Received: 22 August 2018 | Revised: 5 November 2018 | Accepted: 7 November 2018

DOI: 10.1111/cpr.12559

ORIGINAL ARTICLE

WILEY Cell Proliferation

Comparison of the efficiency of different enemas on patients with distal ulcerative colitis

Yujie Zhang[1] | Di Chen[1] | Fang Wang[2] | Xiaowei Li[3] | Xianmin Xue[1] | Mingzuo Jiang[1] | Bing Xu[1] | Yi Chu[1] | Weijie Wang[1] | Kaichun Wu[1] | Ren Mao[4] | Jun Shen[5] | Shaoqi Yang[2] | Jie Liang[1]

[1]State Key Laboratory of Cancer Biology, National Clinical Research Center for Digestive Diseases and Xijing Hospital of Digestive Diseases, Air Force Military Medical University, Xi'an, China

[2]Department of Gastroenterology, General Hospital of Ningxia Medical University, Yinchuan, China

[3]Department of Gastroenterology, Navy General Hospital, Beijing, China

[4]Department of Gastroenterology, The First Affiliated Hospital of Sun Yat-sen University, Guangzhou, China

[5]Department of Digestive Diseases, Shanghai RenJi Hospital, Shanghai, China

Correspondence
Ren Mao, Department of Gastroenterology, The First Affiliated Hospital of Sun Yat-sen University, Guangzhou, China.
Email: 160179556@qq.com
Jun Shen, Department of Digestive Diseases, Shanghai RenJi Hospital, Shanghai, China.
Email: shenjun@vip.163.com
Shaoqi Yang, Department of Gastroenterology, General Hospital of Ningxia Medical University, Yinchuan, China.
Email: shaoqiynh@163.com
Jie Liang, Xijing Hospital of Digestive Diseases, Air Force Military Medical University, Xi'an, China.
Email: liangjie@fmmu.edu.cn

Funding information
National Science-technology Support Plan, Grant/Award Number: 2015BAI13B07; National Natural Science Foundation of China, Grant/Award Number: 81301804, 81322037, 81772650, 81572302, 81421003, 81627807 and 91542000; National Key Research and Development Plan, Grant/Award Number: 2017YFC0908300 ; independent Funds of the Key Laboratory, Grant/Award Number: CBSKL2015Z12

Abstract

Objectives: Rectal application of steroids and 5-aminosalicylic acid (5-ASA) is associated with few side effects and has a high therapeutic efficacy in left-sided colitis. Previous studies have shown that rectal administration of both steroids and 5-ASA is superior to one single alone. However, some reports are still controversial. Therefore, it is necessary to investigate the treatment choice and efficacy of these different enemas in distal ulcerative colitis (UC) patients.

Materials and Methods: Questionnaire survey and a retrospective study were carried out in Chinese hospitals to investigate the efficacy of 5-ASA or hydrocortisone/dexamethasone or their combination enema in patients with distal active UC. Dextran sodium sulphate (DSS)-induced colitis model in mice was also utilized to evaluate the effects in vivo.

Results: The results from questionnaire survey showed that majority of physicians would prefer oral 5-ASA with topical 5-ASA therapy for distal UC patients. However, 43.01% of physicians would like to choose oral 5-ASA and topical hydrocortisone/dexamethasone with or without 5-ASA enema. A retrospective study demonstrated that 5-ASA enema or 5-ASA combined with hydrocortisone/dexamethasone enema therapy was superior to hydrocortisone/dexamethasone enema to decrease C-reactive protein, erythrocyte sedimentation rate (ESR), Mayo score and induce clinical remission and clinical response. No superiority of combination therapy was further proved in DSS-induced colitis in mice.

Conclusions: Although 43.01% of physicians would like to choose hydrocortisone/dexamethasone with or without 5-ASA enema for the treatment of distal UC, the combination was not superior to 5-ASA enema. Hydrocortisone/dexamethasone enema with 5-ASA enema is not recommended for distal active UC patients.

Yujie Zhang, Di Chen, Fang Wang and Xiaowei Li contribute equally to this paper.

1 | INTRODUCTION

Ulcerative colitis (UC), one type of inflammatory bowel disease (IBD), is a chronic relapsing inflammatory disorder which usually involves in the rectum and extends to the left side or entire colon, with the inflammation characteristically limited to the mucosa.[1] Although the aetiology and pathogenesis of UC remain unclear, a number of studies have shown that it is related with environment, inheritance, immunity and microorganism.[2] 5-aminosalicylic acid (5-ASA) (systemic or topical) is one of the major medicines for the treatment of UC,[3] and has different forms including oral preparations, liquid, foam, suppositories and enemas.[4] According to therapeutic aims, the extent and severity of the disease, and the tolerance and compliance of patients, doctors will choose disparate therapeutic regimen-single therapy or combination treatment.

Results from a population-based inception cohort study have shown that the overwhelming majority of the UC patients (70%) exhibit only a proctitis/proctosigmoiditis or left-sided colitis, only 30% have extended diseases.[5] Precisely, because the majority of UC patients suffer a type of distal UC,[6,7] a topical therapy should be applied due to the higher success rates and fewer side effects compared with oral therapy. Rectal application of steroids and 5-ASA is associated with fewer side effects and has a higher therapeutic efficacy in left-sided colitis, which was underused.[3,8] On one hand, some results have proved that rectal 5-ASA is superior to rectal corticosteroids in the management of distal UC.[9,10] On the other hand, however, other studies demonstrated a similar efficacy of both treatments.[11-16] Although it is clearly stated that rectal 5-ASA is superior to steroid in recent European Crohn´s and Colitis Organisation (ECCO) guideline,[17,18] the steroids used are beclomethasone dipropionate (BDP) or budesonide[15,19-27] in their study, which is not available in China.

In addition, researchers also investigated whether rectal combination application of steroids and 5-ASA was superior to one single alone. Intriguingly, the results are controversial.[19,28] Considering combination application of steroids and 5-ASA is widely used by Chinese doctors, we are interested in studying the effect of hydrocortisone/dexamethasone enema treatment with or without 5-ASA.

Therefore, a questionnaire survey was carried out in 72 gastrointestinal (GI) centres to investigate the preference of choice for the treatment of distal UC in Chinese doctors. Then, a retrospective study was carried out in four IBD centres to evaluate the efficacy of hydrocortisone/dexamethasone with or without 5-ASA enema in patients with distal active UC. Finally, Dextran sodium sulphate (DSS)-induced colitis model in mice was used to prove the effect. In this study, we try to find which is the best enema through clinical and experimental research, then it might provide a decisive evidence for Chinese doctors for their treatment of distal active UC.

