Increased platelet responsiveness following coronary stenting

Heparin as a possible aetiological factor in stent thrombosis

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Aims Platelet activation may be a determinant of thrombotic and restenotic complications following intracoronary stenting. In order to measure the effect of stenting on platelet activation antigen expression we used whole blood flow cytometry in 18 patients undergoing Palmaz–Schatz stenting (treated with full anticoagulation) and compared these with a group of 18 patients undergoing elective angioplasty. The effects of low molecular weight heparin and unfractionated heparin on platelet behaviour were also studied, both in vitro and in vivo to determine the contribution of prolonged heparin therapy to platelet activation following stenting.

Methods and Results Fibrinogen binding to activated GPIIb-IIIa, and surface expression of P-selectin, GPIb and GPIIb-IIIa antigens were measured in unstimulated peripheral blood samples (rest) and on stimulation with adenosine diphosphate ($0 \cdot 1$–$10 \, \mu mol \cdot l^{-1}$) and thrombin ($0 \cdot 02$–$0 \cdot 16 \, U \cdot ml^{-1}$). No changes were seen in resting samples following angioplasty or stenting. Agonist responsiveness was unaltered after angioplasty, but in stented patients antigen expression in response to thrombin was significantly reduced ($P \leq 0 \cdot 04$), whilst the adenosine diphosphate response was significantly increased ($P = 0 \cdot 01$). Similar effects were observed in patients with unstable angina treated with either low molecular weight heparin or unfractionated heparin in vivo. In vitro, both unfractionated and low molecular weight heparin inhibited thrombin-induced platelet activation, but stimulation of adenosine diphosphate responses was more marked with unfractionated than low molecular weight heparin.

Conclusions There was a significant increase in platelet responsiveness to adenosine diphosphate following intracoronary stenting in patients treated with conventional anticoagulants. This was probably a consequence of treatment with heparin. Activation of platelets by heparin may explain the increased rate of stent thrombosis in patients treated with anticoagulant therapy. Low molecular weight heparins stimulate platelets less than unfractionated heparin.
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Key Words: Stents, angioplasty, platelets, heparin.

Introduction

Stent thrombosis is an important complication of intracoronary stent implantation, occurring in a significant minority of patients in early studies[1], despite the use of aggressive anticoagulation. The incidence of stent thrombosis has declined as implantation techniques have improved[2], and patients treated with antiplatelet (aspirin and ticlopidine), rather than anticoagulant (heparin and warfarin) therapy after stenting have been shown to be at reduced risk of stent thrombosis[3, 4].

It is not surprising that antiplatelet therapy should reduce the incidence of stent thrombosis. Pathological studies[5] in both porcine coronary arteries and in human saphenous vein grafts have shown that the earliest vascular response to stent implantation is extensive deposition of platelets. The beneficial clinical effects of ticlopidine suggest an important role for adenosine diphosphate-dependent, shear stress-induced platelet activation since ticlopidine, but not aspirin[6] inhibits the effect of adenosine diphosphate on platelets[7].

Table 1   Patient characteristics

Characteristic	Angioplasty patients n=18	Stent patients n=18
Mean age in years	56 ± 9·3 (39–79)*	63 ± 7·9 (49–79)
Males	17 (94%)	13 (72%)
Previous MI	9 (50%)	5 (28%)
Angiography		
One vessel disease	10 (56%)	6 (33%)
Two vessel disease	6 (33%)	10 (56%)
Three vessel disease	2 (11%)	2 (11%)
Impaired LV function	3 (17%)	1
Risk factors		
Smoking history	14 (78%)*	7 (39%)
Hypertension	4 (22%)	7 (39%)
Diabetes	0	1
Cholesterol ≥5·5 mmol . l⁻¹	11 (61%)	13 (72%)

$*=P<0.05$.

However, differences in outcome between patients treated with anticoagulant and antiplatelet therapy might be explained not only on the basis of the favourable pharmacological actions of ticlopidine, but also by adverse platelet effects of anticoagulant drugs. In particular, heparin has been reported to activate platelets[8–10] both in vitro and in vivo.

Direct assessment of expression of activation antigens on individual platelets can be measured by fluorescence-activated flow cytometry[11]. Platelet activation results in fibrinogen binding to the platelet membrane glycoprotein GPIIb-IIIa complex[12–14], and translocation of granule membrane glycoproteins such as a-granule P-selectin[15] to the platelet surface, both of which can be recognised with specific antibodies. Whole blood flow cytometry of unfixed blood minimizes handling artefacts and platelet losses during washing procedures, and allows the response of platelets to ex vivo stimulation with agonists such as thrombin and adenosine diphosphate to be studied in the presence of other blood components. Using whole blood flow cytometry we investigated platelet behaviour in patients treated with low molecular weight heparin and warfarin following coronary stenting, in comparison with a control group of angioplasty patients. We compared this with the platelet effects of unfractionated heparin and low molecular weight heparin in vivo in patients with unstable angina, and in vitro.

Methods

Subjects

Patients undergoing angioplasty or stenting
Thirty six patients were studied. All had stable angina pectoris and were scheduled for elective coronary angioplasty. Antianginal medication was standardized prior to admission so that all patients were taking a beta-

blocker and aspirin only. No patients were taking long-acting nitrates. Patient characteristics are shown in Table 1. Stented patients were slightly older than those having angioplasty (PTCA) ($P=0.02$), but there was no significant difference between the groups in terms of distribution of coronary artery disease or cardiovascular risk factors, with the exception of smoking history which was more common in the PTCA group ($P=0.041$). Patients underwent blood sampling on the morning prior to the procedure, and 24 h later on the following morning, at the same time after antianginal drug administration and approximately 2 h after administration of low molecular weight heparin. No patients smoked periprocedurally.

