Effects of low molecular weight heparin on platelet surface P-selectin expression and serum interleukin-8 production in rats with trinitrobenzene sulphonic acid-induced colitis

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AIM: To observe the effects of low molecular weight heparin (LMWH) on platelet surface P-selectin expression and serum interleukin-8 production in rats with trinitrobenzene sulphonate (TNBS) induced colitis.

METHODS: Colitis was induced in female Sprague-Dawley rats by colonic administration of 2, 4, 6-TNBS. LMWH, a dalteparin (150 U/kg, 300 U/kg) was subcutaneously administrated one hour before induction of colitis and went on once a day for 6 days. Then a half dose was given for the next 7 days. Control animals received the same volume of normal saline once a day for 14 days after treated by TNBS. Animals were sacrificed at 24 h, days 7 and 14 after induction of colitis. The colon was excised for the evaluation of macroscopic and histological findings and TNF-α immunohistochemical assay. Platelet surface P-selectin expression was determined by radioimmunoassay and serum IL-8 production was assayed by ELISA method.

RESULTS: LMWH treatment in a dose of 300 U/kg for 14 days significantly improved colonic inflammation by histological examination. Serum IL-8 production in the 300 U/kg treatment group was more significantly decreased at day 14 than that at 24 h (P<0.05). However, platelet surface P-selectin expression and TNF-α staining in colonic tissue were not significantly different among the three groups.

CONCLUSION: LMWH has an anti-inflammatory effect on TNBS induced colitis in rats. The effect is possibly related to inhibition of proinflammatory cytokine IL-8, but not involved platelet surface P-selectin expression.

CONCLUSIONS: The limited studies demonstrated that it was possibly associated with the increase of several growth factors and the decrease of nitric oxide synthesis (NOS) and myeloperoxidase[9,13,14,22,23]. The effects of heparin on platelet surface P-selectin and IL-8 have not been studied. The present study was designed to observe the effects of LMWH on platelet surface P-selectin expression, serum IL-8 production, and TNF-α expression in mucosa of rats with trinitrobenzene sulphonate acid (TNBS)-induced colitis, and to clarify the anti-inflammatory mechanisms of LMWH in the treatment of colitis.

Materials and Methods

Animals

Female Sprague-Dawley rats weighing 200-250 g were used in the study. The animals were fasted for 24 h before the experiment and allowed food and water ad libitum after induction of colitis. The study was approved by the Ethic Committee of Wuhan University Medical School.

Induction of TNBS-induced colitis

Colitis was induced by a method of hapten-induced colonic inflammation as previously described[24]. Rats were anaeasthetized by intraperitoneal administration of 100g/L urethane. A small
volume of 2, 4, 6-TNBS (Sigma Company) was dissolved in 50% ethanol to a final concentration of 100 mg/mL, and 0.3 mL of TNBS solution was intracolonically administered with a polypropylene catheter by inserting 8 cm via the anal canal. Rats in a group were given 150 U/kg LMWH (dalteparin made by Pharmacia & Upjohn Company) subcutaneously 1 h before induction of colitis, and went on once a day for 6 d. Then a half dose of LMWH was given for the next 7 d. Treatment of the LMWH 300 U/kg group was the same as the 150 U/kg group, but the dose of LMWH was as high as 300 U/kg. Control animals received the same volume of normal saline once a day instead of LMWH after treated by TNBS.

Animals in each group were sacrificed at 24 h, d 7 and 14 after induction of colitis. The colon was isolated and a segment of colon was excised for the evaluation of macroscopic and histological findings and also for TNF-α immunohistochemical assay. A blood sample was drawn from heart of the rats for determination of platelet surface P-selectin and serum IL-8 before the rats were sacrificed.

**Macroscopic evaluation of colonic damage**

Macroscopic evaluation of colonic damage was conducted by two authors (HH, LLG) and mucosal hyperemia, ulcers, inflammatory exudation and bleeding were recorded. The most damaged site of the colon was chosen as the part for histological studies. For control group the colon at 8 cm above anus was excised for histological studies.

**Histological studies of colonic damage**

For histological examination, formalin-fixed tissues were embedded in paraffin and 5 μm-thick sections were stained with hematoxylin and eosin, and evaluated under light microscope by a pathologist (GSG) blinded to the experimental protocol. Colonic damage was assessed by the grades described by Fedorak et al.[25]. Mucosal ulceration: 0: no-ulceration; 1: focal ulceration; 2: multifocal-ulceration; 3: diffuse ulceration. Depth of injury was graded as follows: 0: no staining; 1: a few maple granules, or a few fine brown granules, not exceeding the 1/4 total area of cytoplasm; 2: uniformity maple in the whole cytoplasm or wide brown granules in the cytoplasm, not exceeding the 1/2 area of cytoplasm; 3: the cytoplasm was full of brown granules with a lower density; 4: the total cytoplasm was full of crassitude dark brown granules, and covered whole nuclei of the cell. One hundred cells were counted under oil microscope, positive cells were recorded and positive cell rate was calculated. The scores of expression of TNF-α were calculated by sum of each grade multiplying its positive cell rate.