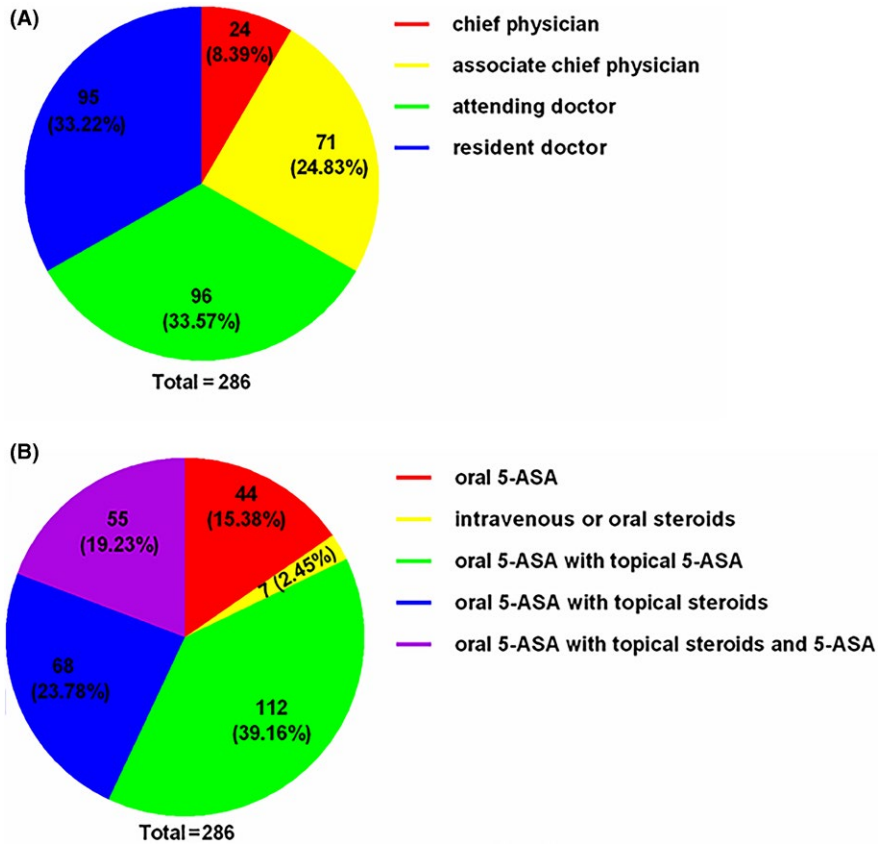


FIGURE 1 The professional title composition of doctors involving the questionnaire and treatment choices for distal active ulcerative colitis. A, A total of 286 doctors with different professional title were enrolled in the survey, and the composition was shown in the pie chart with different colour. B, In the survey, doctors chose different therapeutic schedule including oral 5-ASA only, intravenous or oral steroids, oral 5-ASA with topical 5-ASA, oral 5-ASA with topical steroids and oral 5-ASA with topical steroids and 5-ASA to treat left-sided colitis patients. The corresponding number and proportion chosen by doctors were shown in the pie chart with different colour

2 | MATERIALS AND METHODS

2.1 | Questionnaire survey

Questionnaire survey was carried out in 72 GI centres and 286 doctors with their informed consent. A self-administered and structured questionnaire, as described in Table S1, was used to collect data.

2.2 | Clinical observation parameters

Mayo score, as described in Table S2, also known as the Mayo Clinic Score and the Disease Activity Index, was used to evaluate the effect on intra-group (before vs after) and inter-group (5-ASA enemas vs hydrocortisone/dexamethasone enemas vs combination enemas).[29]

The laboratory parameters including RBC, WBC, PLT, Hb, CRP and erythrocyte sedimentation rate (ESR) were collected and analyzed before and after treatment.

2.3 | Animal model

C57BL/6 mice (female, aged 6-8 weeks) were from the Animal Center of the Air Force Military Medical University. All experimental procedures were approved by the Experimental Animal Welfare and Ethics Committee, the Air Force Military Medical University. Animal experiments were performed in accordance with National Institutes of Health Animal Care and Use Committee guidelines.

Acute colitis was induced with 3.5% DSS for 5 days, and then regular water for another 5 days.

The mice were divided into five groups as follows:

TABLE 1 Baseline characteristics of enrolled 101 UC patients

Groups	5-ASA enema group	Hydrocortisone/dexamethasone enema group	Combination enema group
Number	34	38	29
Sex			
Female	15	25	16
Male	19	13	13
Age (y) (mean ± SEM)	43.50 ± 2.46	39.68 ± 2.71	47.66 ± 2.74
Duration time (y)	1 y-15 y	7 d-16 y	1 y-20 + y
Severity			
Mild	3	10	1
Moderate	21	18	14
Severe	10	10	14

TABLE 2 Mayo score of UC patients

Groups	5-ASA enema group	Hydrocortisone/dexamethasone enema group	Combination enema group
Before			
Stool frequency	2.15 ± 0.74	1.89 ± 1.03	2.52 ± 0.57
Rectal bleeding	2.38 ± 0.85	1.74 ± 0.89	2.45 ± 0.74
Mucosa	2.09 ± 0.79	2.29 ± 0.65	2.28 ± 0.70
Physician's global assessment	2.00 ± 0.85	2.00 ± 0.77	2.24 ± 0.74
Mayo score	8.62 ± 2.36	7.92 ± 2.89	9.45 ± 2.35
After			
Stool frequency	0.71 ± 0.80	1.24 ± 0.94	1.00 ± 0.71
Rectal bleeding	0.41 ± 0.50	1.03 ± 0.91	0.55 ± 0.51
Mucosa	1.12 ± 0.69	1.53 ± 0.69	0.93 ± 0.26
Physician's global assessment	0.71 ± 0.68	1.55 ± 0.76	0.69 ± 0.54
Mayo score	2.97 ± 1.82	5.34 ± 2.40	3.14 ± 1.48
P			
Before vs After	<0.0001	<0.0001	<0.0001

Annotation: The data were presented as mean ± SD.

1. Wild type (WT) group (without induction of colitis) (n = 7) which treated intrarectally with phosphate-buffered saline (PBS) (200 μL).
2. DSS group I (n = 16) which treated intrarectally with PBS (200 μL).
3. DSS group II (n = 16) which treated intrarectally with 5-ASA enema (Salofalk) (200 μL).
4. DSS group III (n = 16) which treated intrarectally with hydrocortisone sodium succinate (Tianjin Biochem Pharmaceutical Co., Tianjin, China) (50 mg dissolved in 32 mL Solution physiologique (1×); 200 μL).
5. DSS group IV (n = 16) which treated with mixtures of 5-ASA enema (200 μL) and hydrocortisone sodium succinate (50 mg dissolved in 16 mL Solution physiologique (2×; 100 μL).

During the course of the experiment, body weight, blood stool and stool consistency were recorded daily, and the latter two indications were scored as follows: for stool consistency: 0-well-formed pellets; 2-pasty and semi-formed stools which did not adhere to the anus; 4-liquid stools that did adhere to the anus; for blood stool: 0-no blood (using hemoccult-Bechman Coulter); 2-positive hemoccult; 4-gross bleeding.

Drug administration via anus to the mice was implemented on the 5th-10th day. Before every treatment, mice should be fasting for about 8 hours and anaesthetized by inhalation of ethylic ether. On the 10th day, mice were sacrificed and the intestinal tract from anus to ileocecus was removed and stool within it was cleared away. After measurement of the length of intestinal tract, parts of intestinal tract were either fixed with 4% paraformaldehyde and paraffin embedded for histological analyses or frozen in −80°C icebox for RNA and protein extraction.