In all patients PTCA was performed from the right femoral artery using an 8 French sheath and guiding catheter. Visipaque (Nycomed (U.K.) Ltd., Birmingham, U.K.), a non-ionic contrast agent, was used in all patients. A single bolus of heparin (15 000 units) was administered at the start of the procedure. Procedural variables are shown in Table 2.

After initial dilatation, Palmaz–Schatz (PS153, Johnson and Johnson Interventional Systems, Warren, N.J., U.S.A.) stents were inserted if there was a large dissection visible at angiography, or if the result was considered suboptimal (with or without a small dissection). In one case, a stent was not implanted, despite a large dissection, because of diffuse distal disease. Balloons of 3·0 or 3·5 mm diameter were used predominantly in patients undergoing stenting, and 2·5 or 3·0 mm balloons in those undergoing PTCA alone. Patients undergoing stenting had a significantly longer procedural time ($P=0.06$), higher incidence of dissection ($P=0.002$) and greater contrast load ($P=0.07$) compared with PTCA patients. PTCA/stenting was successful in all cases and there were no major complications in any patient.

No further intravenous heparin was administered after the procedure and patients had their femoral sheaths removed 6 h after the procedure. Stent patients

Table 2 *Procedural variables*

	Angioplasty patients	Stent patients
Vessel dilated		
LAD	9 (50%)	8 (45%)
RCA	6 (33%)	4 (22%)
Circumflex	3 (17%)	6 (33%)
Coronary dissection		
Absent	15 (83%)	5 (28%)*
Small	2 (11%)	4 (22%)
Large	1	9 (50%)*
Balloon size		
1·5/2 mm	2 (11%)	0
2·5 mm	5 (28%)	0
3 mm	11 (61%)	10 (56%)
3·5 mm	0	7 (39%)
4 mm	0	1
Procedure time (min)	48 ± 17·4 (18–74)	74 ± 33·1 (38–151)*
Volume of contrast (ml)	190 ± 84 (50–375)	257 ± 127 (70–500)*

$*P<0.007$.

were started on warfarin, dipyridamole 100 mg three times daily (for 3 months) and low-molecular weight heparin (Fragmin, Pharmacia and Upjohn, Milton Keynes, U.K.) 5000 units twice daily subcutaneously (for 5 days) on the evening of the procedure. PTCA patients continued aspirin and the beta-blocker only.

Unstable angina patients receiving Heparin

The effects of heparin were studied in 11 patients admitted to the Royal Brompton Hospital with a clinical diagnosis of unstable angina. All patients had angiographic evidence of significant coronary artery disease (>70% stenosis in a major epicardial artery). Prior to sampling, medication was standardized to atenolol 50 mg, amlodipine 5 mg, aspirin 150 mg and either intravenous or oral nitrate. No patient had experienced chest pain or had heparin administered for the 24 h before the study, and all baseline clotting profiles were within normal limits.

Sampling was performed at baseline. Patients were then started on either intravenous unfractionated heparin (Heparin, Leo Pharmaceuticals, Princes Risborough, Bucks, U.K.) 1000 U/hour for 24 h (five male patients; mean age 59 years (range 49–66)) or 5000 units of low molecular weight heparin (Fragmin, Pharmacia and Upjohn, Milton Keynes, U.K.) subcutaneously twice daily for 24 h (six patients; five male, mean age 65 years (range 50–81)). The unfractionated heparin group were sampled at 24 h and the low molecular weight heparin group were sampled 4 h after the morning injection of low molecular weight heparin, coincident with the peak activity of the drug[16].

All patients gave written consent to participate in the studies, which were approved by the Ethical Practices Committee of the Royal Brompton Hospital.

Normal subjects (in vitro effects of Heparin)

The effects of unfractionated and low molecular weight heparins were studied in vitro using blood samples from six normal volunteers (two male, mean age 35 (range 24–59)). All were staff or students of the Royal Free Hospital and School of Medicine and all gave informed consent. All denied taking any platelet active drugs in the 2 weeks preceding the investigation. This part of the study was approved by the Ethical Practices Committee of the Royal Free Hospital.

Blood sampling

In all subjects, blood was drawn after 30 min rest. All blood samples were obtained by clean venepuncture, via a 21 gauge butterfly needle, into Monovette tubes (Sarstedt, Beaumont Leys, Leicester, U.K.). Minimal stasis was used and any repeated venepunctures were made distal to the initial site; conditions designed to avoid artefactual activation of platelets during phlebotomy. On each occasion the first 2·7 ml was drawn into an EDTA tube and used to obtain blood counts. The next 5 ml was aspirated into 1/10th volume 0·106 mmol . l^{-1} tri-sodium citrate and used, within 10 min, for the flow cytometric analysis. A final 10 ml of blood was collected into a serum/gel tube for analysis of cholesterol and lipoproteins.