**Statistical analysis**

Data were expressed as mean±SD. Data in different groups were analyzed by ANOVA and post multiple tests (Tukey-Kramer or Student-Newman-Keuls). A P value less than 0.05 was considered statistically significant.

**RESULTS**

**Macroscopic evaluation of TNBS-induced colitis**

Control animals subjected to intracolonic administration of TNBS in 500ml/l ethanol showed colonic mucosal injury with ulceration. Signs of the mucosal damage were monitored for 14 d. As early as 24 h after TNBS treatment, colonic mucosa was shown to have hyperemia, congestion, erosion, hemorrhagic ulcers and partial epithelial necrosis. On d 14, the colonic ulceration still existed. In both doses of LMWH treated groups, mucosal hyperemia, congestion and ulcerations in the injured site were slighter than those in control group. However, mucosal hemorrhage in both LMWH treated groups was severer than that in control group at 24 h, but was resolved on d 7 and 14, respectively.

**Histological examination of TNBS-induced colitis**

In control group a large number of neutrophils, monocytes and eosinophils infiltrated in mucosa and submucosa at 24 h and the damage reached peak on d 7. As shown in Table 1, the histological grades according to Fedorak et al.[25] were greatly higher on d 7 compared with those at 24 h (P<0.01). On d 14, the damage of colon was mild, but still had ulceration, chronic inflammatory cell infiltration, vesiculitis and granulation tissue formation. In contrast, in both 150 U/kg and 300 U/kg LMWH treated groups colonic damage was mild. Table 1 shows that the histological injury grades in the 300 U/kg LMWH group were decreased at 24 h and on d 7 after treatment, and much obviously on day 14 compared with the control group (P<0.01).

**Expression of TNF-α in colonic mucosa of TNBS-induced colitis**

As shown in Table 2, the scores of expression of TNF-α in mucosa were greatly higher in the first 24 h and decreased on d 7 and 14 after induction of colitis, but there were no
significant differences among the three groups except a difference at 24 h between 300 U/kg LMWH treatment group and 150 U/kg LMWH treatment group.

| Table 1 Effect of low molecular weight heparin (dalteparin) in Fedorak grades on mucosa of TNBS-induced colitis in rats |
| --- |
| n | 24 h | Day 7 | Day 14 |
| --- | --- | --- | --- |
| Control group | 5 | 1.33±0.54 | 4.33±0.81 | 3.67±2.13 |
| 150 U/ kg LMWH group | 4 | 1.00±0.00 | 3.33±2.08 | 1.67±1.15 |
| 300 U/ kg LMWH group | 5 | 0.57±0.33 | 2.00±1.33 | 0.66±0.32 |

DISCUSSION

Control group showed more histological improvement of colitis, lower TNF-α expression in mucosa and serum IL-8 production than 150 U/kg LMWH treated group. With a continuing LMWH treatment these effects were gradually demonstrated on d 7 and 14. Our result was slightly different from that of Dotan et al. They showed that a single dose of 80 µg/kg of LMWH (enoxaparin) was more optimal for amelioration of dinitrobenzene sulphinic acid- and iodoacetamide-induced colitis in rats than a dose of 200 µg/kg and 40 µg/kg of enoxaparin. The mechanism is not known, but may be related to optimal interactions between LMWH fragments and their receptors.

Platelet surface P-selectin expression and serum interleukin 8 production

Expression of platelet surface P-selectin was increased on d 7 and 14 in all three groups as shown in Table 3, but reached a significant level only in control group. There were no significant differences among these three groups at the three time points, 24 h, d 7 and 14. Production of serum interleukin 8 was higher at 24 h in the three groups, but significantly decreased on day 14 compared with that at 24 h in 300 U/kg LMWH treated group as shown in Table 4.

| Table 3 Effects of low molecular weight heparin (dalteparin) on platelet surface P-selectin expression (moleculars/ per platelet) in rats with TNBS-induced colitis |
| --- |
| n | 24 h | d 7 | d 14 |
| --- | --- | --- | --- |
| Control group | 5 | 217±44 | 48±11 | 50±18 |
| 150 U/ kg LMWH group | 4 | 264±42 | 46±21 | 48±16 |
| 300 U/ kg LMWH group | 5 | 198±38 | 26±18 | 16±8 |