2.4 | Pathological analysis

Histological assessments of colitis and severity scores were made in a double-blinded manner after H&E staining as described in Table S3.[30]

2.5 | Other methods

For other Section 2, please see Supporting Information.

2.6 | Statistical analysis

The results were analyzed with different statistical methods, and *P* value <0.05 was considered statistically significant. A majority of our data was described with the mean and standard deviation and those data were dealt with Student's *t* test between any two groups. As for comparison of rate, we employed chi-square test to evaluate differences between two groups. We consulted professional teachers of statistics about the choice of specific statistical methods.

3 | RESULTS

3.1 | General information of questionnaire survey

Of 286 doctors enrolled in the survey, 8.39% (24/286) were chief physician, 24.83% (71/286) were associate chief physician, 33.57% (96/286) were attending doctor and 33.22% (95/286) were resident doctor (Figure 1A). Among these doctors, 8.39% (24/286) treated

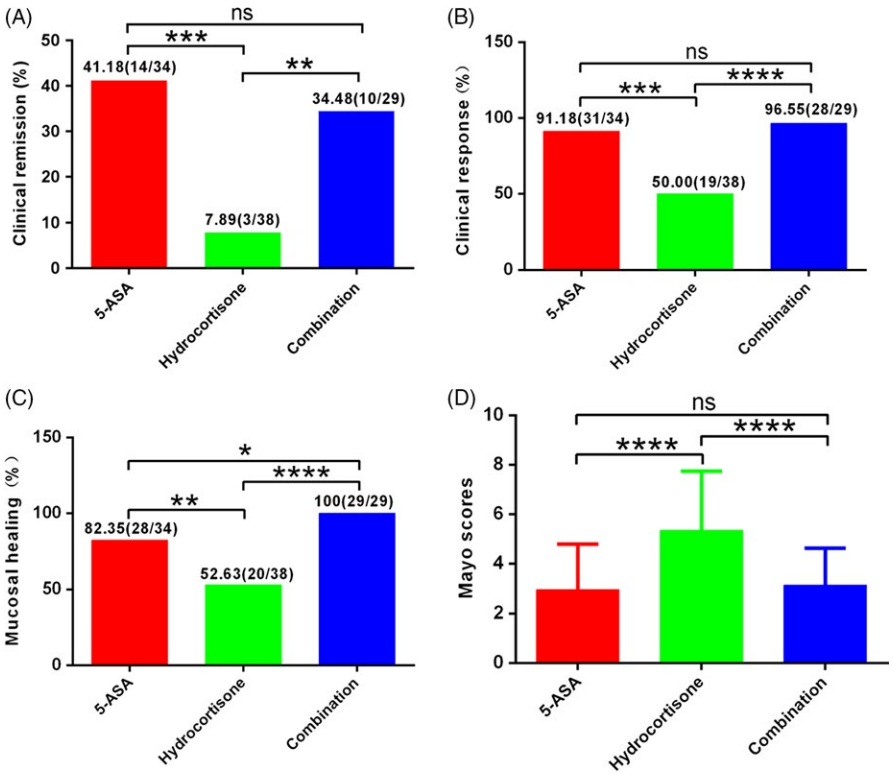


FIGURE 2 The efficiency of 5-ASA or hydrocortisone/dexamethasone or their combination in patients with distal active UC. The duration of treatment was 4 wk for UC patients in each group. A, Clinical remission; B, clinical response; C, Mucosal healing; D, Mayo scores (after treatment). **P* < 0.05, ***P* < 0.01, ****P* < 0.005, *****P* < 0.0001

TABLE 3 The analysis of laboratory indexes

(1)

Groups	RBC			WBC			PLT		
	Before	After	*P*	Before	After	*P*	Before	After	*P*
5-ASA enema group	4.29 ± 0.56	4.51 ± 0.57	0.136	7.16 ± 3.39	6.49 ± 3.23	0.594	251.58 ± 79.69	233.74 ± 84.22	0.436
Hydrocortisone/dexamethasone enema group	4.09 ± 0.71	4.15 ± 0.57	0.831	8.66 ± 2.77	8.09 ± 2.35	0.418	313.34 ± 108.81	314.74 ± 112.64	0.975
Combination enema group	4.39 ± 0.61	4.47 ± 0.57	0.895	8.71 ± 4.15	7.13 ± 1.83	0.184	252.97 ± 84.55	244.62 ± 69.25	0.803

(2)

Groups	Hb			CRP			ESR		
	Before	After	*P*	Before	After	*P*	Before	After	*P*
5-ASA enema group	125.68 ± 19.86	132.42 ± 19.09	0.6935	18.93 ± 13.03	13.62 ± 8.98	0.010	20.74 ± 16.61	12.11 ± 11.52	0.000
Hydrocortisone/dexamethasone enema group	122.00 ± 20.19	123.55 ± 18.87	0.9273	27.34 ± 15.97	25.14 ± 15.51	0.433	20.23 ± 11.86	18.05 ± 10.22	0.429
Combination enema group	126.59 ± 18.83	132.59 ± 16.29	0.6978	15.43 ± 7.41	11.01 ± 5.02	0.003	24.28 ± 16.12	14.72 ± 8.78	0.018

Annotation: The data were presented as mean ± SD.

more than five patients monthly with UC, 17.83% (51/286) treated 3-5 patients, 58.04% (166/286) treated 1-2 patients and 15.73% (45/286) had no chance to treat patients.

3.2 | Treatment choices for distal active UC

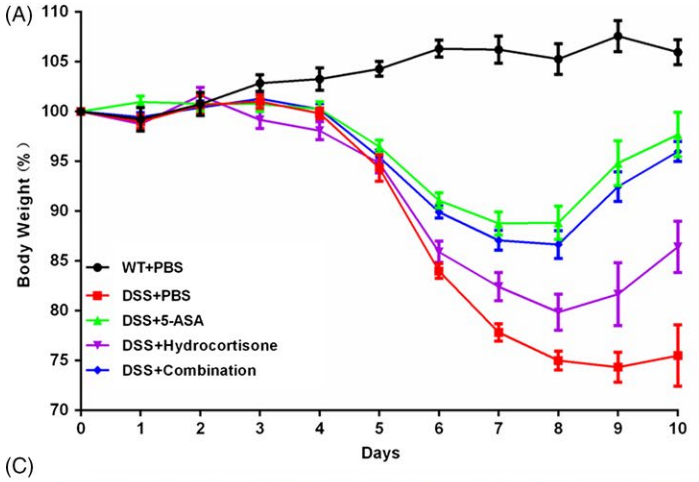
According to the extent and severity of UC patients, doctors will choose different therapeutic schedules. Sometimes, the willingness of doctors and the tolerance and compliance of patients are responsible for the therapeutic choice. In our survey, we mainly paid attention to the choice of doctors when they treated left-sided colitis patients. As shown in Figure 1B, oral 5-ASA only was chosen by 15.38% (44/286) doctors, intravenous or oral steroids by 2.45% (7/286) doctors, oral 5-ASA with topical 5-ASA (enema or suppository) by 39.16% (112/286) doctors, oral 5-ASA with topical steroids (enema or suppository) by 23.78% (68/286) doctors and oral 5-ASA with topical steroids and 5-ASA (enema or suppository) by 19.23% (55/286) doctors, respectively. Obviously, major doctors would prefer oral 5-ASA with topical 5-ASA (enema or suppository) treatment. However, 43.01% of the doctors would like to choose oral 5-ASA with topical hydrocortisone/dexamethasone with or without 5-ASA enema in China.

