Comparison of the effect of a conventional heparin and a low molecular weight heparinoid on platelet function

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1 In a study comparing the *in vitro* effects of heparin and a low molecular weight heparinoid (Organon 10172) on aggregation of platelets from normal subjects, we have demonstrated that whereas heparin markedly enhances platelet aggregation induced by other aggregators and inhibits the anti-aggregatory effect of epoprostenol (prosta-cyclin, PGI₂), heparinoid does not produce such effects.

2 The use of heparinoid may thus have a significant advantage over that of heparin in situations where enhanced platelet aggregation is the main factor leading to thrombosis or where heparin treatment is followed by thrombocytopaenia.

Keywords heparin heparinoid platelet function

Introduction

Treatment with heparin can induce thrombocytopaenia in some patients (Hussey *et al.*, 1979). The platelets of these patients, but not those of controls, have been shown to aggregate *in vitro* following the addition of heparin (Hussey *et al.*, 1979). This phenomenon may result from the ability of heparin to enhance platelet aggregation (Macintyre *et al.*, 1981), and to oppose the anti-aggregatory activity of epoprostenol (prosta-cyclin, PGI₂), prostaglandin E₁ (PGE₁) and adenosine (Eldor & Weksler, 1979; Macintyre *et al.*, 1981; Saba *et al.*, 1979).

Since these properties of heparin are potentially hazardous, a search for 'safer' heparins and heparin-like substances is essential. It is therefore of interest that a low molecular weight heparin derivative has been shown to possess less platelet aggregation enhancing capacity than conventional heparin (Holmer *et al.*, 1980).

We undertook the present study to establish whether a new preparation of a low molecular weight heparinoid (Organon 10172) was potentially less pro-aggregatory *in vitro* than a commercially available heparin.

Methods

Materials

Heparin (Thromboliquine) and a low molecular weight heparinoid (Organon 10172, specific activity approximately 8 anti-Xa u/mg), in the form of solutions, as well as the diluent used in these solutions, were obtained from Organon International B.V., Oss, Holland. PGI₂ was obtained from Upjohn, Kalamazoo, U.S.A. Adenosine diphosphate (ADP) and fatty acid free human albumin were obtained from Sigma Chemicals, Poole, U.K.

Platelet aggregation

Blood was collected from healthy volunteers who had not ingested any drugs for at least 2 weeks prior to sampling.

Citrated platelet rich plasma (PRP) and platelet poor plasma (PPP) were prepared as previously described (Mikhailidis *et al.*, 1982b). Platelet aggregation was carried out at 37°C, in a Chronolog aggregometer (Mikhailidis *et al.*, 1982b). Aggregation was measured as the per-

centage fall in optical density 3 min after inducing aggregation. The concentrations of the aggregating agents used are recorded below.

Evaluation of the effect of the heparin/heparinoid diluent solution on platelet aggregation

The diluent solution was further diluted with physiological saline and then added to PRP. After 5 min incubation at 37°C aggregation was induced by adding ADP (final concentrations 1, 2 and 5 $\mu\text{mol/l}$). The amounts of diluent added were such as to mimic those added in the experiments described below.

Control experiments

Incubation with saline was selected for control experiments since addition of appropriate volumes of either saline or saline with diluent exerted no significant effect on platelet aggregation. The structure of these experiments is described below.

Structure of individual experiments

In all experiments, controls were tested simultaneously with the samples containing heparin/heparinoid.

1. Assessment of spontaneous platelet aggregation in the presence of heparin or heparinoid

Heparin and heparinoid solutions were further diluted in physiological (0.9%) saline and then added to PRP in the aggregometer. Spontaneous aggregation was then assessed for a period of 10 min by monitoring changes in the baseline aggregometer tracing. Twenty subjects were tested at each of the following final concentrations of heparin/heparinoid:

Heparin: 0.6, 1.0, 1.6, 3.2, 6.4, 12.5 u/ml
Heparinoid: 16, 32, 64, 128, 250, 500 $\mu\text{g/ml}$
Controls: Appropriate volumes of physiological saline