Flow cytometric analysis of platelet activation

Reagents

Platelets were identified with a monoclonal antibody to GPIba (RFGP37) and GPIIb-IIIa was identified with a CD41 monoclonal antibody (RFGP56), both raised in our laboratory[17]. The monoclonal antibodies were purified from ascites on Protein G Sepharose (Pharmacia LKB, Milton Keynes, U.K.) and coupled to fluorescein isothiocyanate (FITC) using standard techniques[18]. Platelet-bound fibrinogen was detected with a FITC-conjugated polyclonal antibody to human fibrinogen (R$a$fgn-FITC), purchased from Dako Ltd (High Wycombe, UK) as described by and Janes *et al.*[14]. P-selectin was identified with a FITC conjugated IgG$_1$ mouse monoclonal antibody (CLB-thromb/6) obtained from Immunotech (Coulter Immunology, Luton, U.K.). All antibodies were used at optimum concentration for maximum fluorescence with minimum non-specific binding, determined by titration.

Adenosine diphosphate and human a-thrombin were purchased from Sigma Chemical Co. Ltd. (Poole, U.K.). Samples incubated with thrombin also contained 0·125 mmol . l^{-1} glycyl-L-prolyl-L-arginyl-L-proline (GPRP) peptide, (Sigma) to inhibit fibrin polymerization and consequent clot formation[19]. The dilution buffer used was N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid (HEPES)-buffered saline (145 mmol . l^{-1} NaCl, 5 mmol . l^{-1} KCl, 1 mmol . l^{-1} MgSO$_4$, and

Table 3   *The effect of angioplasty and stenting on platelet counts and size*

Patient group	Platelet count ($\times 10^9$ dl^{-1})		Mean platelet volume (fl)	
	Pre	Post	Pre	Post
Stented patients	219 ± 45	215 ± 51	9.5 ± 1.1	9.6 ± 0.9
Angioplasty patients	220 ± 41	221 ± 45	9.2 ± 0.9	9.4 ± 0.8

10 mmol . l^{-1} HEPES; pH 7·4) that had been passed through a 0·22 μm filter to remove dust particles.

Assay procedure

Blood samples were prepared for flow cytometric analysis using the whole blood method essentially as described by Janes *et al.*[14]. Five microlitres of citrated blood were added to LP3 tubes containing 50 μl of HEPES-buffered saline and 5 μl of appropriate concentrations of antibodies and agonists. After gentle mixing, the samples were incubated for 20 min, then diluted with 0·5 ml of 0·2% (v/v) formyl saline, to inhibit further activation. Incubations were carried out at room temperature (20–22 °C) and all samples were run in duplicate. Fibrinogen binding was measured in response to adenosine diphosphate (0·1–10 μmol . l^{-1}) and thrombin (0·02–0·16 units . ml^{-1}). P-selectin expression was measured in response to maximal adenosine diphosphate stimulation (10 μmol . l^{-1}) and to thrombin (0·02–0·16 units . ml^{-1}).

The effects of unfractionated and low molecular weight heparin were studied in vitro. In these experiments, blood from normal volunteers was diluted and incubated as described above but parallel tubes also contained unfractionated heparin or low molecular weight heparin at 0–5 units . ml^{-1}. Fibrinogen binding was measured in unstimulated samples and in response to adenosine diphosphate (0·1–10 μmol . l^{-1}) and thrombin (0·04–0·16 units . ml^{-1}).

Samples were analysed, within 2 h of collection, in a Coulter EPICS Profile II flow cytometer (Coulter Electronics Ltd., Luton, U.K.). The instrument was aligned daily with 'Immuno Check' and 'Standard Brite' beads (Coulter Immunology) to calibrate the light scatter and fluorescence parameters respectively. The platelet population was identified from its light scatter characteristics and its identity confirmed using the anti-GPIba monoclonal antibody. An electronic bitmap was set around the platelet population and adjusted for each sample to ensure that >98% of the particles analysed were positive for GPIba. The negative cut-off levels for fluorescence in unstimulated samples were set at 1%[14]. Five thousand platelets were analysed and the results represent the means of duplicate samples. Results are expressed as a binding index (BI) calculated from the percentage of platelets positive for the marker and the mean fluorescence intensity (MFI) for each sample from the following equation: BI = (percent positive × MFI) × 100^{-1}.

Statistical analysis

All data are shown as mean ± standard deviation. Differences between patient/procedural variables were calculated using either the Student's t test or Fisher's exact test. Differences between flow cytometric dose-response curves were calculated using non-parametric (Wilcoxon) tests. Data were analysed on Graph Pad InStat software.

Results

Haematology

Haemoglobin and blood cell counts were normal in all patients. Platelet counts were normal in all patients and were not significantly affected by stenting or PTCA (Table 3) or heparin administration (data not shown).

The effect of angioplasty and stenting

Baseline platelet activation status

There was no significant change in either the level of fibrinogen binding, expression of P-selectin, GPIba or GPIIb-IIIa before and after PTCA or stent implantation (Table 4).

Platelet responsiveness to agonist stimulation

Fibrinogen binding in response to stimulation with thrombin (0·02–0·16 units . ml^{-1}), was significantly reduced ($P=0.02$) after stenting (Fig. 1(a)) but not after PTCA (Fig. 1(b)). A different pattern for fibrinogen binding was seen, however, in response to stimulation with adenosine diphosphate (0·1–10 μmol . l^{-1}), with significant enhancement ($P=0.01$) of adenosine diphosphate responsiveness following stenting (Fig. 2(a)), but no effect after PTCA (Fig. 2(b)).