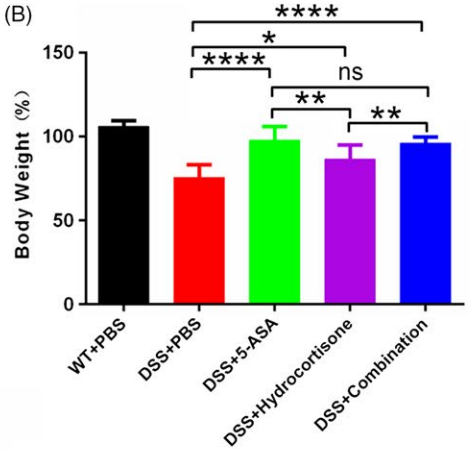
3.3 | General information of patients

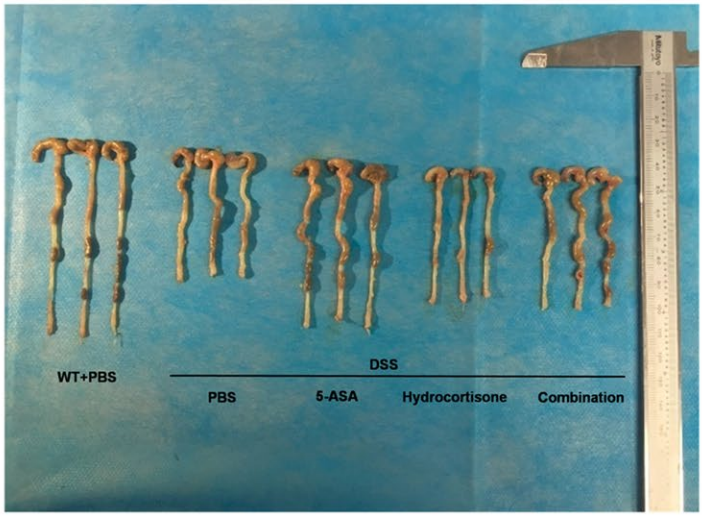
General information of UC patients was summarized in Table 1. One hundred and one patients (34 for 5-ASA, 38 for hydrocortisone/dexamethasone and 29 for hydrocortisone/dexamethasone with 5-ASA enema treatment) were selected and analyzed. Baseline demographics including case number, sex, age and duration time were found to have no significant differences among three groups. But, the severity of the disease was different between groups. The group of 5-ASA enema and hydrocortisone/dexamethasone enema had more mild to moderate patients and their combination enema group had more moderate and severe patients (Table 1). Limited by the number of the cases, no significant difference was observed.

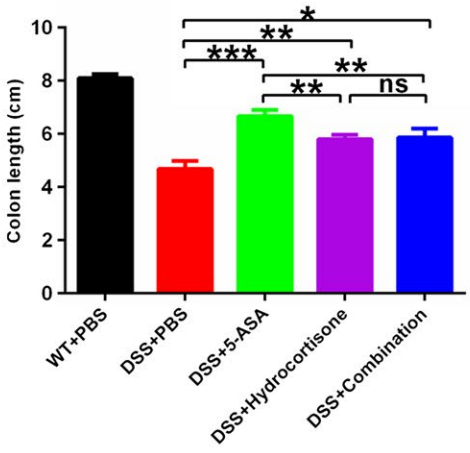
3.4 | The efficiency of 5-ASA or hydrocortisone/dexamethasone or their combination enema treatment in patients with distal active UC

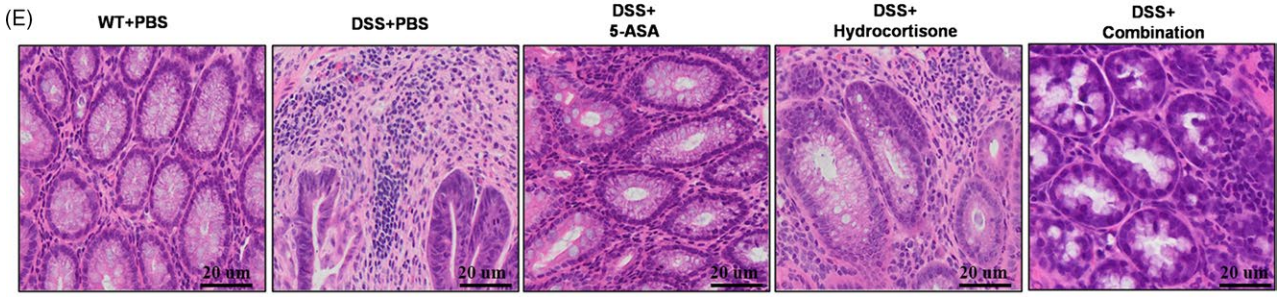
In order to evaluate the efficiency of 5-ASA or hydrocortisone/dexamethasone or their combination in patients with distal active UC, Mayo score was employed and detailed results were presented in Table 2. Compared with before treatment, the Mayo score after treatment was found to be reduced significantly in each group ($P < 0.001$) (Table 2). Clinical remission[31-33] (Mayo Clinic score ≤ 2, with no subscore > 1) occurred in 41.18% (14/34) in 5-ASA enema group, 7.89% (3/38) in hydrocortisone/dexamethasone enema group and 34.48% (10/29) in combination enema group (Figure 2A), the clinical response[31-33] (reduction in the Mayo Clinic score of ≥3 points and ≥30% from baseline, with a decrease in the rectal bleeding subscore of ≥1 point or a subscore


(A)
Body Weight (%)
Days
WT+PBS
DSS+PBS
DSS+5-ASA
DSS+Hydrocortisone
DSS+Combination


(B)
Body Weight (%)
****
*
****
ns
**
**
WT+PBS
DSS+PBS
DSS+5-ASA
DSS+Hydrocortisone
DSS+Combination


(C)
WT+PBS
DSS
PBS
5-ASA
Hydrocortisone
Combination


(D)
Colon length (cm)
*
**
***
**
**
ns
WT+PBS
DSS+PBS
DSS+5-ASA
DSS+Hydrocortisone
DSS+Combination


