

Treatment of corticosteroid-resistant ulcerative colitis with oral low molecular weight heparin

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INTRODUCTION

The etiology and pathogenesis of ulcerative colitis (UC) have remained unclear. Treatment is nonspecific based on the anti-inflammatory agents corticosteroid and sulfasalazine. A significant proportion fail to respond to this therapy^[1]. As the relapse, refractory or serious UC patients had a hypercoagulable state and an increased incidence of thromboembolic events^[2-4], heparin has been used by some authors^[5-7]. Yet, its half-life period is short, needing long-term injection, which restricts its further clinical application. Our previous studies have demonstrated oral LMWH not only overcomes the shortcomings of common heparin^[8,9], but also has anti-inflammatory effects^[10,11]. The aim of this paper is to study the therapeutic effects and mechanism of oral LMWH in patients with corticosteroid-resistant UC.

MATERIALS AND METHODS

Clinical materials

There were eight men and twelve women aged 21 years to 56 years (mean 33 years). All cases were histologically confirmed and met the diagnostic standard of chronic non-infectious intestinal disease of China (Taiyuan meeting, 1993), including seventeen cases of severe, and three moderate UC. Duration of diseases ranged from 8 months to 11 years (mean 4.1 years). Rectal bleeding, diarrhea, mucus stool, abdominal pain were the main symptoms. Four patients were associated with thromboembolic diseases. All patients were treated

with high-dose corticosteroid and sulfasalazine for more than 4 weeks without effect, sulfasalazine was maintained in combination with oral LMWH (366U/kg, twice daily) for more than 4 weeks. Prednisolone was tapered and stopped.

Monitoring parameters

Assessment of platelet activation and aggregability^[2,4]

We used a sensitive flow cytometric technique designed to minimize sample handling and render fixation unnecessary to quantify platelet activation. Blood samples were incubated by 10 minutes of venesection with fluorescein isothiocyanate (FITC) conjugated antibodies to the platelet surface antigens, P-selectin (CD_{62P}) and CD₆₃ (Immunotech, Marseilles, France). Analysis was made within 15 minutes of venesection using a BD (Becton Dickinson Immunocytometry Systems) FAC Scan. TXA-2 (Suzhou Medical College) was measured using RIA method, samples were taken without tourniquet into chilled tubes containing 1:9 anticoagulant/antiaggregant solution (trisodium citrate 3.8%), centrifuged for 15min-30min, later at 4 °C for 30 minutes to minimize *in vitro* activation, supernatant was decanted off and stored at -20 °C for assay within 3 months. Platelet aggregation rates (PAR) and thrombosis length (TL) *in vitro* were assessed by XSN-R II instrument according to the manufacturer's instruction.

Measurement of CD₅₄. CD₅₄ in blood and tissues were measured using flow cytometric technique according to our previous report^[12].

Assessment of efficacy^[7]

Pre- and post-treatment scores were calculated for the following disease parameters: ① Stool frequency (average number per day for the past week). ② Rectal bleeding (0: absent, 1: streak of blood on stools occasionally, 2: obvious blood on stool frequently, 3: complete bloody stools). ③ Colonoscopic appearance 0: normal vascular pattern, 1: mild lesion (loss of vascular pattern, mucosa edema, no bleeding), 2: moderate lesion (granularity and friability of the mucosa), 3: severe lesion (discrete ulceration and spontaneous bleeding). ④ Histological grading: serial biopsies of the rectum and the colon were taken. Five histological changes seen in UC (cellular infiltrate in the lamina propria, cryptitis, crypt abscess

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formation, goblet cell depletion, and regenerative hyperplasia of the epithelium) each were scored from 0 (absent) to 3 (severe), a total UC score of 5 or less indicated mild disease, a score of 5-10, moderate, and a score of 10-15 severe disease. ⑤ General health status (0: excellent, 1: good, 2: poor, 3: poorer, 4: very poor, 5: poorest).

Statistical analysis

Student's *t* test and Friedman test were used to assess the significance of differences between mean pre- and post-treatment parameters.

RESULTS

Therapeutic effects

Nineteen patients (95.0%) achieved clinical remission (normal stool frequency and no rectal bleeding) on a combination of oral LMWH and sulfasalazine. One patient had reduced rectal bleeding only. The average period of marked improvement was 2.9 weeks (range 1 week-4 weeks), and of remission was 5 weeks (range 1 week-12 weeks). Rectal bleeding ceased in 19 patients (5 patients within 5 days -8 days, the others within 2 weeks-7 weeks). Nineteen patients had general health condition improved earlier on oral LMWH, than bowel symptoms. There were highly significant improvement in mean scores for all disease parameters (Table 1).

Table 1 Therapeutic effects of oral LMWH in corticosteroid-resistant UC patients

Group	Stool frequency (times/day)	Rectal bleeding (score)	Colonoscopy (score)	Histology (score)	Well-being (score)
Pre-treatment	8.6	2.6	2.7	12.0	4.0
Post-treatment	1.5 ^b	0.2 ^b	1.0 ^b	4.0 ^b	0.6 ^b

^b*P*<0.01 vs pre-treatment.

Blood contents of CD_{62P}, CD₆₃, TXA₂, platelet aggregation rate (PAR) and thrombosis length (TL) *in vitro*

All the indexes in corticosteroid-resistant UC patients increased significantly as compared with the normal controls (*P*<0.01). After treatment with oral LMWH, all the parameters of UC patients decreased (*P*<0.01), but CD_{62P} and CD₆₃ remained higher than normal (*P*<0.01), (Table 2).

