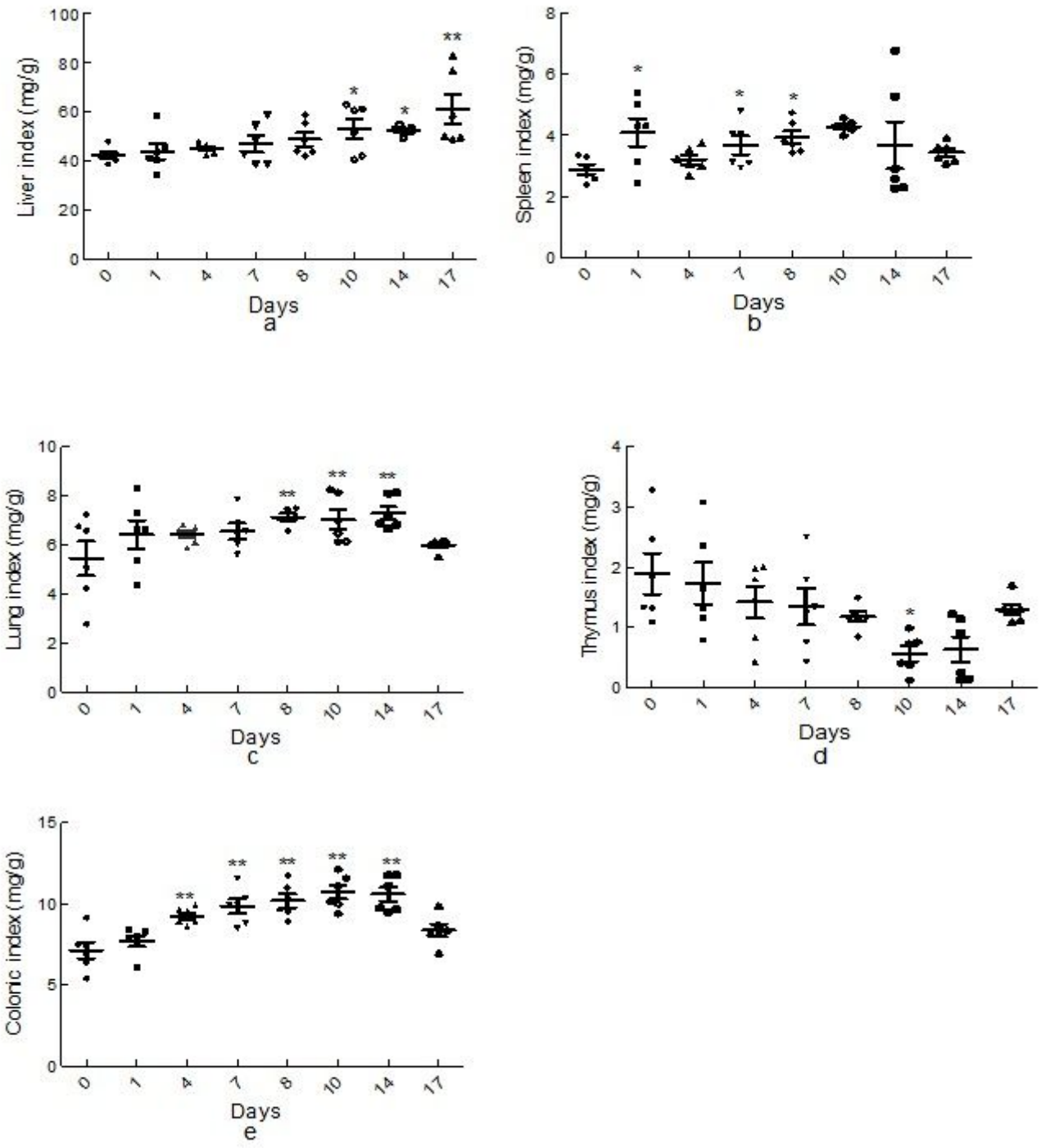

Figure 6

Changes of organ index in model mice at different time points Note: a: Liver index b: Spleen index c: Lung index. d: Thymus index e: Colonic index

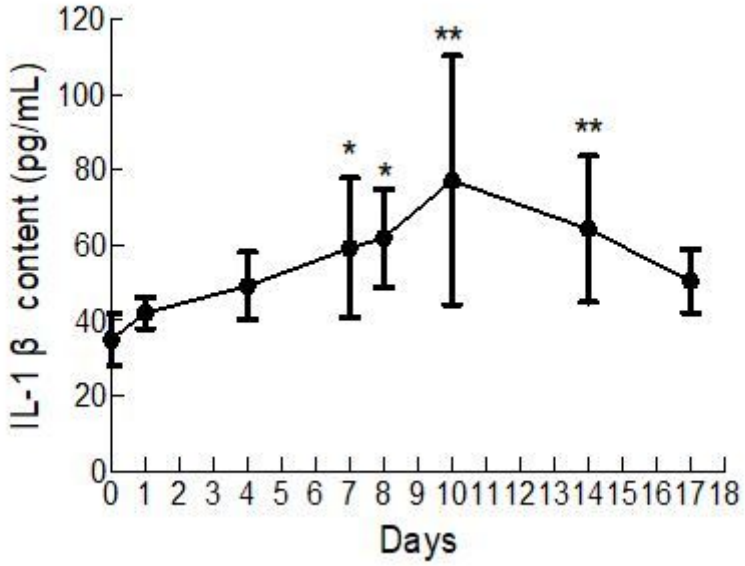

Figure 7

changes of serum IL- β in model mice at different time points

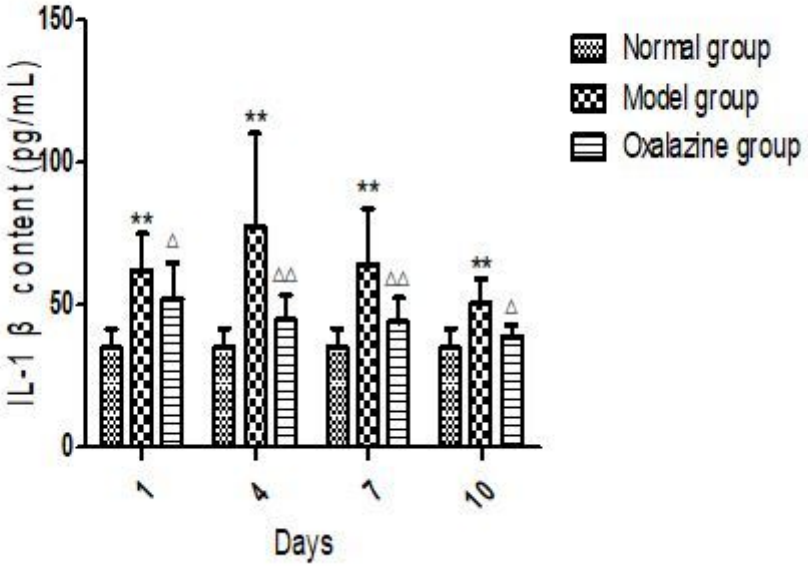

Figure 8

Changes of serum IL-1 β in model mice at different time points after administration.