P-selectin expression showed a similar pattern to that seen for fibrinogen binding. The response to thrombin was inhibited following stenting (Fig. 3(a)), but not following PTCA (Fig. 3(b)), whilst P-selectin expression in response to maximal adenosine diphosphate stimulation (10 μmol . l^{-1}) was significantly increased after stenting ($P=0.01$), but not after PTCA (Fig. 4).

Table 4(a)   *Baseline platelet activation status before and after angioplasty*

Antigen	Before angioplasty	After angioplasty
Fibrinogen	0.08 ± 0.05	0.08 ± 0.08
P-selectin	0.04 ± 0.02	0.04 ± 0.03
GPIb	13.06 ± 2.57	13.24 ± 2.70
GPIIb–IIIa	16.03 ± 2.54	15.77 ± 2.33

Table 4(b)   *Baseline platelet activation status before and after stenting*

Antigen	Before stenting	After stenting
Fibrinogen	0.07 ± 0.03	0.08 ± 0.05
P-selectin	0.05 ± 0.03	0.06 ± 0.02
GPIb	13.97 ± 2.43	13.96 ± 2.15
GPIIb–IIIa	14.03 ± 2.83	14.72 ± 2.02

The effect of unfractionated and low molecular weight heparin in vivo

Both low molecular weight and unfractionated heparin produced similar effects in vivo on fibrinogen binding and P-selectin expression to those seen in the stented patients. Low molecular weight heparin did not affect baseline levels of fibrinogen binding (0.06 ± 0.04 pre and 0.06 ± 0.01 post; $P>0.5$) but unfractionated heparin caused an increase (0.27 ± 0.09 pre and 0.43 ± 0.09 post; $P=0.01$). As shown in Figs 5 and 6, fibrinogen binding induced by thrombin was inhibited, whilst adenosine diphosphate-induced fibrinogen binding was increased. Unfractionated heparin had a greater effect than low molecular weight heparin. Similar effects were seen for P-selectin expression (data not shown). There were no changes in the total expression of GPIIb-IIIa or GPIb.

In vitro studies

When unfractionated heparin and low molecular weight heparin were added to the whole blood assay at concentrations ranging from 1 to 5 units . ml^{-1}, in the absence of agonist (Fig. 7(a)), unfractionated heparin caused a dose-dependent increase in fibrinogen binding to a maximum at concentrations of 2.5 units . ml^{-1} and above. These levels represent approximately 10% of platelets positive for bound fibrinogen. In contrast, low molecular weight heparin had no effect on platelet fibrinogen binding at any concentration in the absence of other agonists.

In the presence of a low concentration of adenosine diphosphate ($0.1\ \mu$mol . l^{-1}) sufficient to cause an increase in the percentage of positive cells from 2.3 ± 1.2 to 18.5 ± 6.1%, unfractionated heparin further enhanced activation, reaching a maximum of 60% positive at 1 unit . ml^{-1} and above (Fig. 7(b)), whereas low molecular weight heparin had no significant effect. The difference between the effects of the two agents was statistically significant at all concentrations above 0.5 units . ml^{-1} ($P<0.5$).

At a higher adenosine diphosphate concentration (1 μmol . l^{-1}) unfractionated heparin increased fibrinogen binding from 4.6 ± 3.8 to 82.3 ± 13.2% at 1 unit . ml^{-1}. At this adenosine diphosphate concentration low molecular weight heparin also caused a small, dose-dependent rise in activation, although this difference did not reach statistical significance (Fig. 7(c)). At adenosine diphosphate concentrations of 10 μmol . l^{-1} neither unfractionated nor low molecular weight heparin increased the already maximal stimulation of fibrinogen binding (data not shown).

Both unfractionated heparin and low molecular weight heparin caused a total inhibition of platelet activation induced by thrombin (0.16 units . ml^{-1}) over the same concentration range that caused activation of unstimulated and adenosine diphosphate-stimulated samples (Fig. 7(d)).

Discussion

We have shown a significant increase in platelet adenosine diphosphate responsiveness, 24 h following elective coronary Palmaz–Schatz stenting in patients treated with low molecular weight heparin, dipyridamole and warfarin. This increase was not observed after PTCA alone.