(E)
WT+PBS
DSS+PBS
DSS+ 5-ASA
DSS+ Hydrocortisone
DSS+ Combination
20 um

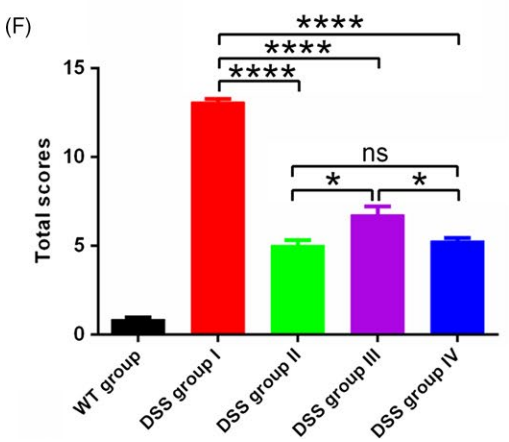

(F)
Total scores
****
****
****
ns
*
*
WT group
DSS group I
DSS group II
DSS group III
DSS group IV

FIGURE 3 The treatment of 5-ASA or hydrocortisone/dexamethasone or combination enema treatment alleviated DSS-induced colitis. A-F, Acute colitis was induced with 3.5% DSS for 5 d, and then regular water for another 5 d. Drug administration via anus to the mice was implemented on the 5th to 10th day. Changes in body weight (A,B), colon shortening (C,D), mucosal histology examined by H&E staining (E, scale bar, 20 μm) and colitis severity scores (Table 4 and Figure F) were determined in a double-blind manner. *P < 0.05, **P < 0.01,***P < 0.005 (Student's t test)

of ≤1) occurred in 91.18% (31/34) in 5-ASA enema group, 50.00% (19/38) in hydrocortisone/dexamethasone enema group and 96.55% (28/29) in combination enema group (Figure 2B) and mucosal healing (endoscopy subscore ≤1 point) occurred in 82.35% (28/34) in 5-ASA enema group, 52.63% (20/38) in hydrocortisone/dexamethasone enema group and 100% (29/29) in combination enema group (Figure 2C). Moreover, the efficiency of different groups after treatment was analyzed and compared. After the treatment, the 5-ASA enema group and combination enema group were superior to hydrocortisone/dexamethasone enema group (Figure 2D; P < 0.001). Finally, we analyzed and compared clinical remission and clinical response according to the severity of disease and the results are showed in Table S5 and Figure S2.

3.5 | Laboratory characteristics for UC patients

In addition, the laboratory indexes including red blood cell (RBC), white blood cell (WBC), platelet (PLT), haemoglobin (Hb), C-reactive protein (CRP) and ESR were also collected and analyzed before and after treatment. After treatment, CRP and ESR were observed to be markedly improved in 5-ASA enema group and combination enema group (P < 0.05), but not in hydrocortisone/dexamethasone enema group (P > 0.05). No differences in RBC, WBC, PLT and Hb were found to be altered before and after the enema treatment in each group (Table 3).

3.6 | The efficiency of 5-ASA or hydrocortisone/dexamethasone or their combination enema treatment in DSS-induced colitis in mice

Seventy-one C57BL/6 mice (female) were enrolled in the experiment. About 66 mice completed the experiment and five mice died during the study, including one mouse in DSS group II, two mice in group III and two mice in group IV. The body weight recovered in each group after the enema treatment. It was more obvious in 5-ASA enema group and combination enema group compared with hydrocortisone/dexamethasone enema group. However, no statistical differences were found between 5-ASA enema group and combination enema group (Figure 3A,B). Similar to the changes of body weight, the shorten length of colon improved most significantly in 5-ASA enema group compared with hydrocortisone/dexamethasone enema group or combination enema group (Figure 3C,D), while there were no significant differences in blood stool and stool consistency among three enema groups (see Figure S1).

In agreement, pathological analysis showed that the therapeutic effect of 5-ASA enema group that had no statistical differences compared with combination enema group, was superior to the hydrocortisone group based on haematoxylin (H&E) staining (Figure 3E) and the pathological assessment of colitis severity scores (Table 4 and Figure 3F), though each enema group had significant improvement after treatment.

3.7 | Cytokines and signalling pathways-related molecules measurement

It is well documented that cytokines secretion and persistent activation of inflammation-associated signalling pathways are prominent feature of IBD,[2,34] so we measured the change of these related molecules including cyclooxygenase (COX)-2, tumour necrosis factor (TNF)-, interleukin (IL)-1β, IL-6, intercellular cell adhesion molecule-1 (ICAM-1), p-NF-κB/p65 by the means of polymerase chain reaction (PCR), western blot (WB) and immunohistochemistry (IHC), respectively. Expression of these molecules was found to be markedly increased in mice of DSS-induced group and significantly decreased after enema treatment. PCR results showed that the expression of COX-2, TNF-, IL-1β, IL-6 and a downstream factor of NF-κB signalling pathway, ICAM-1 was reduced much more significantly in 5-ASA enema group and combination enema group compared with hydrocortisone/dexamethasone enema group (Figure 4A-E), which was also verified by means of WB and IHC analysis (Figure 4F,G).

TABLE 4 The pathological assessment of colitis severity scores

	Score parameter				
Groups	Inflammation severity	Inflammation extent	Crypt damage	Per cent of involvement	Total scores
WT group	0.49 ± 0.18	0.36 ± 0.20	0.00 ± 0.00	0.00 ± 0.00	0.85 ± 0.12
DSS group I	3.00 ± 0.00	3.00 ± 0.00	4.00 ± 0.00	3.15 ± 0.32	**13.09 ± 0.18**
DSS group II	1.53 ± 0.33	1.50 ± 0.44	1.50 ± 0.52	0.60 ± 0.26	**5.03 ± 0.29**
DSS group III	2.10 ± 0.13	1.54 ± 0.33	1.78 ± 0.31	1.41 ± 0.19	**6.74 ± 0.47**
DSS group IV	1.48 ± 0.22	1.45 ± 0.22	1.67 ± 0.45	0.68 ± 0.23	**5.28 ± 0.16**

Annotation: Total scores were the sum of score of the four parameters, and the data were presented as mean ± SD. The significance of Bold values is shown in the (Figure 3F).

