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Original Article

Efficacy of single vs multiple doses of 5-aminosalicylic acid (5-ASA) in the treatment of mild-moderate ulcerative colitis: an open randomized clinical trial

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Abstract: This trial aimed to investigate the difference between once-daily dosing and three times-daily dosing of 5-ASA. In this study, patients (n = 210) with active UC were recruited, and randomly assigned to two groups, 3 g OD (n = 96) and 1 g TID (n = 114), in which, 3 g 5-ASA was administrated once daily at morning and 1.0 g 5-ASA three times daily for 6 weeks. Visits were scheduled at week 0, 2, 4, and 6 (final visit), and vital signs, hematology, biochemistry and urinalysis tests and clinical signs were recorded/performed at each visit. Endoscopy and histology were compared between baseline and the final visit. Simultaneously, colonic biopsies were obtained for evaluation of inflammatory cytokines by qRT-PCR. Once-daily dosing regimen was efficacious for the induction of clinical remission and endoscopic remission in mild-moderate UC as well as three times-daily treatment. After a 6-week treatment, histological grading was markedly improved in both 3 g OD group and 1 g TID group. In addition, proinflammatory cytokines and some routine biochemical parameters (CRP and ESR) were markedly decreased in both groups after treatment with 5-ASA for 6 weeks. Only 11.8% and 13.7% of the patients in each group were reported adverse events. Among these results, there was no significant difference between the two groups. In conclusion, a 3.0 g once-daily dose of 5-ASA is efficacious to induce the clinical and endoscopic remission compared with 1.0 g TID in active UC patients, and once-daily dose of 5-ASA is well-tolerated and safe.

Keywords: 5-aminosalicylic acid (5-ASA), ulcerative colitis, colon, inflammatory bowel disease (IBD), clinical trial

Introduction

Ulcerative colitis (UC) is a chronic inflammatory bowel disease (IBD) that causes inflammation and ulcers in the colon, characterized by bloody diarrhea, tenesmus, and abdominal cramps [1]. Inflammation can be detected in the rectum alone or extending continuously upwards into any part of the colon, and as so-called backwash ileitis even into the terminal ileum [2]. According to the Montreal classification of extent of UC [3], there are three types of UC - proctitis (E1), left sided UC (E2, distal UC) and extensive UC (E3, pancolitis), which is limited to the rectum, limited to a proportion of the colorectum distal to splenic flexure and extends proximal to the splenic flexure, respectively.

Hospital-based studies demonstrated that the incidence and prevalence were 1.0-2.0 and 11.4 per 100,000 person-years in China, which they are both much smaller than those (7.6-14.3, 145-238) in Western countries [4-9]. According to the more recent population-based studies, the incidences of UC in Wuhan and Zhongshan cities in China are 1.45 and 2.05, respectively, and they are also lower than in Western countries [10, 11]. Although the incidence and prevalence of UC in China at present are relatively low compared with Western countries, the incidence of IBD is dramatically increasing in China as well as other Asian countries [12].

5-ASA is usually a clinically first-line agent used for mild-moderate active UC, because it is able to induce the remission of active mild-moderate UC, maintain the remission in patients, and prevent UC relapse and colorectal cancer (CRC) [13]. Compared with salicylazosulfapyridine

(SASP), another drug commonly used in inflammatory bowel disease, 5-ASA is better tolerated and has less toxicity in renal/hepatic impairment [14]. Additionally, the therapeutic action of 5-ASA is multifactorial. It has strong anti-inflammatory effect and significant influence on synthesis of inflammatory lipid mediators, and chemo-preventive function on CRC as well [15].