2. Assessment of the enhancement of platelet aggregation by heparin or heparinoid (Tables 1 and 2)

Heparin and heparinoid solutions were further diluted and added to PRP as described above. Appropriate volumes of physiological saline were added to PRP to provide controls. Following a 5 min incubation, platelet aggregation was induced by the addition of ADP. The concentration of ADP was adjusted (up to 1.2 $\mu\text{mol/l}$) so as to produce 5–12% aggregation in the control

experiments. This 'low' percentage aggregation would allow the observation of an enhancing effect by heparin or heparinoid (Barradas *et al.*, 1982; Eldor & Weksler, 1979; Macintyre *et al.*, 1981). These experiments were repeated using low-dose adrenaline (0.1–0.5 $\mu\text{mol/l}$) or collagen (0.1–0.4 $\mu\text{g/ml}$) to induce aggregation. Only the lowest effective dose of heparin (0.8 u/ml) was compared with a high concentration of heparinoid (250 $\mu\text{g/ml}$, i.e. approximately 2 anti-Xa u/ml).

The concentrations of heparin or heparinoid and the number of subjects evaluated are shown in Tables 1 and 2. The concentrations of heparin and heparinoid were selected as to approximate to the plasma levels achieved during treatment (Harenberg *et al.*, 1983; Henny *et al.*, 1983; Meulemann *et al.*, 1982; Olsson *et al.*, 1963).

3. Assessment of the effects of heparin and heparinoid on PGI₂-mediated inhibition of platelet aggregation (Table 3)

Heparin, heparinoid or saline were added to PRP as described above. Following a 5 min incubation PGI₂ was added and 1 min later platelet aggregation was induced by the addition of ADP (final concentration 10 $\mu\text{mol/l}$). The concentration of PGI₂ (1–4 $\mu\text{g/l}$) was adjusted to produce a 'low' percentage aggregation (10–20%) in the control experiments. This would allow heparin or heparinoid to further enhance aggregation (Barradas *et al.*, 1982; Eldor & Weksler, 1979; Macintyre *et al.*, 1981). The concentrations of heparin and heparinoid and the numbers of subjects tested are shown in Table 3.

4. Assessment of the effect of heparin and heparinoid on the rate of decay of PGI₂ activity in PPP and in fatty acid free human albumin

Heparin, heparinoid or saline were added to PPP and equilibrated at 37°C. PGI₂ was then added to these incubates. Samples from this incubate were then added to PRP and after 1 min incubation in the aggregometer, ADP (10 $\mu\text{mol/l}$) was added to induce platelet aggregation. Using this bioassay technique, described in detail elsewhere (Mikhailidis *et al.*, 1981; 1982a, b; 1983), the decay in PGI₂ activity was monitored.

The heparin concentrations in the PPP incubate were 1, 1.6, 3.2 and 6.4 u/ml. The heparinoid concentrations tested were 16, 32, 64, 125 and 250 $\mu\text{g/ml}$. Controls consisted of PPP incubates with the appropriate volumes of saline only added.

The final concentration in the PRP which was used to bioassay PGI₂-like anti-aggregatory activity were:

Heparin: 0.04–0.26 u/ml
 Heparinoid: 0.6–10 µg/ml
 Controls: appropriate volumes of saline only

PGI₂: 1–3 µg/l

The heparin and heparinoid in the PPP incubate were adjusted so as to achieve therapeutic and supratherapeutic concentrations (Harenberg *et al.*, 1983; Henny *et al.*, 1983; Meulemann *et al.*, 1982; Olsson *et al.*, 1963). The heparin and heparinoid concentrations in the PRP used to bioassay the PGI₂-like anti-aggregatory activity were of an order that they did not enhance platelet aggregation, or interfere with the bioassay.

These experiments were repeated using fatty acid free human albumin solution (40 g/l) in phosphate buffer (0.1 mol/l; pH 7.4) instead of PPP. Two subjects were tested at each heparin and heparinoid concentration.

Statistical analysis of results

The percentage aggregation obtained in control samples was compared with that obtained in the samples containing heparin/heparinoid. A paired Wilcoxon rank sum test was used to obtain the *P* values which are shown in the appropriate tables.

Results

Evaluation of the effect of the heparin/heparinoid diluent on platelet aggregation

The diluent solution, in the amounts used in the experiments, did not have any significant inhibitory or enhancing effect on platelet aggregation.

