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• *BRIEF REPORTS* •

Effects of sulfasalazine on biopsy mucosal pathologies and histological grading of patients with active ulcerative colitis

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Abstract

AIM: To investigate the mechanisms of sulfasalazine (SASP) in the treatment of ulcerative colitis (UC).

METHODS: Changes of pathological signs and histological grading of 106 patients with active UC were observed before and after the treatment with SASP, 1 g, thrice daily for 6 wk.

RESULTS: The effect of SASP on the vasculitis in lamina propria was 48.2% and 17.4% in the mild active UC ($P<0.001$) and 68% and 26.7% in the moderate active UC ($P<0.001$) before and after treatment. Fibroid necrosis of vessel wall was found in one case of mild UC and two cases of moderate UC before treatment and was not found after treatment. No thrombosis was found in mild UC before and after treatment, while thrombosis was found in one case of moderate UC before treatment. The effect on mucosal glandular abnormality was 30.4% and 13.0% in mild UC ($P<0.05$), and 42% and 40% in moderate UC ($P>0.05$) before and after treatment. The rate of eosinophil infiltration was 98.2% and 80.4% in mild UC ($P<0.01$), and 100% and 91.1% in moderate UC ($P<0.05$) before and after treatment. The effect on crypt abscess was 21.4% and 4.4% in mild UC ($P<0.05$), and 48% and 13.3% in moderate UC ($P<0.001$) before and after treatment. The effect on mucosal pathohistological grading was 2.00±0.84 and 0.91±0.46 in mild UC ($P<0.001$), and 2.49±0.84 and 1.31±0.75 in moderate UC ($P<0.001$) before and after treatment.

CONCLUSION: SASP can improve small vessel lesions and crypt abscesses and reduce neutrophilic and eosinophilic leukocyte infiltration in inflammatory mucosa of UC.

Key words: Ulcerative colitis; Biopsy mucosae; Sulfasalazine; Pathology

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INTRODUCTION

Ulcerative colitis (UC) is an inflammatory bowel disease mainly characterized by inflammatory changes in mucosa and submucosa of the rectum and colon. The continuous inflammatory lesions commonly involve rectum and extend to the proximal colon and even to pancolitis. The main clinical manifestations are chronic relapsing diarrhea, mucopurulent hematochezia and abdominal pain.

Though sulfasalazine (SASP) has been used to treat active UC for more than 60 years, its mechanisms have not been completely clarified[1]. The purpose of the present study was to investigate the mechanisms of SASP in treatment of active UC.

MATERIALS AND METHODS

Patients[2]

Following patients were enrolled in the study: patients aged 14-69 years with chronic or relapsing diarrhea, mucopurulent hematochezia, abdominal pain and systemic symptoms, and extraintestinal presentations of their joints, skin, eyes, mouth, liver, and gall bladder, as well as those with inflammatory lesions of rectum and colon. Patients with bacillary or amoebic dysentery, fungous or tuberculous colitis, ischemic bowel disease, radiation colitis, Crohn's disease and large bowel cancer and those who did not complete the course of treatment due to drug allergy or quitted the study were excluded.

Objects of study

A total of 106 patients (50 males and 56 females) with active UC were enrolled in the study[2]. The ratio of male to female was 0.98:1. Their age was 20-66 years (41.6±11.4 years). Fifty-six cases (22 males and 34 females) had mild UC, and their age was 22-66 years (41.5±10.6 years). Fifty cases (28 males and 22 females) had moderate UC, and their age was 20-65 years (42.8±12.1 years). There were no significant

differences in their age, ratio of male to female and clinical types between the two groups ($P>0.05$). Forty-six of fifty-six cases of mild UC and 45 of 50 cases of moderate UC were followed up.

Treatment

The patients received 1 g SASP thrice daily for 6 wk. Endoscopy was carried out before and after treatment. Biopsy specimens were taken at the same sites before and after treatment.

Grouping

Patients were divided into two groups according to the criteria of mild and moderate UC[2].

Endoscopy and mucosal biopsy

The colon and rectum were examined with electronic colonoscope and 3-5 biopsy specimens were taken from the most obvious inflammatory lesions on the left side of colon or rectum. The specimens were fixed in 40 g/L formaldehyde and embedded in paraffin wax, then cut into continuous sections and stained with hematoxylin and eosin. Pathologic morphometry was analyzed by one pathologist with double blind method. The final morphologic results were obtained from the average of three observations under a high power microscope.