Pharmacokinetic studies showed that 5-ASA moiety is generally not absorbed by the large intestine, but mainly absorbed in the small bowel (80%), and the small amount that is absorbed in the colon is acetylated by N-acetyltransferase-1 and excreted in the urine [16]. In the metabolic pathway of 5-ASA after oral administration, variable intraluminal pH in different parts of gastrointestinal (GI) tract directly affects 5-ASA absorption [17, 18]. Efficacy of 5-ASA depends on achieving a high concentration at the inflamed mucosal areas, clinical response correlates more highly with tissue than with plasma concentrations, and concentrations of 5-ASA and its metabolites in the urine and faeces do not correlate well with those in specific GI tissue regions, so the extremely low level of 5-ASA in the colon brings a challenge for 5-ASA use in clinic. Therefore, special forms of 5-ASA help delivery of 5-ASA moiety to the diseased areas, and suppository or enema is useful in UC confined to the rectum (proctitis). Although currently available 5-ASA preparations have been shown to maintain remission, many patients are poorly compliant. As compliance is such a major factor in disease control, it is important to understand what drives non-adherence and what patients want from their medication. Patients' non-adherence with conventional multi-dose (2 or 3 times daily) treatment regimens may result in reduced efficacy, poor long-term prognosis, and increased costs of care, and 3 times per day (TID) dosing has been found to be the most significant risk factor of partial non-compliance [19, 20].

To optimize adherence, one administration per day would be an advantage compared to two or three applications every day, and alternate dose or route of administration may be necessary to achieve adequate 5-ASA amounts in the colon during acute exacerbations of disease. Therefore, it is necessary to investigate whether there are significant differences between once-daily dosing and three times-daily dosing.

Methods

Patients recruitment

From October, 2013 to August, 2015, 210 patients with newly diagnosed active UC (> 15 cm of rectum to entire colon; or relapsed < 6 weeks (wks), confirmed by endoscopy and histology) were recruited in our study, including 134 patients from Shanghai Tenth Peoples' Hospital (in the charge of Dr. Zhanju Liu) and 76 patients from The First Affiliated Hospital of Kunming Medical University (in the charge of Dr. Yinglei Miao). The active UC with an endoscopic score ≥ 1, a Physician's Global Assessment (PGA) score ≤ 2 and compatible histology was considered as a mild-moderately active UC. The patients aged from 18 to 70 years old, and were diagnosed with negative stool culture (bacteria, parasite, virus, fungi) and negative pregnancy for female ones. This research was approved by the Review Board and Ethics Committees of each affiliation. All patients signed and provided the written informed consent.

Exclusion criteria

The following situations were excluded:

a. Severe UC (Sutherland scores 11-12; PGA > 2; current relapse lasting > 6 wks; current while on maintenance therapy with > 2 g/d of 5-ASA); b. Crohn's disease, indeterminant colitis, proctitis (≤ 15 cm), or other immune disease-associated colitis; Bleeding diseases, active peptic ulcers, asthma, pregnancy; c. Prior bowel resection leading to diarrhea, and/or pouch formation, toxic megacolon, hemorrhagic diathesis, present or past CRC, or serious other secondary diseases; d. Those who used steroids within 4 wks; e. Those who used antibiotics within the previous 1 wk; f. Patients who used immunosuppressants or anti-TNF within 6 wks; g. Those who had abnormal liver/kidney function tests, namely ALT or AKP ≥ 2X and serum creatine > 1.5 mg/dl; h. Patients who used other drugs (e.g., herbal medicine); i. Violators.

Table 1. Primers used for qRT-PCR

Target gene	Forward (5'-3')	Reverse (5'-3')
IFN-γ	AGCGGATAATGGAACTCTTTTCTTAG	AAGTTTGAAGTAAAAGAAGACAATTTGG
TNF-α	TCTCGAACCCCGAGTGACA	GGCCCGGCGGTTCA
GAPDH	TGCACCACCAACTGCTTAG	GATGCAGGGATGATGTTC
IL-10	GCCTGGTCCTCCTGACTGGG	GCAGGTTGCCTGGGAAGTGG
IL-17A	AATCTCCACCGCAATGAGGA	ACGTTCCCATCAGCGTTGA
IL 21	GAGTGGTCAGCTTTTTCCTGTT	AGGAATTCTTTGGGTGGTTTTT
IL-23P19	GGACAACAGTCAGTTCTGCTTGC	GGAGGCTGCGAAGGATTTTG
IL-25	CCAGGTGGTTGCATTCTTGG	TGGCTGTAGGTGTGGGTTCC
RORC	AGAAGACCCACACCTCACAAA	AACTTGACAGCATCTCGGGA

