Heparin and Bleeding: An Association with Lipase Release

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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-

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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

Table 3 Comparison of BT ratios and lipase release

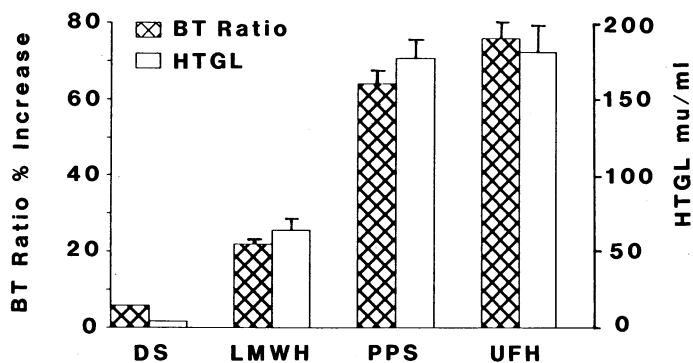| 2.5 mg/kg	BT ratio increase (%)	Total lipase (mu/ml)	HTGL (mu/ml)
Saline	0	0	0
DS	6	9.9	4.4
LMWH	22	127.9	64.4
PPS	64	302.8	177.0
UFH	76	293.0	181.4

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.

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In this book a survey is given on the technical development and reliability of the Holter monitoring technique as well as the clinical relevance of the results obtained. Following the introductory presentations on general technical problems of the Holter monitoring technique, the analysis of the ST segment of the surface ECG were discussed. Thereafter the recovery and clinical significance of cardiac arrhythmias, particularly with respect to their relevance to sudden cardiac death, were debated, based on both experimental and clinical experiences.

In addition to the Holter ECG technique the final session focussed on long-term ambulatory recording of different biological parameters like arterial blood pressure, pulmonary artery pressure, arterial pulse wave contours, and of the electrical activity of the brain.