Observed pathological items[3-6]

The following pathlogical items were observed: small vessel lesions in lamina propria such as vasculitis, fibroid necrosis of vessel wall, thrombosis and focal hemorrhage; mucosal gland lesions including glandular abnormality, epithelial cell regeneration, atypical hyperplasia, goblet cell depletion (GCD) and Paneth cell metaplasia (PCM); inflammatory cell infiltration in mucosal epithelium such as lymphocyte hyperplasia, lymphoid follicular formation, eosinophils (Eos) and plasmacytes; cellular stromal lesions including granulation tissue formation, fiber tissue hyperplasia and pseudo-polyp; crypt abscess.

Pathohistological grading criteria[7,8]

Grade 0: no neutrophilic leukocyte infiltration in lamina propria; grade I: a small number of neutrophilic leukocytes (<10/HPF) in lamina propria with minimal infiltration of crypts; grade II: prominent neutrophilic leukocytes (10-50/HPF) in lamina propria with infiltration of more than 50% of crypts; grade III: a large number of neutrophilic leukocytes (>50/HPF) in lamina propria with crypt abscesses; grade IV: significant acute inflammation with ulcerations in lamina propria.

Statistical analysis

Results were expressed as mean±SD. The data of two groups were analyzed using independent and paired t test. The counted data were expressed as rate or ratio, and analyzed by χ^2 test. All data were analyzed with SPSS10.0 software.

RESULTS

Effects of SASP on small vessel lesions in lamina propria

No significant changes were found in all specimens before and after SASP treatment ($P>0.05$). The effect of SASP on vasculitis in lamina propria was 48.2% and 17.4% in mild active UC ($P<0.001$), and 68% and 26.7% in moderate active UC ($P<0.001$) before and after treatment. Fibroid necrosis of vessel wall was found in one case of mild UC and two cases of moderate UC before treatment but was not found after treatment. Thrombosis was not found in mild UC before and after treatment, but was found in one case of moderate UC before treatment and not found after treatment (Table 1).

Effect of SASP on mucosal gland lesions

The effect of SASP on mucosal glandular abnormality was 30.4% and 13.0% in mild UC ($P<0.05$), and 42% and 40% in moderate UC ($P>0.05$) before and after treatment. No significant effect of SASP on epithelial cell regeneration, atypical hyperplasia, GCD, and PCM was observed before and after treatment ($P>0.05$, Table 2).

Effects of SASP on inflammatory cell infiltration

The rate of Eos infiltration was 98.2% and 80.4% in mild UC ($P<0.01$), and 100% and 91.1% in moderate UC ($P<0.05$)

Table 1 Effect of SASP on small vessel lesions in lamina propria (*n*%)

	n	Focal hemorrhage	Vessel inflammation	Fibroid necrosis	Thrombosis
Before treatment (mild)	56	41 (73.2)	27 (48.2)	1 (1.8)	0
After treatment (mild)	46	25 (54.3)	8 (17.4)	0	0
Before treatment (moderate)	50	32 (64.0)	34 (68.0)	2 (4.0)	1 (2.0)
After treatment (moderate)	45	22 (48.9)	12 (26.7)	0	0

Table 2 Effect of SASP on mucosal gland lesions (*n*%)

	n	Glandular abnormality	Epithelial cell regeneration	Atypical hyperplasia	GCD	PCM
Before treatment (mild)	56	17 (30.4)	19 (34.0)	8 (14.3)	3 (5.4)	0 (0)
After treatment (mild)	46	6 (13.0)	13 (28.3)	2 (4.4)	2 (4.4)	1 (2.2)
Before treatment (moderate)	50	21 (42.0)	15 (30.0)	15 (30.0)	12 (24)	2 (4.0)
After treatment (moderate)	45	18 (40.0)	7 (15.6)	8 (17.8)	6 (13.3)	0 (0)

Table 3 Effects of SASP on inflammatory cell infiltration (*n*%)

	n	Lymphocyte hyperplasia	Lymphoid follicle	Eos infiltration	Plasmacyte infiltration
Before treatment (mild)	56	42 (75.0)	44 (78.6)	55 (98.2)	50 (89.3)
After treatment (mild)	46	30 (65.2)	32 (69.6)	37 (80.4)	37 (80.4)
Before treatment (moderate)	50	37 (74.0)	35 (70.0)	50 (100)	46 (92)
After treatment (moderate)	45	32 (71.1)	26 (57.8)	41 (91.1)	39 (86.7)

before and after treatment. No significant effects of SASP on lymphocyte hyperplasia, lymphoid follicular formation and plasmacyte infiltration were observed before and after treatment ($P>0.05$, Table 3).