Protocols for medications

The patients were randomly divided into two groups, 3 g OD (once daily) and 1 g TID (three times daily). 5-ASA tablets (Salofalk, 500 mg tablet) manufactured by Dr. Falk Pharma (Freiburg, Germany) was administrated once daily at morning or three times daily (TID: morning, noon, and bedtime) for 6 wks. Visits were scheduled at week 0, 2, 4, and 6 (final visit). Vital signs, results of laboratory tests (hematology, biochemistry and urinalysis) and clinical signs were recorded at each visit. Endoscopy and histology were compared between baseline and the final visit (wk 6). Colonic biopsies were performed at baseline and final visits for evaluation of inflammatory cytokines by qRT-PCR. Patients with treatment failure were withdrawn and assigned an appropriate alternative therapy at each center. Rescue medication was not permitted. Compliance was calculated by determining the amount of unused drugs. All patients underwent a physical examination, and the demographics and medical history were recorded. Vital signs and routine laboratory parameters were assessed at each visit. Efficacy was assessed by the research team at baseline and the final visit using the following scores/scales: disease activity index for UC (UC-DAI) (Profs. Liu and Miao), endoscopic scores (Profs. Liu and Miao) and histological scores assessed from biopsies (Prof. Wei).

qRT-PCR

Total RNA of colonic tissues (3 g OD, n = 60; 1 g TID, n = 62) was extracted using Trizol Reagent kits (Thermo Fisher Scientific Inc., USA). The concentration of extracted RNA was determined using an ultraviolet spectrophotometer and 500 ng of total RNA was used to reverse transcribed. The transcript-specific primers used were listed in **Table 1**. The reactions were performed using the SYBR PrimeScript RT-PCR kit (Takara Bio, Inc.) with an ABI 7900 Sequence Detection System (Applied Biosystems). Each experiment was carried out with three biological replicates. As an internal control, levels of GAPDH were quantified in parallel with the target genes. The cycle threshold (Ct) values were normalized to the expression levels of GAPDH. Normalization and fold changes were calculated using the ΔΔCt method.

UC-DAI

UC-DAI was recorded according to the reference [21], total disease activity scores (sum of the item score) were categorized as ≤ 2: remission; 3-5: mild; 6-10: moderately active; and 11-12: severe.

Clinical and endoscopic scores

Clinical remission: ≤ 2, no individual subscore > 1, sum of stool frequent, rectal bleeding, mucosal appearance and physician's rating.

Endoscopic remission: using the Mayo endoscopic sub-score, 0/1 as commonly accepted criterion for remission and mucosal healing; and score decrease by 1 point as response [21].

Histological scores (the Riley scoring system)

The Riley scoring system was used to evaluate histological scores by a specialized GI pathologist (Dr. Qing Wei), who was blinded to the clinical and endoscopic scores. Histological remission was considered as no significant inflammation, which refers to architecture changes in the absence of erosions, crypt abscesses or PMN infiltration [22]. The detailed Riley scoring system was described in the references [22, 23].