Platelet surface P-selectin expression and serum interleukin 8 production

Effect of low molecular weight heparin (dalteparin) in rats with TNBS-induced colitis was demonstrated by improvement of colonic inflammation with macroscopic and histological alterations in a dose of 300 U/kg of heparin treatment for 14 d. The result was similar to that of Fries et al., in which they showed that heparin could prevent TNBS-induced colitis, but steroids could not. Our data showed that serum IL-8 production in 300 U/kg LMWH treated group was significantly lower on d 14 than that at 24 h, but we did not find this variation in other groups. Expression of platelet surface P-selectin in control group was significantly increased consecutively at 24 h, d 7 and 14, but expression of platelet surface P-selectin did not increase in LMWH treatment group. We also did not find differences of expression of TNF-α in colonic mucosa between LMWH treatment group and control group. Our results suggested that LMWH (dalteparin) in a high dose had anti-inflammatory effects. The effects were possibly related to the decrease of proinflammatory cytokine IL-8, but not related to platelet surface P-selectin.

We observed dose and time-dependent effects of LMWH in rats with TNBS-induced colitis. Three hundreds U/kg LMWH treatment group showed more histological improvement of colitis, lower TNF-α expression in mucosa and serum IL-8 production than 150 U/kg LMWH treated group. With a continuing LMWH treatment these effects were gradually demonstrated on d 7 and 14. Our result was slightly different from that of Dotan et al. They showed that a single dose of 80 µg/kg of LMWH (enoxaparin) was more optimal for amelioration of dinitrobenzene sulphinic acid- and iodoacetamide-induced colitis in rats than a dose of 200 µg/kg and 40 µg/kg of enoxaparin. The mechanism is not known, but may be related to optimal interactions between LMWH fragments and their receptors.

REFERENCES

1. Musio F, Oldner SA, Jenkins T, Gregorie EM. Case report: cerebral venous thrombosis as a manifestation of acute ulcerative colitis. Am J Med Sci 1993; 305: 28-35
2. Thornton M, Solomon MJ. Crohn’s disease: in defense of a microvascular aetiology. Int J Colorectal Dis 2002; 17: 287-297
3. Mutu B, Ermeeydan CM, Enc F, Fotbolcu H, Demirkol O, Bayrak F, Basaran Y. Acute myocardial infarction in a young woman with severe ulcerative colitis. Int J Cardiol 2002; 83: 183-185
4. Srivastava AK, Khanna N, Sardana V, Gaekwad S, Prasad K, Behari M. Cerebral venous thrombosis in ulcerative colitis. Natl Med J India 2002; 50: 215-217
5. Crowe A, Taffinder N, Layer GT, Irvine A, Nicholls RJ. Portal vein thrombosis in a complicated case of Crohn’s disease. Postgrad...
6. Nguyen LT, Laberge JM, Guttmann FM, Albert D. Spontaneous deep vein thrombosis in childhood and adolescence. J Pediatr Surg 1986; 21: 640-643

7. Braverman D, Bogoch A. Arterial thrombosis in ulcerative colitis. Am J Dig Dis 1978; 23: 1148-1150

8. Ryan FP, Timperley WR, Preston FE, Holdsworth CD. Cerebral involvement with disseminated intravascular coagulation in intestinal disease. J Clin Pathol 1977; 30: 551-555

9. Manzoni PF, Di Bello M, Masini E. Platelets and inflammation: role of platelet-derived growth factor, adhesion molecules and histamine. Inflamm Res 1997; 46: 4-18

10. Collins CE, Cahill MR, Newland AC, Rampton DS. Platelets circulate in an activated state in inflammatory bowel disease. Gastroenterology 1994; 106: 840-845

11. Schaufelberger HD, Uhr MR, McGuckin C, Logan RP, Misiewicz JJ, Gordon-Smith EC, Beglinger C. Platelets in ulcerative colitis and Crohn’s disease express functional interleukin-1 and interleukin-8 receptors. Eur J Clin Invest 1994; 24: 656-663

12. Collins CE, Rampton DS. Review article: platelets in inflammatory bowel disease—pathogenetic role and therapeutic implications. Aliment Pharmacol Ther 1997; 11: 237-247

13. Levine A, Kenet G, Bruck R, Avni Y, Avinoach I, Aeed H, Matas Z, David M, Yaron A. Effect of heparin on tissue binding activity of fibroblast growth factor and heparin-binding epidermal growth factor in experimental colitis in rats. Pediatr Res 2002; 51: 635-640

