Thrombosis and Haemostasis – © F. K. Schattauer Verlagsgesellschaft mbH (Stuttgart) 60 (3) 434–436 (1988)

Heparin and Bleeding: An Association with Lipase Release

T. W. Barrowcliffe, R. E. Merton, E. Gray, and D. P. Thomas

From the National Institute for Biological Standards and Control, London, UK

Key words

Heparin – Lipase – Haemorrhage

Summary

The effects of four sulphated polysaccharides on bleeding time and lipase release in rabbits have been compared. Unfractionated heparin (UFH) and pentosan polysulphate both gave significant prolongation of bleeding times and high lipase release. Low molecular weight heparin had reduced effects on bleeding time and lipase release, while dermatan sulphate had no influence on either parameter. There was a highly significant correlation (r = 0.97) between these two measurements.

These results suggest that the same structural features influence both the haemorrhagic and lipase-releasing properties of sulphated polysaccharides.

Introduction

The development of low molecular weight (LMW) heparins for clinical use is attracting much interest, partly because of the suggestion that LMW heparins might retain the antithrombotic properties of unfractionated heparin, whilst exhibiting reduced haemorrhagic side-effects (1–6). Although some LMW heparins have been shown to have less haemorrhagic effects than unfractionated heparin in animal models (6–8), several clinical reports have indicated excessive bleeding in patients given LMW heparin (9–11). It is clear from both animal and clinical data that there is little relationship between the haemorrhagic properties of unfractionated and various LMW heparins and their anticoagulant activities as measured by the prolongation of the APTT.

Another property that distinguishes LMW heparins is their reduced lipase releasing effect compared with unfractionated heparin (12, 13). One of the lipase enzymes released, hepatic triglyceride lipase (HTGL) has anticoagulant activity (14, 15) and we therefore decided to examine whether a relationship exists between haemorrhagic properties and lipase release. We have compared the lipase-releasing properties of unfractionated heparin, LMW heparin and two other sulphated polysaccharides with their haemorrhagic activities, as measured by prolongation of the bleeding time.

Subjects, Materials, and Methods

Sulphated Polysaccharides

Unfractionated heparin (UFH) was obtained from Diosynth (Oss, The Netherlands), low molecular weight (LMW) heparin (CY 216) from the Institut Choay, Paris, and pentosan polysulphate (PPS) was from Clin-Midy Paris. Purified dermatan sulphate (DS) was prepared from sheep mucosal residues (16), and kindly provided by Dr Edward Johnson of this Institute.

Bleeding times were measured in a rabbit ear template model as described by Merton and Thomas (17). Three minutes after i. v. injection of 2.5 mg/kg drug or saline, a post-treatment bleeding time was carried out on the contralateral ear. Results were expressed as ratios of the post- to pre-treatment bleeding times for each drug and for the saline control. Groups of 10 rabbits were used for each treatment.

Lipase release was measured in separate groups of 4–6 rabbits, using a tritiated triolein method (18). Hepatic tryglyceride lipase (HTGL) was measured separately from total lipase by use of a substrate with 1 M NaCl and no serum (19). Platelet-free plasma was separated from blood drawn 3 minutes after injection of test substances and results were calculated in mu/ml (1 mu = 1 nmol fatty acid release/minute at 37° C), using an internal post-heparin plasma standard which had been assigned a value based on six independent assays.

Anticoagulant activities. APTT and amidolytic anti-Xa activities were measured as previously described (20, 21).

Results

The in vitro anticoagulant characteristics of the four sulphated polysaccharides are shown in Table 1. LMW heparin had higher activity by anti-Xa than by APTT, whereas PPS and DS had no activity by anti-Xa assays. LMW heparin and PPS had similar activity by APTT assay, but DS had almost no activity.

Table 2 shows the bleeding time ratios obtained with the four polysaccharides and with the saline control. Saline injections consistently gave a slight shortening of the bleeding time. The ratios following the administration of UFH and PPS were significantly higher ($p < 0.05$) than those obtained with the saline control, and also significantly higher ($p < 0.05$) than those obtained with LMW heparin.