Statistical analysis

Descriptive data are reported as percentages and medians and ranges. The paired t test was used to compare average DAI scores at different time points. All analyses used two-sided

Table 2. Demographics and patients' baseline characteristics

Variable	3 g OD (n = 96)	1 g TID (n = 114)	*P* values
Age at diagnosis, yrs	39.8 ± 10.4	40.3 ± 11.6	> 0.05
Gender (Male/Female)	50/46	63/51	> 0.05
Mean time since diagnosis, yrs	3.1 (0.3-8.6)	3.5 (0.2-7.6)	> 0.05
Diagnosis (new/established)	38/58	51/63	> 0.05
Smoking status (n)	18	25	> 0.05
Median duration of present acute episode (days)	15.6 (2-102)	14.2 (2-126)	> 0.05
Mean number of previous episodes/relapses	3.1 (n = 58)	3.6 (n = 63)	> 0.05
Location			
E1, n (%)	44 (45.8)	43 (37.7)	> 0.05
E2, n (%)	36 (37.5)	49 (43.0)	
E3, n (%)	16 (16.7)	22 (19.3)	
Disease activity (mild/moderate), n	52/44	59/55	> 0.05
Pre-study medication, n (%)	62 (64.6)	71 (62.2)	> 0.05
SASP	12 (12.5)	10 (8.8)	
5-ASA	30 (31.2)	40 (35.1)	
5-ASA enema	14 (14.6)	13 (11.4)	
5-ASA suppository	10 (10.4)	14 (12.3)	
Steroids	32 (33.3)	35 (30.7)	
Immunosuppressant	8 (8.3)	12 (10.5)	

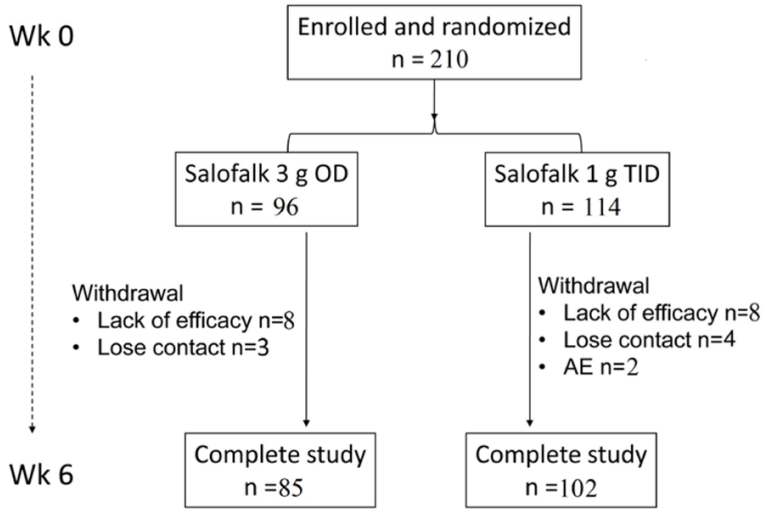

Figure 1. Outcome of all patients enrolled in the trial.

tests of statistical significance with a significance level of 0.05. Statistical analyses were performed using Data Processing System software (DPS, Zhejiang University, China).

Results

Demographics and patients' baseline characteristics

As shown in **Table 2**, the age at diagnosis, gender ratio, mean time since diagnosis, new/established diagnosis ratio, smoking status, median duration of present acute episode, median number of previous episodes/relapses, location, disease activity (mild/moderate), pre-study medication of the patients showed non-significant ($P > 0.05$) differences between the two groups. Additionally, 25 patients were withdrawn during the course of this trial because of lack of efficacy (3 g OD, n = 8; 1 g TID, n = 8), losing contact (3 g OD, n = 3; 1 g TID, n = 4) and AE (1 g TID, n = 2) (**Figure 1**). Despite of these lost information, these results could still suggest a solid foundation for the following research in this study.

Once daily 5-ASA efficaciously induced clinical remission and endoscopic remission in mild-moderate UC

In the treatment of mild-moderate UC, once-daily 5-ASA was demonstrated to be efficacious for the induction of clinical remission and endoscopic remission as good as 1 g TID, and there