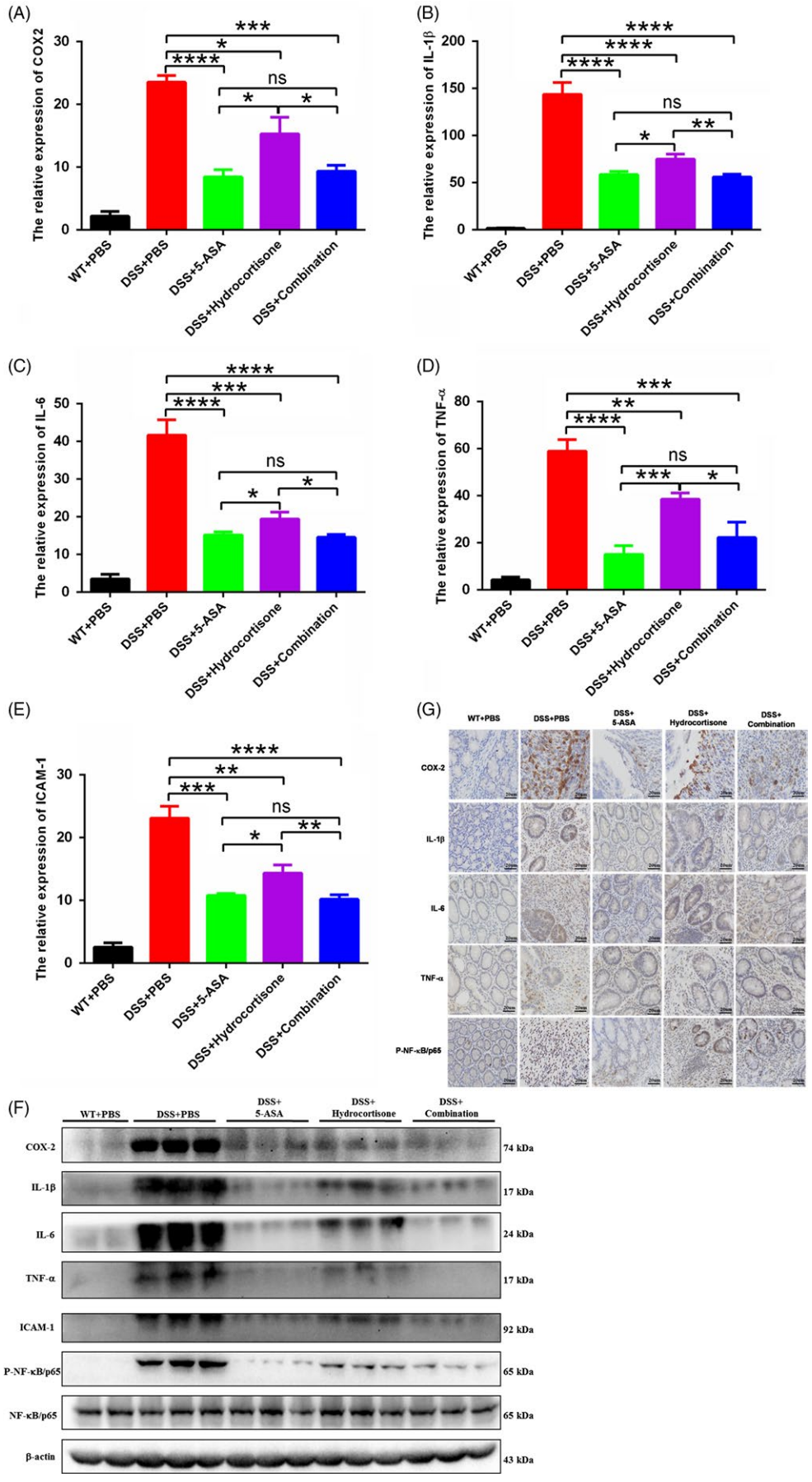

(A)
The relative expression of COX2
(B)
The relative expression of IL-1β
(C)
The relative expression of IL-6
(D)
The relative expression of TNF-α
(E)
The relative expression of ICAM-1
WT+PBS
DSS+PBS
DSS+5-ASA
DSS+Hydrocortisone
DSS+Combination
ns
(G)
COX-2
IL-1β
IL-6
TNF-α
P-NF-κB/p65
(F)
74 kDa
17 kDa
24 kDa
17 kDa
92 kDa
65 kDa
65 kDa
43 kDa
NF-κB/p65
β-actin
ICAM-1

FIGURE 4 The treatment of 5-ASA or hydrocortisone/dexamethasone or their combination enema decreased COX-2, IL-1β, IL-6, TNF- and ICAM-1 expression through NF-κB signalling pathway. A-E, mRNA expression of cytokines and signalling pathway-related molecule were examined by qRT-PCR, including COX-2 (A), IL-1β (B), IL-6 (C), TNF- (D) and ICAM-1 (E). F, Protein expression of cytokines and signal pathway-related molecule were examined by Western blotting. β-actin served as a loading control. G, Representative IHC-stained sections of COX-2, TNF-, IL-1β, IL-6 and p-NF-κB/p65. Scale bars, 20 μm. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.005$, $^{****}P < 0.0001$ (Student's *t* test).

NF-κB signalling is a key process during inflammation and thus constitutes an attractive target for anti-inflammatory interventions.[35] To clarify the possible molecular mechanisms of the enema treatment, we evaluated their effects on NF-κB activation in mice with DSS-induced colitis. As shown in Figure 4F,G, WB and IHC analysis demonstrated that the enema treatment markedly suppressed the protein expression of p-NF-κB/p65 and the 5-ASA enema group and combination enema had better effects than the hydrocortisone/dexamethasone enema group.

4 | DISCUSSION

As is known to all, topical 5-ASA and steroids are common therapies for UC. However, which is more effective of topical 5-ASA, steroids or their combination for the treatment of left-sided UC is still controversial. Especially, the steroids used for enema are different from those in western country. Therefore, our study aims at comparing the efficacy of 5-ASA, hydrocortisone/dexamethasone or combination enema in patients with distal active UC. The most meaningful finding of our study is that 43.01% of Chinese doctors would like to choose hydrocortisone/dexamethasone with or without 5-ASA enema for the treatment of distal UC. In addition, the results from a retrospective study and an animal experiment showed that two are no better than one-the combination enema was not superior to 5-ASA enema.

Firstly, in order to investigate the preference of GI doctors for topical treatment of distal UC, we introduced a questionnaire survey which was carried out in 72 GI centres of China. The results showed that 43.01% of the doctors would like to choose topical hydrocortisone/dexamethasone with or without 5-ASA enema. In China, some doctors think that systemic steroids are mainly used for implosive therapy for severe UC and the efficacy is superior to 5-ASA, so they take it for granted that steroid enema is better than 5-ASA enema, although it is clearly stated that topical 5-ASA is more effective than topical steroids in recent ECCO guideline.[17,18] Besides, some foreign researches have investigated the efficiency of rectal combination application of steroids and 5-ASA compared with single drug, the results are controversial and the steroids used are BDP or budesonide, which is not available in China. Even so, combination application of 5-ASA and other steroids, such as hydrocortisone or dexamethasone is still an alternative for many doctors in our country, which affirmed the necessity of our study. Intriguingly, there are 28.71% (29/101) patients with distal UC who were treated with combination enema in the retrospective study mentioned below.