Table 2 Effects of oral LMWH on CD_{62P} and CD₆₃, TXA₂, platelet aggregation rate (PAR) and thrombosis length (TL) *in vitro* in UC patients ($\bar{x}\pm s$)

Group	CD _{62P} (%)	CD ₆₃ (%)	TXA ₂ (ng/L)	PAR(%)	TL(cm)
UC patients					
Pre-treatment	8.1±3.2 ^b	6.2±2.2 ^b	541.7±82.4 ^b	44.5±10.1 ^b	2.4±0.5 ^b
Post-treatment	4.2±1.9 ^{a,d}	3.1±1.7 ^{ab}	396.4±75.8 ^d	35.2±8.7 ^d	1.9±0.4 ^d
Normal controls	1.9±0.4	1.6±0.8	340.2±40.4	34.1±9.1	

^a*P*<0.05, ^b*P*<0.01 vs normal person; ^d*P*<0.01 vs pretreatment.

CD₅₄ in blood and tissues

CD₅₄ elevated in both blood and tissues in corticosteroid-resistant UC patients (*P*<0.01), CD₅₄ in tissues being higher than in blood. After oral LMWH, CD₅₄ lowered significantly in both blood and tissues (*P*<0.01), but still higher than that of normal controls (*P*<0.05), (Table 3).

Table 3 Effects of oral LMWH on CD₅₄ in UC patients

Group	Blood CD ₅₄	Tissue CD ₅₄
UC patients		
Pre-treatment	28.7±6.1 ^b	50.7±6.8 ^b
Post-treatment	14.6±5.2 ^{a,d}	22.8±4.7 ^{a,d}
Normal controls	6.2±3.7	8.8±3.2

^a*P*<0.05, ^b*P*<0.01 vs normal; ^d*P*<0.01 vs post-treatment.

Complications

No serious complications were associated with the use of oral LMWH.

DISCUSSION

Heparin, a group of sulphated glycosaminoglycans, in addition to its physiological effects and anticoagulant, antithromboembolic, antiallergic, antiviral, antiendotoxic and immunoregulative biological activities, has a wide range of potentially anti-inflammatory effects, including inhibition of neutrophil elastase and inactivation of chemokines^[5,13]. Compared with heparin, LMWH has an enhanced antithromboembolic effect, longer half life period, less bleeding tendency, higher bioavailability, easier absorption by oral administration^[8,9], and has the anti-inflammatory effects as well^[10,11]. Previous reports^[5-7] on improvement in UC patients treated with heparin prompted us to perform a pilot study of oral LMWH to find a more convenient and effective drug for patients with corticosteroid-resistant UC. The observed response to oral LMWH is paradoxical. Nineteen of 20 patients with corticosteroid-resistant UC achieved clinical remission and became asymptomatic on oral LMWH combined with sulfasalazine. Opposite to the traditional idea that heparin can enhance bleeding, rectal bleeding was the first symptom to be improved by oral LMWH. The results are similar to other reports of heparin treatment^[5-7].

If oral LMWH has a therapeutic effect in UC, its mechanism of action should shed some light on the elusive pathogenesis of this disease. There are several thrombophilic features of UC that suggest the effect of oral LMWH on colitic symptoms may be attributable to its anticoagulant and antithrombotic properties. Evidence of a

thrombotic process in UC includes: reports of a hypercoagulable state^[2-4], an increased incidence of thromboembolic event^[14], and ischemic complications such as toxic megacolon and pyoderma gangrenosum. In this study, the membrane marks of platelet activity CD_{62P} and CD₆₃ increased significantly, and the derivative of active platelet TXA-2 also elevated, suggesting that the blood platelet was in an active state, which not only led to a hypercoagulable state and an increased incidence of thromboembolic events, but also enhanced inflammatory reaction^[24]. Activated hyperaggregable platelets in the mesenteric circulation could amplify the inflammatory cascade by promoting neutrophil recruitment and chemotaxis. P-selectin has an established action as the adhesion molecule for neutrophils, and circulating platelet aggregates may contribute to ischemic damage and infarction by occluding the intestinal microvasculature. Platelet derived thromboxane A₂ may also contribute to the ischemia by inducing local vasoconstriction. After treatment with oral LMWH, all these parameters dropped markedly, suggesting that the therapeutic effect of LMWH is partly related to inhibition of platelet activity^[9]. CD₅₄ antigen reacts with the 85 kD-110 kD integral membrane glycoprotein, is also known as an intercellular adhesion molecule-1 (ICAM-1) expressed on endothelial cells and both resting (weak) and activated (moderate) lymphocytes and monocytes. CD₅₄ is ligand for the leukocyte function antigen-1 (CD_{11a}). Its expression is up-regulated upon stimulation by inflammatory mediators such as cytokines and LPS, and it is involved in B cell-T cell co-stimulatory interactions. In this study, CD₅₄ elevated significantly in blood and tissues of UC patients, being in tissues higher than in blood^[12]. Therefore, it could reflect the inflammation of intestinal mucosa. After oral LMWH, CD₅₄ dropped significantly in both blood and tissues, indicating that oral LMWH could relieve the inflammatory activity in these patients who received prednisolone for a long period (more than 4 weeks) and had no significant improvement and were regarded as corticosteroid-resistant refractory cases of UC. In other reports^[5], heparin

can also inhibit c-reactive protein (CRP), tumor necrosis factor (TNF) and L-selectin of UC patients. The detailed mechanisms by which the anti-inflammatory properties of oral LMWH are mediated in UC remain to be elucidated further.

From these results, we conclude that oral LMWH may play a role in treating corticosteroid-resistant UC, the mechanism is partly related to inhibition of platelet activity, hypercoagulable state and anti-inflammatory effects. No serious complications were found associated with the use of oral LMWH.

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