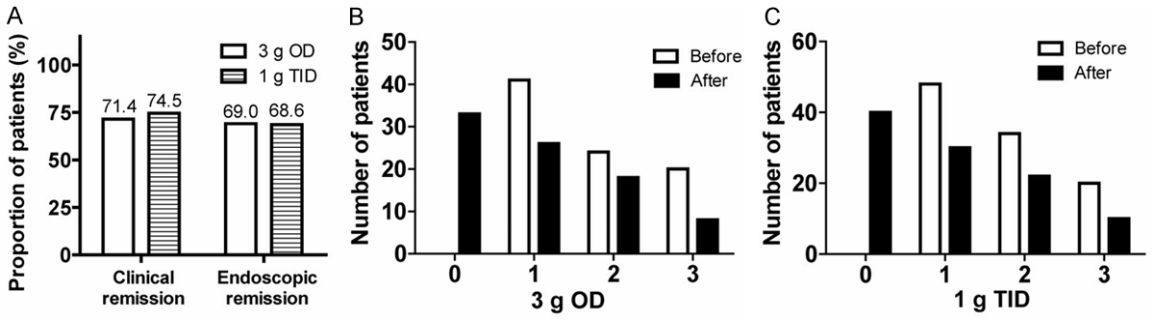

Figure 2. A. 3 g OD and 1 g TID of 5-ASA equally induced the clinical and endoscopic remission in UC. B, C. Endoscopic grading at 1-3, was markedly decreased after 6 weeks of treatment in both 3 g OD and 1 g TID groups.

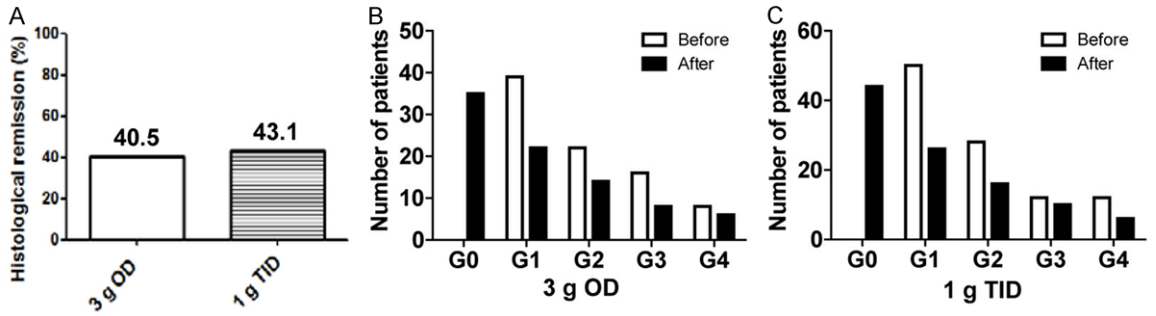

Figure 3. A. 3 g OD and 1 g TID 5-ASA treatments showed approximate histological remission after 6-week treatment. B, C. Based on the Riley scoring system, both groups displayed markedly healing effect.

was no marked difference in the number of the patients who had clinical remission or endoscopic remission between the two groups (**Figure 2A**).

The results of endoscopic grading also demonstrated that, after different treatments with 5-ASA for 6 weeks, the numbers of the patients who were at 1-3 grades were remarkably decreased in both 3 g OD and 1 g TID groups (**Figure 2B**, **2C**).

Histological grading was markedly improved after 3 g OD 5-ASA treatment

After 6-week therapy, remarkable histological remission was found in both of the two groups, in which the number of the patients who were detected to have histological remission in 3 g OD and 1 g TID groups were 40.5% and 43.1%, respectively (**Figure 3A**). Using the Riley scoring system, we found the number of patients at grading G0 was markedly improved after 6-week treatment in both groups.

Proinflammatory cytokines, C reactive protein (CRP) and erythrocyte sedimentation rate (ESR) were significantly decreased after treatment in both groups

It has been reported by many researchers that proinflammatory cytokine production correlates well with activity of ulcerative colitis [24-26]. In our present study, we found significantly higher levels of proinflammatory cytokine mRNAs in mild-moderate UC patients' colon compared with healthy colon. After continuous treatment with 5-ASA (3 g OD, or 1 g TID) for 6 wks, most of the mRNA levels of the detected proinflammatory cytokines and RORC (retinoic acid receptor-related orphan receptor), which is a member of the nuclear receptor family of transcription factors and closely associated with Th17 cell-mediated inflammation [27], were significantly ($P < 0.05$) decreased, including IFN-γ, TNF, IL-17A, IL-21 and IL-23p19 (**Table 3**).