14. Tyrrell DJ, Horne AP, Holme KR, Preuss JM, Page CP. Heparin in inflammation: potential therapeutic applications beyond anticoagulation. Adv Pharmacol 1999; 46: 151-208

15. Cahalon L, Lider O, Schor H, Avron A, Gilat D, Hershkoviz R, Margalit R, Eshel A, Shoseyov O, Cohen IR. Heparin disaccharides inhibit tumor necrosis factor-alpha production by macrophages and arrest immune inflammation in rodents. Int Immunol 1997: 9: 1517-1522

16. Vrij AA, Jansen JM, Schoon EJ, de Bruijn A, Hemker HC, Stockbrugger RW. Low molecular weight heparin treatment in steroid refractory ulcerative colitis: clinical outcome and influence on mucosal capillary thrombi. Scand J Gastroenterol Suppl 2001; 234: 41-47

17. Dotan I, Hallak A, Arber N, Santo M, Alexandrowitz A, Knaani Y, Hershkoviz R, Brazowski E, Halpern Z. Low-dose low-molecular weight heparin (enoxaparin) is effective as adjuvant treatment in active ulcerative colitis: an open trial. Dig Dis Sci 2001; 46: 2239-2244

18. Dotan I, Hershkoviz R, Karmeli F, Brazowski E, Peled Y, Rachmilewitz D, Halpern Z. Heparin and low-molecular-weight heparin (enoxaparin) significantly ameliorate experimental colitis in rats. A Liment Pharmacol Ther 2001; 15: 1687-1697

19. Torkvist L, Thorlacius H, Sjoqvist U, Bohman L, Lapidus A, Flood L, Agren B, Raud J, Lofberg R. Low molecular weight heparin as adjuvant therapy in active ulcerative colitis. A Liment Pharmacol Ther 1999; 13: 1323-1328

20. Folwaczny C, Wiebecke B, Loescheke K. Unfractioned heparin in the therapy of patients with highly active inflammatory bowel disease. A Liment Pharmacol Ther 1999; 94: 1551-1555

21. Evans RC, Wong VS, Morris AI, Rhodes JM. Treatment of corticosteroid-resistant ulcerative colitis with heparin—a report of 16 cases. A Liment Pharmacol Ther 1997; 11: 1037-1040

22. Michell NP, Lelor P, Langman MJ. Heparin therapy for ulcerative colitis? Effects and mechanisms. Eur J Gastroenterol Hepatol 2001; 13: 449-456

23. Papa A, Danese S, Gasbarrini A, Gasbarrini G. Review article: potential therapeutic applications and mechanisms of action of heparin in inflammatory bowel disease. A Liment Pharmacol Ther 2000; 14: 1403-1409

24. Morris GP, Beck PL, Herridge MS, Depew WT, Szewczuk MR, Wallace J. Hapten-induced model of chronic inflammation and ulceration in the rat colon. Gastroenterology 1989; 96: 795-803

25. Fedorak RN, Empey LR, MacArthur C, Jewell LD. Misoprostol provides a colonic mucosal protective effect during acetic acid-induced colitis in rats. Gastroenterology 1990; 98: 615-625

26. Fries W, Pagiaro E, Canova E, Carraro P, Gasparini G, Pomeri F, Martin A, Carlotto C, Mazzeno E, Sturlioni GC, Longo G. The effect of heparin on trinitrobenzene sulphonic acid-induced colitis in the rat. A Liment Pharmacol Ther 1998; 12: 229-236

27. Chowers Y, Lider O, Schor H, Barshack I, Tal R, Ariel A, Bar- Meir S, Cohen IR, Cahalon L. Disaccharides derived from heparin or heparan sulfate regulate IL-8 and IL-1 beta secretion by intestinal epithelial cells. Gastroenterology 2001; 120: 449-459

28. Salas A, Sans M, Soriano A, Reverter JC, Anderson DC, Pique JM, Panes J. Heparin attenuates TNF alpha induced inflammatory response through a CD11b dependent mechanism. Gut 2000; 47: 88-96

29. Nelson RM, Cecconi O, Roberts WG, Aruffo A, Linhardt RJ, Bevilacqua MP. Heparin oligosaccharides bind L- and P-selectin and inhibit acute inflammation. Blood 1993; 82: 3253-3258

30. Panes J, Esteve M, Cabre E, Hinojosa J, Andreu M, Sans M, Fernandez-Banas F, Feu F, Gassull MA, Pique JM. Comparison of heparin and steroids in the treatment of moderate and severe ulcerative colitis. Gastroenterology 2000; 119: 903-908

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