Then, a retrospective study involved four major IBD centres in China was carried out. One hundred and one left-sided UC patients were enrolled, and general information was similar within three groups. Oral medicine was treated in suitable patients without significant difference in each group. After the treatment, colitis was relieved in three groups according to the Mayo score and inflammatory indexes. 5-ASA enema and combination enema therapy were superior to hydrocortisone/dexamethasone enema evaluated by clinical remission, clinical response, mucosal healing and Mayo score. Except mucosal healing, no significant differences were seen between 5-ASA enema alone and combination therapy. It is worth noting that in combination enema group, there were more moderate and severe patients compared to 5-ASA or hydrocortisone/dexamethasone enema group, which may have certain effect on the results. Therefore, we evaluated clinical remission and clinical response according to severity of disease in the three groups. Clinical remission and clinical response were worst in hydrocortisone/dexamethasone enema group independent of the severity of the disease and in the other two groups, clinical remission and clinical response seemed to be better in combination enema group compared with 5-ASA enema group in terms of mild patients, whereas they had no significant differences in terms of moderate or severe patients. Surprisingly, these results, which were inconsistent with previous studies,[19,28] indicated that hydrocortisone/dexamethasone enema seemed to have no or minute effect. The main reason may be that for distal UC therapy, BDP and budesonide are very popular in the foreign country, which are not available in China, so we prefer to use hydrocortisone and dexamethasone instead. In addition, BDP and budesonide are characterized with high first-pass hepatic metabolism, conversely, hydrocortisone and dexamethasone metabolize in liver, so it is difficult to ensure adequate drug concentration locally. On the other hand, steroid enema treatment may increase the incidence of opportunistic infections, change the microbial species of the gut and have bad affects on intestinal mucosal permeability because of the ion disorder, which may affect drug efficacy.

Finally, the therapy effect was proved in DSS-induced colitis model in mice. The 5-ASA enema group and combination enema group showed better treatment effect according to the improvement of body weight and the length of colon. H&E staining and the pathological assessment of colitis severity scores also showed preferable treatment in these two enema groups. Notably, lots of reports have demonstrated that NF-κB signalling pathway plays a key role in the development of inflammation-associated diseases, the transcription factor NF-κB can control the expression of many genes involved in cytokines/chemokines and their modulators, immunoreceptors, cell adhesion molecules, acute phase proteins and so on (http://www.bu.edu/nf-kb/gene-resources/target-genes/).[36,37] TNF- and IL-6 are potent activators of NF-κB, and activated NF-κB can in turn induce TNF- expression,[38,39] while COX-2, IL-1 and ICAM-1 are downstream effectors of NF-κB activation.[36] To further

explore the potential mechanism by which the drugs played a role, inflammation and signalling pathways-related molecules mentioned above were measured. With DSS exposure, inflammation occurred, the expression of related molecules including COX-2, TNF-, IL-1β, IL-6 and ICAM-1 increased and NF-κB signalling pathway was activated. After enema treatment, the inflammation-related molecules decreased to varying degrees compared with PBS enema group and the reduction was worst in hydrocortisone/dexamethasone enema group. As we know, one of the most crucial pharmacological actions of steroids for UC therapy is its powerful anti-inflammatory effect. In the present study, unlike systematical application, topical steroid treatment may seriously influence its anti-inflammatory effect and to some extent, locally limited drug concentration may be another reason, which is responsible for the worst efficacy of hydrocortisone/dexamethasone enema.

One of the strengths about our study is that it is the first evidence of treatment choice in Chinese doctors for the distal UC through a questionnaire survey in 72 GI centres and 286 doctors. Moreover, a retrospective study carried out in four major IBD centres with an animal experiment was used to compare the effect among 5-ASA enema, hydrocortisone/dexamethasone enema and combination enema.

There are several limitations to this study. First, the questionnaire survey was carried out in 72 GI centres and 286 doctors, which might comprehensively represent the choice for Chinese doctors. However, certain number of doctors was not IBD specialist and the preference of hydrocortisone/dexamethasone enema or hydrocortisone/dexamethasone combined with 5-ASA enema treatment might be more widely chosen by none-IBD specialist. Second, a retrospective study was carried out instead of a prospective, controlled, randomized, double-blind study. To some certain extent, it had a few disadvantages, such as relative poor representativeness, incomplete information, lost follow-up and so on. Nevertheless, a randomized controlled trial (RCT) had already registered by these IBD centres (NCT03110198) to make up for the shortcomings. Third, unlike BDP and budesonide, which are characterized with high first-pass hepatic metabolism, hydrocortisone/dexamethasone might be metabolized in liver. The plasma concentration or the side effects were not investigated in this study. Even though, the practical use of different steroids in China might just reflect the value and significance of our study. Finally, the aim of our study was to investigate the therapeutic effects of hydrocortisone/dexamethasone and its combination with 5-ASA to treat left-sided UC, therefore, the side effects of hydrocortisone/dexamethasone are not be paid much more attention.

ACKNOWLEDGEMENTS

We are thankful for clinical data provided by State Key Laboratory of Cancer Biology, National Clinical Research Center for Digestive Diseases and Xijing Hospital of Digestive Diseases, Air Force Military Medical University. We are also thankful for Prof. Bing Bai to provide us with professional statistics instruction. This work was funded by National Natural Science Foundation of China [81301804, 81322037, 81772650, 81572302, 81421003, 81627807 and 91542000], Independent Funds of the Key Laboratory [CBSKL2015Z12], National Key Research and Development Plan [2017YFC0908300] and National Science-technology Support Plan [2015BAI13B07].

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

AUTHOR CONTRIBUTIONS

Conception and design: Kaichun Wu, Jie Liang; Acquisition of data: Yujie Zhang, Di Chen, Fang Wang, Xiaowei Li, Xianmin Xue; Analysis and interpretation of the data: Mingzuo Jiang, Bing Xu, Yi Chu and Weijie Wang; Drafting of the article: Di Chen; Critical revision of the article: Shaoqi Yang, Jie Liang; Final approval of the article: Kaichun Wu, Ren Mao, Jun Shen, Jie Liang.

ORCID

Jie Liang https://orcid.org/0000-0002-6792-8941

REFERENCES

1. Ordas I, Eckmann L, Talamini M, Baumgart DC, Sandborn WJ. Ulcerative colitis. *Lancet*. 2012;380:1606-1619.
2. Neurath MF. Cytokines in inflammatory bowel disease. *Nat Rev Immunol*. 2014;14:329-342.
3. Seibold F, Fournier N, Beglinger C, Mottet C, Pittet V, Rogler G. Topical therapy is underused in patients with ulcerative colitis. *J Crohns Colitis*. 2014;8:56-63.
4. Banks C, Forbes A. Topical treatment of ulcerative colitis. Some reflections. *Dig Liver Dis*. 2007;39:338-341.
5. Solberg IC, Lygren I, et al. Clinical course during the first 10 years of ulcerative colitis: results from a population-based inception cohort (IBSEN Study). *Scand J Gastroenterol*. 2009;44:431-440.
6. Rogler G. Medical management of ulcerative colitis. *Dig Dis*. 2009;27:542-549.
7. Lakatos PL, Lakatos L. Ulcerative proctitis: a review of pharmacotherapy and management. *Expert Opin Pharmacother*. 2008;9:741-749.
8. Reddy SI, Friedman S, Telford JJ, Strate L, Ookubo R, Banks PA. Are patients with inflammatory bowel disease receiving optimal care? *Am J Gastroenterol*. 2005;100:1357-1361.
9. Cohen RD, Woseth DM, Thisted RA, Hanauer SB. A meta-analysis and overview of the literature on treatment options for left-sided ulcerative colitis and ulcerative proctitis. *Am J Gastroenterol*. 2000;95:1263-1276.
10. Marshall JK, Irvine EJ. Rectal corticosteroids versus alternative treatments in ulcerative colitis: a meta-analysis. *Gut*. 1997;40:775-781.
11. Manguso F, Balzano A. Meta-analysis: the efficacy of rectal beclomethasone dipropionate vs. 5-aminosalicylic acid in mild to moderate distal ulcerative colitis. *Aliment Pharmacol Ther*. 2007;26:21-29.
12. Lemann M, Galian A, Rutgeerts P, et al. Comparison of budesonide and 5-aminosalicylic acid enemas in active distal ulcerative colitis. *Aliment Pharmacol Ther*. 1995;9:557-562.