We also detected routine biochemical parameters before and after the treatment, and the

Table 3. Proinflammatory cytokines in colon before and after treatment

	3 g OD (n = 60)		1 g TID (n = 62)	
	wk 0	wk 6	wk 0	wk 6
IFN-γ	112.1 ± 31.5	41.5 ± 21.1*	115.2 ± 29.1	45.2 ± 19.3*
TNF	59.2 ± 14.2	18.3 ± 7.8*	57.9 ± 21.5	17.7 ± 6.9*
IL-17A	32.6 ± 10.7	12.5 ± 5.1*	35.2 ± 9.1	15.4 ± 6.3*
IL-21	20.4 ± 6.2	8.4 ± 4.2*	19.3 ± 8.4	6.5 ± 3.1*
IL-23p19	24.7 ± 8.2	11.6 ± 5.8*	22.9 ± 6.8	9.5 ± 4.6*
IL-10	10.9 ± 3.5	14.5 ± 4.5	11.2 ± 4.4	15.2 ± 5.1
IL-25	8.7 ± 3.2	15.5 ± 5.4	9.6 ± 2.5	14.5 ± 4.7
RORC	25.6 ± 8.4	9.6 ± 3.4*	24.1 ± 7.5	8.8 ± 3.6*

NOTE: Colonic biopsies from the Shanghai Tenth People's Hospital were used in mRNA detection using qRT-PCR. *$P < 0.05$ indicated a statistical difference compared with the status at wk 0.

Table 4. Routine biochemical parameters before and after treatment

	3 g OD (n = 85)		1 g TID (n = 102)	
	wk 0	wk 6	wk 0	wk 6
CRP (mg/dl)	25.6 ± 5.9	7.1 ± 2.1*	28.4 ± 6.3	6.5 ± 2.5*
ESR (mm/h)	26.6 ± 8.9	15.9 ± 5.4*	26.4 ± 7.4	16.8 ± 6.7*
Hb (g/l)	89.6 ± 12.5	95.2 ± 13.4	87.1 ± 14.8	94.7 ± 15.1
ALT (U/ml)	24.5 ± 9.2	25.8 ± 7.4	28.1 ± 8.7	24.1 ± 6.9
AST (U/ml)	22.8 ± 6.7	23.2 ± 6.5	21.2 ± 5.3	20.7 ± 6.8
AKP (U/ml)	71.4 ± 13.8	69.0 ± 14.4	67.8 ± 13.6	62.1 ± 11.2
GGT (U/ml)	36.5 ± 12.5	31.5 ± 10.2	32.3 ± 11.1	32.1 ± 9.3
BUN (mmol/dl)	4.18 ± 1.68	3.87 ± 1.38	3.86 ±1.72	3.34 ± 1.49
Cr (μmol/ml)	59.7 ± 14.1	56.8 ± 12.4	53.8 ± 11.6	54.4 ± 12.5

NOTE: *$P < 0.05$ indicated a statistical difference, compared with the status at wk 0.

Table 5. Treatment-related adverse events

Variable	3 g OD (n = 85)	1 g TID (n = 102)
Any AE	10 (11.8%)	14 (13.7%)
AE leading to withdrawal	0	0
SAE, n (%)	0	0
Gastrointestinal disorders		
Vomiting	1	2
Nausea	2	2
Abdominal pain	2	1
Diarrhea	1	2
Headache	2	2
Skin allergy	1	1
Abnormal liver function	0	0
Abnormal kidney function	0	0

results showed a significant ($P < 0.05$) decrease in CRP and TNF after 6 weeks of treatment with 5-ASA no matter 3 g once daily or 1 g three times daily (**Table 4**).