Assessment of spontaneous platelet aggregation in the presence of heparin or heparinoid

There was no significant spontaneous platelet aggregation detected with either heparin or heparinoid at all the concentrations tested.

Assessment of the enhancement of platelet aggregation by heparin or heparinoid (Tables 1 and 2)

Heparin, at concentration of 0.8 U/ml and above, significantly enhanced ADP-induced platelet aggregation, while heparinoid, at concentrations of up to 250 µg/ml, did not show such an effect. Similar results were obtained with adrenaline and collagen-induced aggregation (see Table 2). In all experiments, a few subjects showed appreciable enhancement of aggregation at high heparinoid concentrations.

Assessment of the effect of heparin and heparinoid on PGI₂-mediated inhibition of platelet aggregation (Table 3)

Heparin at concentration above 1 u/ml significantly antagonised the inhibitory effect of PGI₂ on ADP-induced platelet aggregation. No such effect was observed with heparinoid.

Assessment of the effect of heparin and heparinoid on the rate of decay of PGI₂ activity in PPP and fatty acid free human albumin

The decay curves of PGI₂ obtained and the PGI₂ half-life were similar to those already described by us (Mikhailidis *et al.*, 1981; 1982a, b; 1983). There was no significant change in the rate of decay of PGI₂ activity in PPP or fatty acid free human albumin solutions when heparin or heparinoid were added. A slight tendency for more rapid decay at the highest concentration of heparin (6.4 u/ml) tested was noted.

Discussion

Our results show that heparinoid, *in vitro*, at concentrations likely to be achieved *in vivo* does not induce platelet aggregation or enhance platelet aggregation induced by ADP, adrenaline or collagen; neither did it antagonise the inhibitory activity of PGI₂. In contrast, heparin, at therapeutic concentrations, significantly enhanced platelet aggregation and antagonised PGI₂ activity. Neither heparin nor heparinoid induced platelet aggregation on their own.

Heparin is known to accelerate the biosynthesis of thromboxane A₂ (TXA₂) by platelets (Mohammed *et al.*, 1981). This may account for its enhancing effect on platelet aggregation on the one hand, and its antagonism of the anti-aggregatory effect of PGI₂ on the other (Eldor & Weksler, 1979; Holmer *et al.*, 1980; Hussey *et al.*, 1979; Macintyre *et al.*, 1981; Saba *et al.*, 1979). The absence of an effect on platelet function by heparinoid in our experiment would suggest that this agent, at the concentrations evaluated, does not significantly alter TXA₂ biosynthesis by platelets.

Our observations with heparin are in agreement with those of others who have reported that whereas heparin does not induce platelet aggregation, it does enhance aggregation and antagonise PGI₂ activity (Eldor & Weksler, 1979; Holmer *et al.*, 1980; Hussey *et al.*, 1979; Macintyre *et al.*, 1981; Saba *et al.*, 1979). Furthermore, it has been shown that a low molecular weight fragment of heparin appears to possess less ability

Table 1 Enhancement of platelet aggregation by heparin (thromboliquine) and heparinoid (Org 10172)

ADP induced platelet aggregation				
Heparin concentration (u/ml)	Median % aggregation obtained with heparin added	Median % aggregation obtained in control experiments	P	Number of subjects studied
0.5	9	7	NS	8
0.8	14	6	0.05	6
1.6	30	10	< 0.01	8
3.2	52	8	< 0.01	8
12.5	36	6	< 0.01	7

Heparinoid concentration (µg/ml)	Median % aggregation obtained with heparinoid added	Median % aggregation obtained in control experiments	P	Number of subjects studied
6.2	8	6	NS	7
16.0	6	10	NS	7
32.0	16	9	NS	8
64.0	6	6	NS	8
125.0	9	5	NS	7
250.0	16	7	NS	7

to enhance platelet aggregation (Holmer *et al.*, 1980).