Whole blood flow cytometry has been shown previously to be able to detect changes in platelet behaviour in a variety of clinical settings[20] and in response to physiological stress[21] and pharmacological treatment[22]. Despite the sensitivity of the technique, the lack of a measurable rise in platelet activation in peripheral blood samples following PTCA is perhaps not surprising, given the small size of the plaque event and the rapid clearance of activated platelets from the
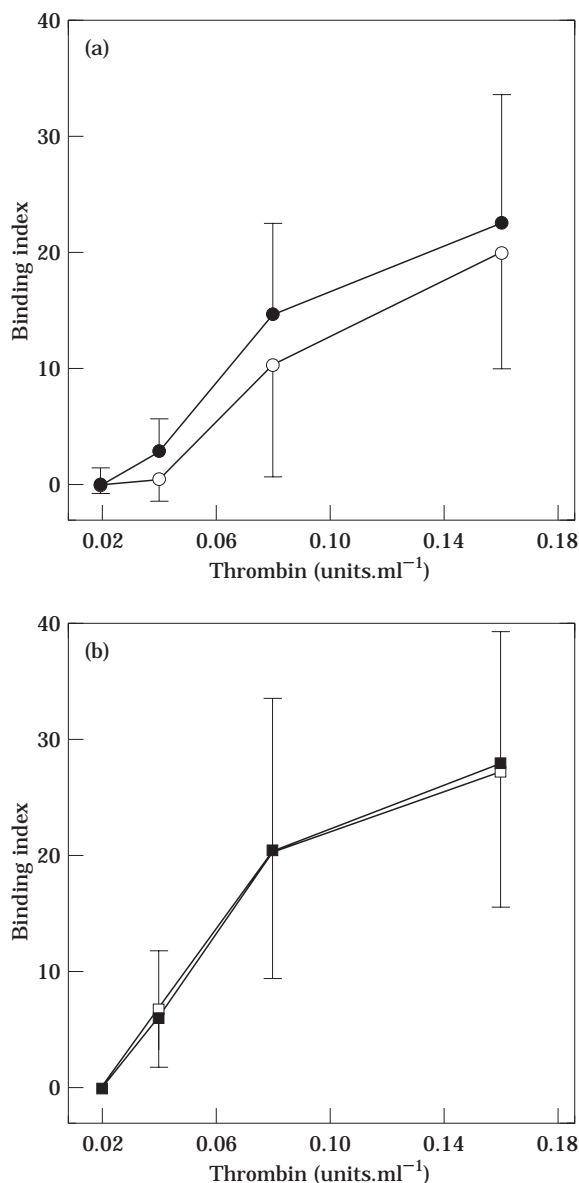
Figure 1 **Fibrinogen binding to platelets stimulated in whole blood with thrombin. Data expressed as mean ± SD. (a) Fibrinogen binding in 18 patients before (●) and after (○) elective stent implantation; (b) fibrinogen binding in 18 patients before (■) and after (□) angioplasty.**
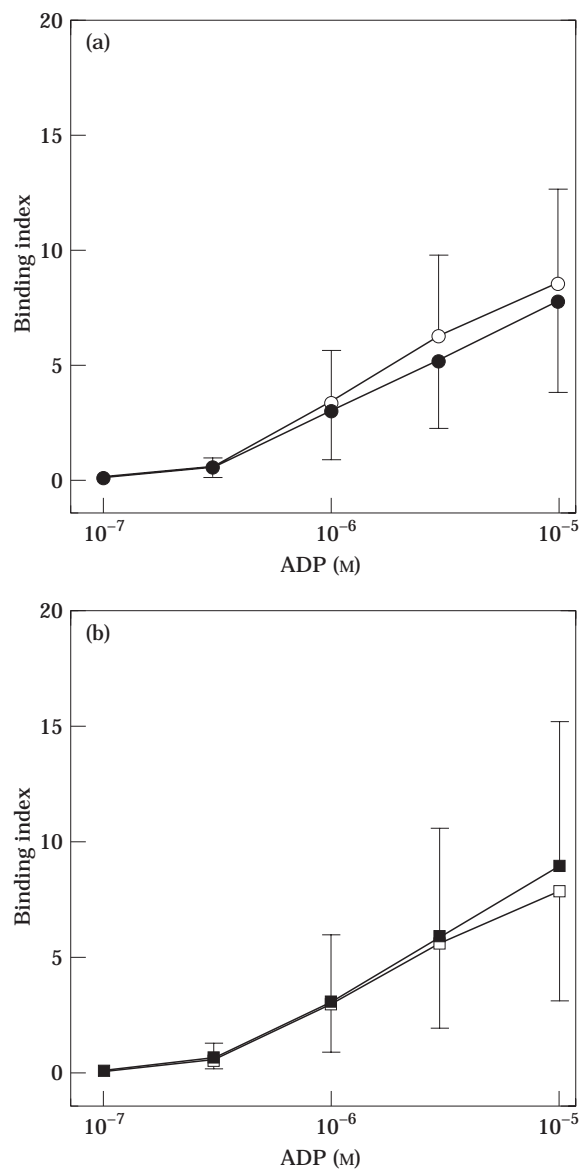
Figure 2 **Fibrinogen binding to platelets stimulated in whole blood with adenosine diphosphate (ADP). Data expressed as mean ± SD. (a) Fibrinogen binding in 18 patients before (●) and after (○) elective stent implantation; (b) fibrinogen binding in 18 patients before (■) and after (□) angioplasty.**

circulation. The majority of flow cytometric studies that have previously reported platelet activation following PTCA have used local sampling techniques, either from the coronary sinus[23,24], or directly from the coronary artery[25]. In contrast to our present findings, Kolarov *et al.*[26], using flow cytometry in peripheral blood, reported both a significant reduction in platelet count and increased platelet activation following PTCA, but sampling was performed much earlier (2 h post PTCA), at a time when platelet behaviour might still be affected by periprocedural anticoagulation. Gawaz *et al.*[27] showed no significant changes in platelet activation in