Treatment-related adverse events (AE)

During the study, no patient reported serious adverse events (SAE) and there was no AE leading to withdrawal. Totally, there were 10 (11.8%) patients in 3 g OD group and 14 (13.7%) in 1 g TID group reported AE. No patient developed abnormal liver function or abnormal kidney function during the course of the study. Other detailed information about the AE that patients reported was shown in **Table 5**.

Discussion

Both a high 5-ASA concentration at the inflamed mucosal areas and patients' compliance are important in disease control. In the presence of available 5-ASA preparations that can effectively maintain remission, compliance seems particularly important for the treatment for UC. The reasons for poor compliance or non-adherence probably mainly include forgetting to take medication, too many pills, dosing required too many times each day, medication too inconvenient, no symptoms present, lack of insight into illness, perceptions and beliefs about the illness, poor physician-patient relationship, insurance/costs/income and education [13, 28]. The patients expect high efficacy, low toxicity, convenient (easy to take), simple dosing regimens (fewer tablets), less often and insurance cover (cheap) for the administration of the drugs in the whole course of the treatment [19, 28]. Presently, the major problem for UC treatment is compliance/adherence, and improving patient adherence may be of great importance for clinical therapy. In our present study, we demonstrated that 3 g OD treatment could effectively induce and maintain clinical remission and endoscopic remission, markedly improve histological grading, and significantly decrease proinflammatory cytokines, CRP and ESR in mild-moderate UC as well as 1 g TID

treatment. We hope these results are expected to provide a solid foundation for the patients to take medicine, and doctors to prescribe in a simple way.

Simulation of colonic levels of 5-ASA seems to be necessary in the treating mild-moderate UC. It was reported that, after 7 days of treatment, the amount of colonic 5-ASA in two groups were equal, where one of them was given ASA 800 mg, tid, and the other was given 2400 mg, once daily [29-31]. Combined with the similar phenomenon found in our present study from different angles, it is concluded that alternate dose or route of administration are necessary to achieve adequate 5-ASA amounts in the colon during the therapy of mild-moderate UC, and single dose of 5-ASA is efficacious for the induction of clinical and endoscopic remission and is able to improve endoscopic grading [32]. Therefore, 5-ASA can be administered as a single daily dose, OD dosing is convenient, because of which, it may be a better choice for the treatment of mild-moderate UC.

There are several high-strength formulations of 5-ASA that have been used, including MMX mesalamine and mesalazine granules (Salofalk). MMX mesalamine (SPD476 marketed as Lialda® [US] and Mezavant® [EU]; Shire Pharmaceuticals Inc., Wayne, PA) is a high-strength formulation of 5-ASA (1.2 g per tablet), which uses MMX Multi Matrix System technology designed to release 5-ASA throughout the colon. Two double-blind, placebo-controlled studies have been performed to evaluate the efficacy and tolerability of MMX mesalazine [32, 33]. This delivery system uses lipophilic and hydrophilic matrices enclosed within a gastro-resistant, pH dependent coating to facilitate prolonged exposure of the colonic mucosa to 5-ASA. It has been proved to be a qualified drug use in alternate route of 5-ASA administration [32, 33]. Likewise, Salofalk granules, a multiparticulate formulation of mesalazine, prolong the release of the active ingredient throughout the entire colon because of their enteric and acid-resistant film coating [30, 34]. Jointly, our present study and others' reports showed that Salofalk was a useful drug that can be taken once daily for the patients, and prescribed for doctors as well [30, 35].

Despite of the aforesaid positive results, there were still some limitations in our study. The number of patients is small and it needs more recruitment, analysis of mucosal and serum concentrations of 5-ASA and Ac-5-ASA may be needed, and other parameters may be necessary (e.g., epithelial cell permeability, mucosal barrier) as well. Besides, genetic variants (N-acetyltransfease polymorphism) should be considered. In short, these aspects are expected in the design of the future researches.

Disclosure of conflict of interest

None.

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