The mean total lipase released by the sulphated polysaccharides is shown in Table 3, together with the percentage increase in bleeding time ratio. There was a highly significant correlation between these two measurements (r = 0.97). The

Table 1 In vitro anticoagulant activities

Material	Potency (iu/mg) Anti-Xa	APTT
UFH	163	175
LMWH	102	22
PPS	<1	18
DS	<1	2.5

Table 2 Bleeding time ratios

	Saline	DS	LMWH	PPS	UFH
Geometric mean	0.81	0.86	0.99	1.33	1.43
95% conf. limits	0.6–1.09	0.61–1.23	0.75–1.31	0.89–1.98	1.02–2.0

Correspondence to: Dr. T. W. Barrowcliffe, National Institute for Biological Standards and Control, Blanche Lane, South Mimms, Potters Bar, Herts. EN6 3QG, UK

Table 3 Comparison of BT ratios and lipase release

2.5 mg/kg	BT ratio increase (%)	Total lipase (mu/ml)	HTGL (mu/ml)
Saline	0	0	0
DS	6	9.9	4.4
LMWH	22	127.9	64.4
PPS	64	302.8	177.0
UFH	76	293.0	181.4

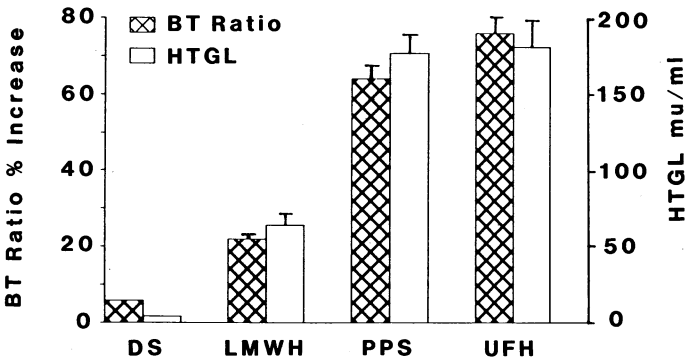

Fig. 1 Comparison of bleeding time ratios and release of HTGL in rabbits given 2.5 mg/kg of sulphated polysaccharides. DS – dermatan sulphate; LMWH – low molecular weight heparin; PPS – pentosan polysulphate; UFH – unfractionated heparin. BT ratios are expressed as percentage increase over the saline control and data are means + s.e.m. from 10 rabbits. HTGL data are means + s.e.m. from 4 to 6 rabbits

correlation between bleeding time ratio increase and HTGL was even more striking (r = 0.99) (Fig. 1).

Discussion

We have confirmed that a LMW heparin gives less enhancement of haemorrhage than UFH (6–8), though this is not necessarily the case for all LMW heparins (22). However, the decreased haemorrhagic effect of LMW heparins may not be due to their lower anticoagulant activity in assays such as the APTT. Pentosan polysulphate, which has similar APTT activity to LMW heparin, caused a greater prolongation of bleeding time and indeed was equal in haemorrhagic effect to unfractionated heparin (Fig. 1).

In a template bleeding time model, impairment of primary haemostasis by inhibition of platelet function is probably more important than anticoagulant activity. Several studies have found that LMW heparin gives less inhibition of platelet aggregation than UFH (23–26). A correlation between inhibition of platelet aggregation and degree of sulphation of sulphated polysaccharides has recently been observed (27), and the degree of sulphation may be the common factor for both lipase release and bleeding in the association we have observed. However, it is possible that lipase release itself could have a direct effect on haemorrhage by interfering with platelet function. Inhibition of platelet aggregation by heparin and LMW heparins ex vivo is greater than that observed for the same concentration in vitro (6, 28), suggesting the release of a component that enhances the platelet inhibitory effect.

Whatever the reasons for the association between bleeding time and lipase release, our results demonstrate that measurement of an indirect action of sulphated polysaccharides, i.e. lipase release, may be more relevant in predicting their haemorrhagic properties than measurement of their anticoagulant activities.