peripheral blood samples from patients undergoing elective PTCA, although changes were observed in patients undergoing PTCA for acute myocardial infarction. Our results would therefore concur with most of these studies, in that peripheral blood sampling, using flow cytometry, fails to detect increased platelet activation as a consequence of the arterial injury caused by elective PTCA. However, in situations where there is a greater degree of intraluminal thrombus, such as PTCA in acute myocardial infarction, or where blood samples are drawn from close to the plaque, increased platelet activation is detectable with this method.
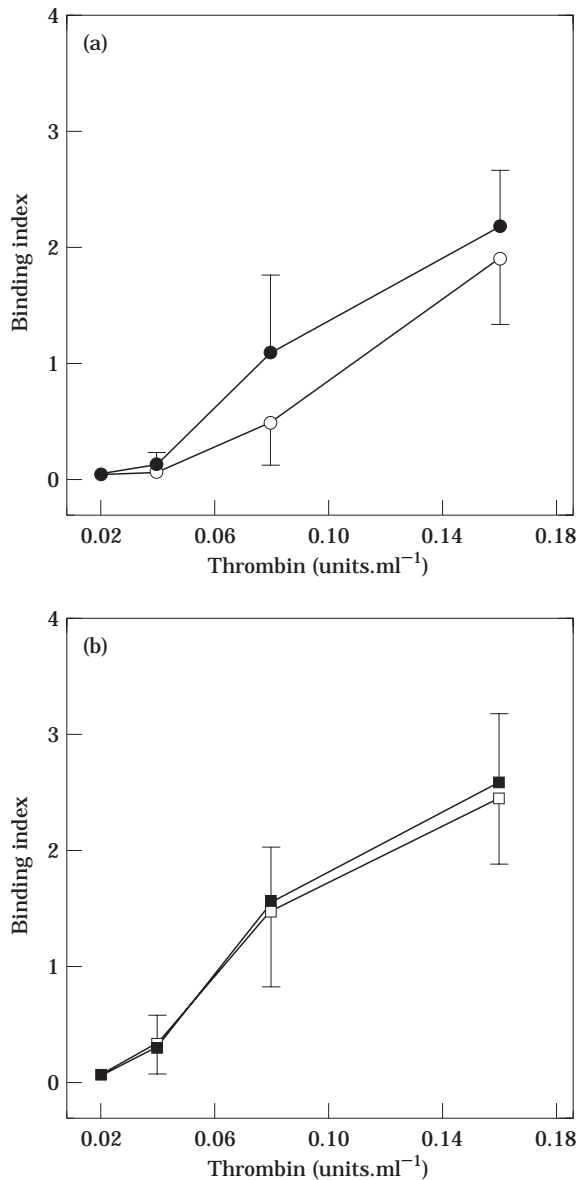
Figure 4 P-selectin expression on platelets stimulated in whole blood with $10 \, \mu mol . l^{-1}$ adenosine diphosphate. Data expressed as median $\pm$ interquartile range (box) and range (whisker).

Figure 3 **P-selectin expression on platelets stimulated in whole blood with thrombin. Data expressed as mean $\pm$ SD. (a) P-selectin expression in 18 patients before (●) and after (○) elective stent implantation. (b) P-selectin expression in 18 patients before (■) and after (□) angioplasty.**

In contrast, in those patients undergoing intracoronary stenting, a significant reduction was observed in the platelet response to thrombin, but platelet responses to adenosine diphosphate were enhanced. This might be a consequence of the stenting procedure itself or of the different drug treatment used in stented patients.

Patients who had stents implanted received more non-ionic contrast agent than those patients undergoing PTCA alone. Non-ionic agents may cause platelet degranulation to a greater extent than low osmolar ionic agents[28]. However, the pattern of activation see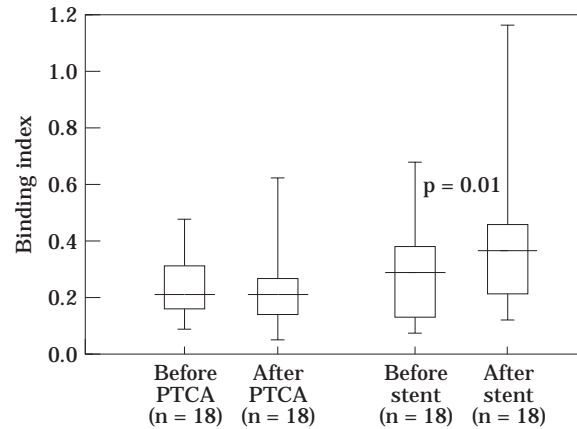n with such contrast agents (increased P-selectin expression without significant rises in fibrinogen binding) differs from that seen in the present study and their effects are only seen at high concentration. It is also unlikely that these effects would persist for 24 h. Increased platelet activation in the stented patients might reflect the higher incidence of coronary dissection in these patients. We did not, however, find any increase in platelet responsiveness in stented patients with large dissections compared to those without. It seems unlikely therefore that procedural factors can account for the changes that we observed in platelet behaviour after stenting.