13. Bar-Meir S, Fidder HH, Faszczyk M, et al. Budesonide foam vs. hydrocortisone acetate foam in the treatment of active ulcerative proctosigmoiditis. *Dis Colon Rectum*. 2003;46:929-936.
14. Rufle W, Fruhmorgen P, Huber W, Kimmig JM. [Budesonide foam as a new therapeutic principle in distal ulcerative colitis in comparison with mesalazine enema. An open, controlled, randomized and prospective multicenter pilot study]. *Z Gastroenterol*. 2000;38:287-293.
15. Gionchetti P, D'Arienzo A, Rizzello F, et al. Topical treatment of distal active ulcerative colitis with beclomethasone dipropionate or mesalamine: a single-blind randomized controlled trial. *J Clin Gastroenterol*. 2005;39:291-297.
16. Biancone L, Gionchetti P, Blanco GV, et al. Beclomethasone dipropionate versus mesalazine in distal ulcerative colitis: a multicenter, randomized, double-blind study. *Dig Liver Dis*. 2007;39:329-337.
17. Gionchetti P, Dignass A, Danese S, et al. 3rd European Evidence-based Consensus on the Diagnosis and Management of Crohn's Disease 2016: Part 2: surgical management and special situations. *J Crohns Colitis*. 2017;11:135-149.
18. Magro F, GionchettiP, et al. Third European Evidence-Based Consensus on Diagnosis and Management of Ulcerative Colitis. Part 1: Definitions, diagnosis, extra-intestinal manifestations, pregnancy, cancer surveillance, surgery, and ileo-anal pouch disorders. *J Crohns Colitis*. 2017;11:649–670.
19. Crispino P, Pica R, Unim H, et al. Efficacy of mesalazine or beclomethasone dipropionate enema or their combination in patients with distal active ulcerative colitis. *Eur Rev Med Pharmacol Sci*. 2015;19:2830-2837.
20. Frei P, Biedermann L, Manser CN, et al. Topical therapies in inflammatory bowel disease. *Digestion*. 2012;86(Suppl 1):36-44.
21. Hartmann F, Stein J. Clinical trial: controlled, open, randomized multicentre study comparing the effects of treatment on quality of life, safety and efficacy of budesonide or mesalazine enemas in active left-sided ulcerative colitis. *Aliment Pharmacol Ther*. 2010;32:368-376.
22. Souza MM, Aguilar-Nascimento JE, Dock-Nascimento DB. Effects of budesonide and probiotics enemas on the systemic inflammatory response of rats with experimental colitis. *Acta Cir Bras*. 2007;22(Suppl 1):40-45.
23. Gross V, Bar-Meir S, Lavy A, et al. Budesonide foam versus budesonide enema in active ulcerative proctitis and proctosigmoiditis. *Aliment Pharmacol Ther*. 2006;23:303-312.
24. Hammond A, Andus T, et al. Controlled, open, randomized multicenter trial comparing the effects of treatment on quality of life, safety and efficacy of budesonide foam and betamethasone enemas in patients with active distal ulcerative colitis. *Hepatogastroenterology*. 2004;51:1345-1349.
25. Sambuelli A, Boerr L, Negreira S, et al. Budesonide enema in pouchitis—a double-blind, double-dummy, controlled trial. *Aliment Pharmacol Ther*. 2002;16:27-34.
26. D'Arienzo A, Manguso F, Castiglione GN, et al. Beclomethasone dipropionate (3 mg) enemas combined with oral 5-ASA (2.4 g) in the treatment of ulcerative colitis not responsive to oral 5-ASA alone. *Ital J Gastroenterol Hepatol*. 1998;30:254-257.
27. Hanauer SB, Robinson M, Pruitt R, et al. Budesonide enema for the treatment of active, distal ulcerative colitis and proctitis: a dose-ranging study. U.S. Budesonide enema study group. *Gastroenterology*. 1998;115:525-532.
28. Mulder CJ, Fockens P, Meijer JW, van der Heide H, Wiltink EH, Tytgat GN Beclomethasone dipropionate (3 mg) versus 5-aminosalicylic acid (2 g) versus the combination of both (3 mg/2 g) as retention enemas in active ulcerative proctitis. *Eur J Gastroenterol Hepatol*. 1996;8:549-553.
29. Dignass A, Eliakim R, Magro F, et al. Second European evidence-based consensus on the diagnosis and management of ulcerative colitis part 1: definitions and diagnosis. *J Crohns Colitis*. 2012;6:965-990.
30. Williams KL, Fuller CR, Dieleman LA, et al. Enhanced survival and mucosal repair after dextran sodium sulfate-induced colitis in transgenic mice that overexpress growth hormone. *Gastroenterology*. 2001;120:925-937.
31. Rutgeerts P,Sandborn WJ, Feagan BG, et al. Infliximab for induction and maintenance therapy for ulcerative colitis. *N Engl J Med*. 2005;353:2462-2476.
32. D'Haens G, Sandborn WJ, Feagan BG et al. A review of activity indices and efficacy end points for clinical trials of medical therapy in adults with ulcerative colitis. *Gastroenterology*. 2007;132:763-786.
33. Sandborn WJ, Feagan BG, Wolf DC, et al. Ozanimod induction and maintenance treatment for ulcerative colitis. *N Engl J Med*. 2016;374:1754-1762.
34. Zhang JM, An J. Cytokines, inflammation, and pain. *Int Anesthesiol Clin*. 2007;45:27-37.
35. Uwe S. Anti-inflammatory interventions of NF-kappaB signaling: potential applications and risks. *Biochem Pharmacol*. 2008;75:1567-1579.
36. Baker RG, Hayden MS, Ghosh S. NF-kappaB, inflammation, and metabolic disease. *Cell Metab*. 2011;13:11-22.
37. Zhang X, Shen J, Man K, et al. CXCL10 plays a key role as an inflammatory mediator and a non-invasive biomarker of non-alcoholic steatohepatitis. *J Hepatol*. 2014;61:1365-1375.
38. Tak PP, Firestein GS. NF-kappaB: a key role in inflammatory diseases. *J Clin Invest*. 2001;107:7-11.
39. Miura K, Kodama Y, Inokuchi S, et al. Toll-like receptor 9 promotes steatohepatitis by induction of interleukin-1beta in mice. *Gastroenterology*. 2010;139:323-334.

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

How to cite this article: Zhang Y, Chen D, Wang F, et al. Comparison of the efficiency of different enemas on patients with distal ulcerative colitis. *Cell Prolif*. 2019;52:e12559. https://doi.org/10.1111/cpr.12559