References

1 Salzman E W. Low molecular weight heparin – is small beautiful? N Engl J Med 1986; 315: 957–9.
2 Thomas D P. Current status of low molecular weight heparin. Thromb Haemostas 1986; 56: 241–2.
3 Andersson L-O, Barrowcliffe T W, Holmer E A, Johnson E A, Sims G E C. Anticoagulant properties of heparin fractionated by affinity chromatography on matrix-bound antithrombin III and by gel filtration. Thromb Res 1976; 9: 575–83.
4 Barrowcliffe T W, Johnson E A, Eggleton C A, Kemball-Cook G, Thomas D P. Anticoagulant activities of high and low molecular weight heparin fractions. Br J Haematol 1979; 41: 573–83.
5 Thomas D P. Heparin in the prophylaxis and treatment of venous thrombosis. Semin Haematol 1978; 15: 1–17.
6 Carter C J, Kelton J G, Hirsh J, Cerskus A, Santos A V, Gent M. The relationship between the hemorrhagic and antithrombotic properties of low molecular weight heparin and heparin. Blood 1982; 59: 1239–45.
7 Esquivel G O, Bergqvist D, Björk C G, Nilsson B. Comparison between commercial heparin, low-molecular-weight heparin and pentosan polysulphate on hemostasis and platelets in vivo. Thromb Res 1982; 8: 389–99.
8 Cade J F, Buchanan M R, Boneu B, Ockelford P, Carter C J, Cerskus A L, Hirsh J. A comparison of the antithrombotic and haemorrhagic. Effects of low molecular weight heparin fractions: the influence of the method of preparation. Thromb Res 1984; 35: 613–25.
9 Schmitz-Huebner U, Bünte H, Freise G, Reers B, Rüschmeyer C, Scherer R, Schulte H, van de Loo J. Clinical efficacy of low molecular weight heparin in postoperative thrombosis prophylaxis. Klin Wochenschr 1984; 62: 349–53.
10 Koller M, Schoch U, Buchmann P, Largiadèr F, von Felten A, Frick P G. Low molecular weight heparin (Kabi 2165) as thromboprophylaxis in elective visceral surgery. A randomized, double-blind study versus unfractionated heparin. Thromb Haemostas 1986; 56: 243–6.
11 Bergqvist D, Burmark U S, Frisell J, Hallibook I, Lundblad B, Risberg B, Torngren S, Wallin G. Low molecular weight heparin once daily compared with conventional low dose heparin twice daily. A prospective double-blind multicentre trial on prevention of postoperative thrombosis. Br J Surg 1986; 73: 204–8.
12 Kakkar V V, Murray W J G. Efficacy and safety of low-molecular-weight heparin (CY 216) in preventing postoperative venous thromboembolism: a cooperative study. Br J Surg 1985; 72: 786–91.
13 Persson E, Nordenström J, Nilsson-Ehle P, Hagenfeldt L. Lipolytic and anticoagulant activities of a low molecular weight fragment of heparin. Eur J Clin Invest 1985; 15: 215–20.
14 Barrowcliffe T W, Gray E, Merton R E, Dawes J, Jennings C A, Hubbard A R, Thomas D P. Anticoagulant activities of pentosan polysulphate (Hémoclar) due to release of hepatic triglyceride lipase (HTGL). Thromb Haemostas 1986; 56: 202–6.
15 Gray E, Bengtsson-Olivecrona G, Olivecrona T, Barrowcliffe T W. Anti-Xa activity of human hepatic triglyceride lipase. J Lab Clin Med 1987; 109: 653–9.
16 Johnson E A, Paterson M S. Lead precipitation: an aid to separation of dermatan and mucokeratan sulfates from glycosaminoglycan mixtures. Anal Biochem 1986; 158: 111–6.
17 Merton R E, Thomas D P. Experimental studies on the relative effectiveness of dermatan sulphate and heparin as antithrombotic agents. Thromb Haemostas 1987; 58: 839–42.
18 Nilsson-Ehle P, Schotz M C. A stable radioactive substrate emulsion for the assay of lipoprotein lipase. J Lipid Res 1976; 17: 536–41.
19 Williams S P, Barrowcliffe T W. The effects of post-heparin plasma lipases on anti-Xa clotting activity. Thromb Res 1985; 37: 371–7.
20 Proctor R R, Rapaport S I. A partial thromboplastin time with kaolin. A simple screening test for first stage plasma clotting factor deficiencies. Am J Clin Pathol 1961; 36: 121–9.
21 Thomas D P, Merton R E. A low molecular weight heparin compared with unfractionated heparin. Thromb Res 1982; 28: 343–50.
22 Pangrazzi J, Abbadini M, Zametta M, Casu B, Donati M B. Low molecular weight heparins and bleeding. Thromb Haemostas 1985; 53: 158.

23 Salzman E W, Rosenberg R D, Smith M H, Lindon J N, Favrean L. Effect of heparin and heparin fractions on platelet aggregation. J Clin Invest 1980; 65: 64–73.
24 Brace L D, Fareed L. Heparin-induced platelet aggregation: dose/response relationships for a low molecular weight heparin derivate (PK 10169) and its subfractions. Thromb Res 1986; 42: 769–82.
25 Westwick J, Scully M F, Poll C, Kakkar V V. Comparison of the effects of low-molecular-weight heparin and unfractionated heparin on activation of human platelets in vitro. Thromb Res 1986; 42: 435–47.
26 Holmer E, Lindahl U, Bäckström G, Thunberg L K, Sandberg H, Söderström G, Andersson L-O. Anticoagulant activities and effects on platelets of a heparin fragment with high affinity for anti-thrombin. Thromb Res 1980; 18: 861–9.
27 Van Ryn-McKenna J, Ofosu F A, Johnson E A, Hirsh J, Buchanan M R. Increased sulfation increases the bleeding side-effects of glycosaminoglycans. Thromb Haemostas 1987; 58: 7 (Abstr).
28 Borowska A, Lauri D, Dejana E, de Gaetano G, Donati M B, Pangrazzi J. Impairment of primary haemostasis by low molecular weight-heparins in rats. Br J Haematol 1988; 68: 339.

Received May 20, 1988 Accepted after revision July 21, 1988