Following stenting, but not PTCA, patients received a combination of dipyridamole, warfarin and low molecular weight heparin. Whilst the current report cannot differentiate the platelet effects of the individual drugs from this combination, there is powerful indirect evidence that the effects seen after stenting are a consequence of treatment with heparin rather than the other agents, as we found similar changes in platelet agonist responsiveness following the administration of both unfractionated and low molecular weight heparins in vivo in other patient groups, where all other drug therapy was controlled. Furthermore, heparin has been known to activate platelets since Eika reported enhanced platelet aggregation with the drug in 1972[8]. Subsequently, heparin has been shown to induce platelet aggregation in vitro in platelet-rich plasma[9,29] and using the same techniques, similar effects have been observed in vivo[10]. Heparin increases platelet interaction with fibrin-rich clots[30] and augments P-selectin expression in response to agonist stimulation in patients undergoing cardiopulmonary bypass[31]. Heparin-induced platelet activation may be mediated by a number of mechanisms including thromboxane generation[32,33], antagonism of the inhibitory effects of prostaglandins[34,35] and attenuation of the platelet inhibitory effects of endogenous nitric oxide[36].
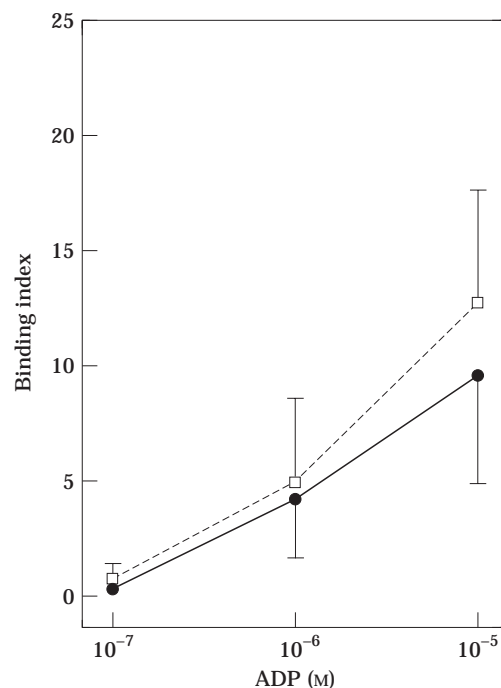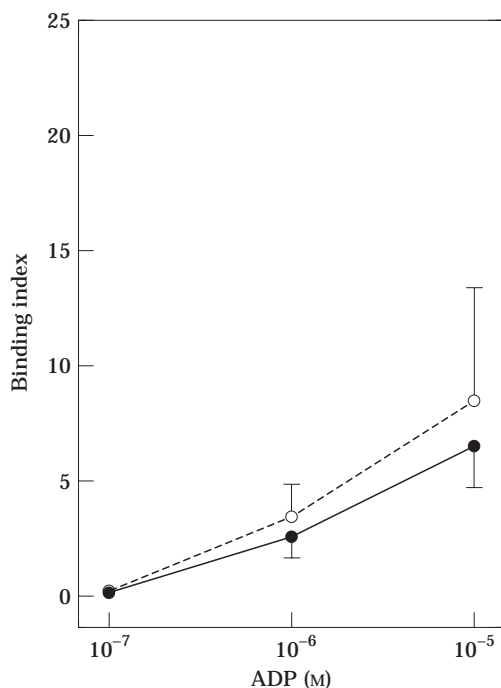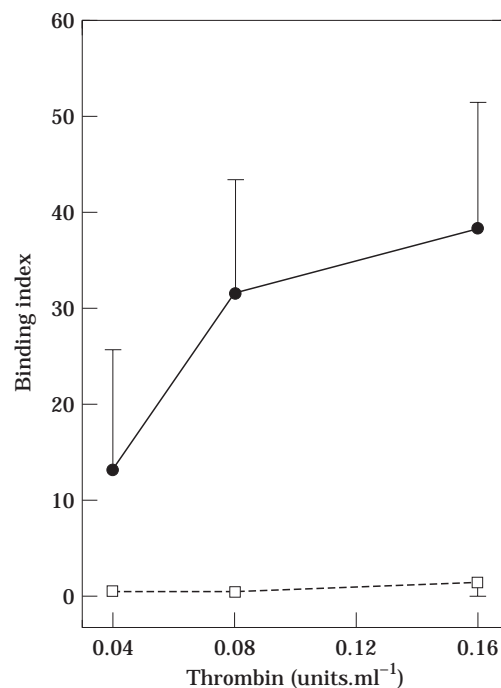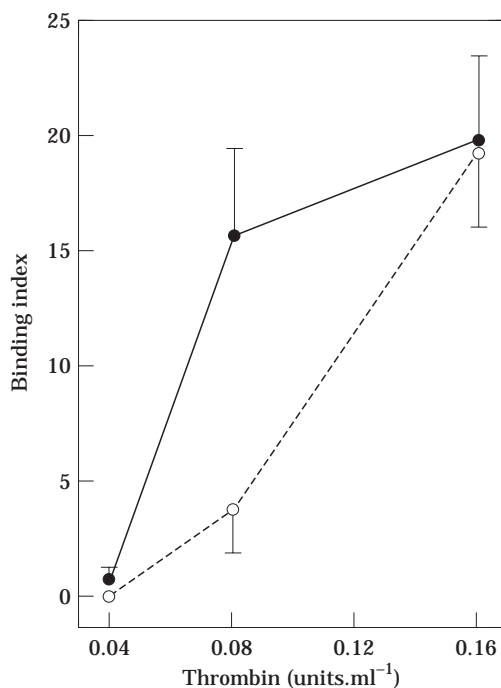
Figure 5 **Fibrinogen binding in response to thrombin and adenosine diphosphate (ADP) to platelets from six patients with unstable angina before (●) and 4 h after (○) administration of 5000 units low molecular weight heparin (Fragmin). Data expressed as meanA ± SD.**

Figure 6 **Fibrinogen binding in response to thrombin and adenosine diphosphate (ADP) to platelets from five patients with unstable angina before (●) and 12 h after (○) administration of unfractionated heparin (1000 units . h⁻¹). Data expressed as mean ± SD.**