Effect of SASP on cellular stromal lesions

No significant effect of SASP on granulation tissue formation, fiber tissue hyperplasia and pseudo-polyp was found before and after treatment ($P>0.05$).

Effect of SASP on crypt abscess

The effect of SASP on crypt abscess was 21.4% and 4.4% in mild UC ($P<0.05$), and 48% and 13.3% in moderate UC ($P<0.001$) before and after treatment.

Effect of SASP on mucosal pathohistological grading

The effect of SASP on mucosal pathohistological grading was 2.00±0.84 and 0.91±0.46 in mild UC ($P<0.001$), and 2.49±0.84 and 1.31±0.75 in moderate UC ($P<0.001$) before and after treatment (Table 4).

Table 4 Effect of SASP on mucosal pathohistological grade (mean±SD)

	n	Mild	*n*	Moderate
Before treatment	46	2.00±0.84	45	2.49±0.84
After treatment	46	0.91±0.46	45	1.31±0.75

DISCUSSION

The common drugs used in treatment of patients with active UC are still SASP and glucocorticoids. Sangfelt *et al*.[9], used prednisolone enema to treat active UC and showed that the release quantity of neutrophil myeloperoxidase (MPO), eosinophil cationic protein (ECP) and eosinophil peroxidase (EPO) in enema liquid is correlated with their clinical, endoscopic and histological activities before treatment. When the patients respond to the treatment, their MPO markedly decreases, but ECP and EPO do not decrease. The results suggest that glucocorticoids can reduce mucosal neutrophil infiltration, but cannot improve Eos infiltration. The increase of MPO is correlated with neutrophil-activated peptide-interleukin 8 and tumor necrosis factor-α[10].

The mechanism of SASP against active UC is that it depresses the production of leukotrienes (LTs), prostaglandin and free radicals[1,11]. SASP plays a role in the treatment of active UC by interfering with synthesis of inflammatory media[12]. SASP can depress immune responses of immune cells and prevent relapse of UC. But the mechanisms have not been completely clarified[1]. Wright *et al*.[13], used 20 mg/100 mL sucralfate once or twice daily to treat distal active UC for 4 wk and showed that it can improve the clinical, endoscopic and histological scores.

The present study using oral SASP to treat active UC for 6 wk showed that it could improve small vessel inflammation and crypt abscess in lamina propria of inflammatory mucosa, decrease neutrophil and Eos infiltration in mucosal epithelium, and decline the histological grading after treatment. There was no fibroid necrosis of vessel wall and thrombosis in all specimens after treatment. Significant effect of SASP on mucosal glandular abnormality, lymphocyte hyperplasia and lymphoid follicular formation, plasmacytes infiltration and cellular stromal lesions was not observed.

The insufficient mucosal blood supply and submucosal vessel lesions are also the pathogenic factors for active UC. SASP could improve mucosal small vessel lesions, crypt abscess, mucopurulent hematochezia and mucosal inflammation in patients with active UC, thus improving and promoting the healing of inflammatory mucosa.

One cause of inflammatory lesions include all kinds of hydrolase and cationic protein released from neutrophil granules of damaged local tissues, which play an important role in the course of inflammation of type III hypersensitivity[14]. The activated neutrophils produce active oxygen and several active molecules that promote occurrence and development of inflammation[15]. SASP could decrease neutrophil infiltration and histological grading, thus decreasing the production of inflammatory factors and improving inflammation.

In this study, SASP obviously decreased mucosal Eos infiltration. Mucosal Eos infiltration is associated with chronic intestine inflammation[16]. Eos collected from the inflammatory mucosa is attracted by immune complex and Eos chemokine releases from mast cells. Eos could synthesize prostaglandins E and E_2, which restrain the synthesis of histamine, neutralize hypersusceptibility and inflammatory reactants. Eos accompanying degranulation phenomena result from allergic reaction by main degranulation way[17]. The number of Eos not only reflects the degree of inflammation[18], but also is an important index of prognosis[19]. There are more acidophil granules in Eos's cytoplasm, containing four toxic cationic proteins: alkalescent protein, ECP, neurotoxin and peroxidase, all of which have toxic effect on normal cells[14]. Raab *et al*.[20], also confirmed that there are more Eos infiltrating lamina propria of active UC, and ECP obviously increases. The activation or degranulation of Eos is correlated with mucosal inflammatory responses. The changes in intestinal mucosa resulted from Eos infiltration in the inflammatory mucosa that are activated and cause mucosal lesions during degranulation and release

of ECP[21].

In conclusion, SASP can effectively treat patients with active UC and improve small vessel lesions, crypt abscesses, and decrease neutrophil and Eos infiltration.

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