Both heparin and heparinoid did not alter the rate of decay of PGI₂ in PPP or fatty acid free human albumin solutions *in vitro*. PGI₂ is stabilised by plasma and the plasma protein responsible for this effect is albumin (Mikhailidis *et al.*, 1981; 1982a, b; 1983; Orchard & Robinson, 1981; Pifer *et al.*, 1981; Wynalda & Fitzpatrick, 1980). Our experiments demonstrating the absence of a heparin or heparinoid effect on PGI₂ decay *in vitro* suggest that neither of these compounds directly interfere with the PGI₂-albumin interaction. This is in agreement with the work of others who, using physico-chemical techniques, concluded that heparin does not bind significantly to the albumin molecule (Marchiniak, 1977). Furthermore, the absence

of an effect by heparin or heparinoid on PGI₂ decay *in vitro* renders it unlikely that these agents directly influence the decay of PGI₂ *in vivo*. However, it is possible that both heparin and heparinoid affect PGI₂ decay *in vivo* by a different mechanism: both are known to activate lipoprotein lipase, which results in lipolysis and a rise of serum non-esterified fatty acid concentrations (Kakkar *et al.*, 1982). An increase in non-esterified fatty acid concentrations has been shown by us to accelerate PGI₂ decay *in vitro* (Mikhailidis *et al.*, 1981, 1983). In this setting fatty acids probably act by interfering with the PGI₂-albumin interaction in a manner similar to that observed in relation to the interference of binding of drugs with albumin following heparin administration (Routledge *et al.*, 1980).

Fatty acid release following lipolysis may also

Table 2 Enhancement of platelet aggregation by heparin (thromboliquine) and heparinoid (Org 10172)

	Median % aggregation with heparin/heparinoid added	Median % aggregation in control experiments	P	Number of subjects studied
<i>Adrenaline-induced aggregation</i>				
Heparin added (0.8 u/ml)	52	5	< 0.01	8
Heparinoid added (250 µg/ml)	6	4	NS	8
<i>Collagen-induced aggregation</i>				
Heparin added (0.8 u/ml)	47	7	< 0.01	8
Heparinoid added (250 µg/ml)	9	6	NS	8

Table 3 Effect of heparin and heparinoid on PGI₂-mediated inhibition of platelet aggregation

Heparin concentration (u/ml)	Median % aggregation obtained with heparin added	Median % aggregation obtained in control experiments	P	Number of subjects studied
0.5	17	13	NS	8
1.0	20	14	0.01	7
1.6	23	12	< 0.01	8
3.2	30	15	< 0.01	9
6.4	35	14	< 0.01	8
12.5	39	12	< 0.01	8

Heparinoid concentration (µg/ml)	Median % aggregation obtained with heparinoid added	Median % aggregation obtained in control experiments	P	Number of subjects studied
16	20	17	NS	7
32	17	15	NS	8
64	20	18	NS	8
125	18	17	NS	7
250	21	18	NS	7

be relevant in two other respects: fatty acids inhibit PGI₂ synthesis (Jeremy *et al.*, 1983) and also activate platelets (Burststein *et al.*, 1978; Connor *et al.*, 1963; Gjesdal *et al.*, 1976; Hoak *et al.*, 1963, 1967). Since heparin has a greater lipolytic effect than low molecular weight heparins (Kakkar *et al.*, 1982), it is likely that the latter would not have as marked adverse effects of PGI₂ metabolism or platelet function.

In conclusion, heparinoid (Organon 10172) did not enhance *in vitro* platelet aggregation, nor did it interfere with the anti-aggregatory effect of PGI₂. In these respects it is superior to the conventional heparin tested. Hence heparinoid may also be superior to heparin in clinical situations with potentially enhanced platelet aggregability. Two recent publications, one (Henny *et al.*, 1983) investigating thrombocytopaenia associated with

haemodialysis, and the other (Harenberg *et al.*, 1983) describing a case of heparin-induced thrombocytopaenia and thrombosis, support the view that in these situations heparinoid (Org 10172) is superior to conventional heparins. Other low molecular weight heparinoids (Huisse *et al.*, 1983; Pourrat *et al.*, 1983) have also recently been shown in various clinical situations to improve thrombocytopaenia induced by conventional heparins, including those where the heparin related thrombocytopaenia was thought to be mediated by an antibody mechanism (Chong *et al.*, 1972; Huisse *et al.*, 1983; Pourrat *et al.*, 1983).

D. P. Mikhailidis is a Wellcome Trust Fellow. The authors thank Mrs M. Elleman and Miss J. Lytle for secretarial assistance.

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(Received March 16, 1983,
accepted September 23, 1983)