Binding of heparin to platelets increases with increasing molecular weight[37], and lower molecular weight heparins may have less effect on platelet aggregation than unfractionated heparin[9,38,39]. Our in vitro studies, using whole blood flow cytometry, would also support the hypothesis that low molecular weight heparin induces less platelet activation to adenosine diphosphate than unfractionated heparins. This was not the result of the lower dose of low molecular weight heparin used as the differences persist at equivalent
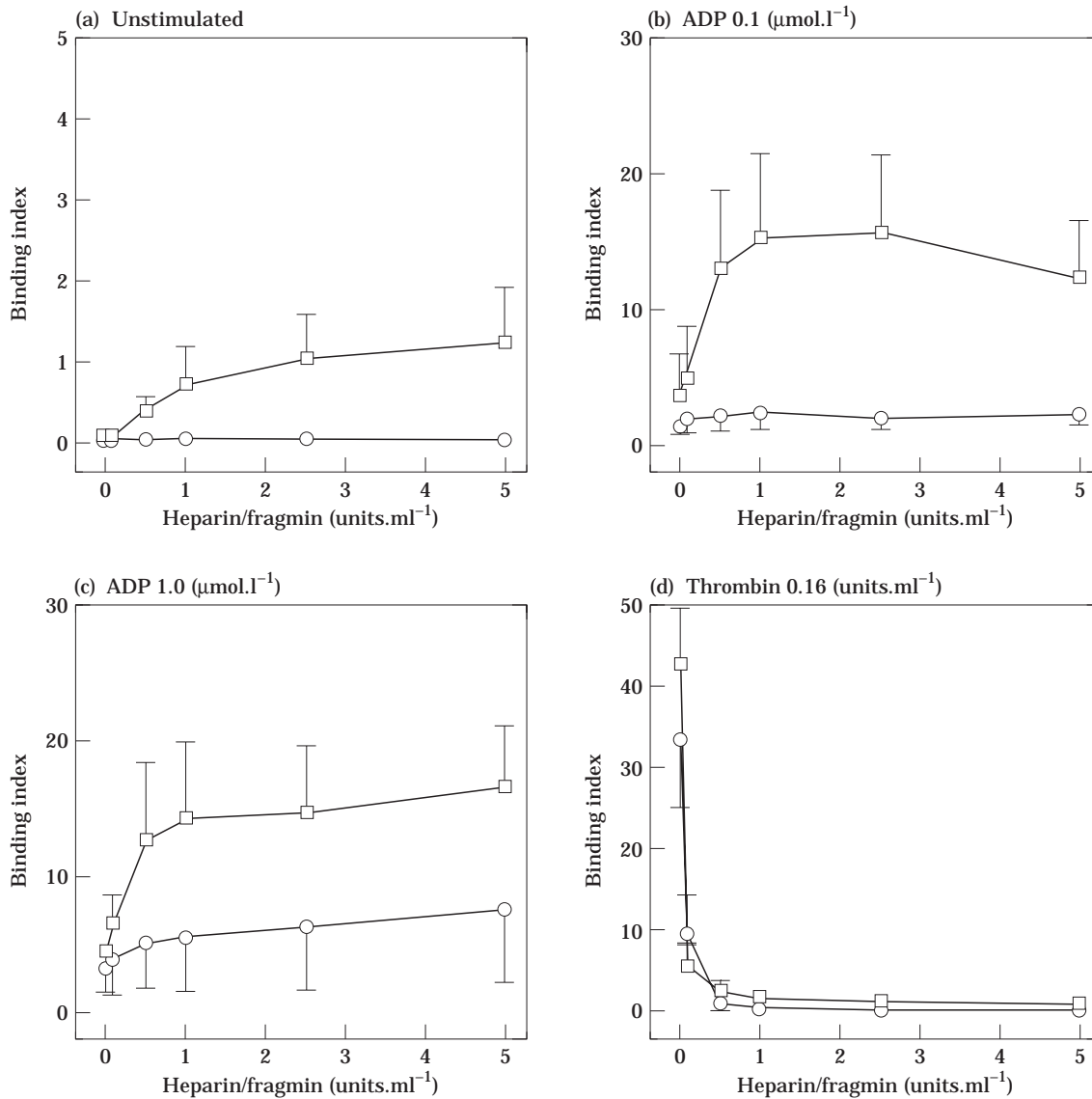
Figure 7 **Fibrinogen binding to platelets from normal subjects (n=6) in (a) unstimulated samples or in response to (b) 0·1 μmol . l^{-1} adenosine diphosphate (ADP) or (c) 1·0 μmol . l^{-1} adenosine diphosphate or (d) 0·16 u . ml^{-1} thrombin in the presence of low molecular weight (○) or unfractionated heparin (□). Data is expressed as mean ± SEM.**

doses of unfractionated and low molecular weight heparins.

Previously, it has been believed that the risk of stent thrombosis in patients receiving anticoagulant therapy is at its highest when heparin is withdrawn a few days after the procedure. However, in a recent study by Schomig *et al.*[3], the majority (71%) of cardiac events in the anticoagulant-treated group occurred within the 10 day period of intravenous heparin therapy. It is possible that some of these events may have occurred, at least in part, as a consequence of the pro-activatory effects of heparin on platelets. The demonstration of a reduction in cardiac events with antiplatelet (aspirin/ticlopidine) compared with anticoagulant regimes in this study[3] also highlights the important role of platelet activation

in the genesis of stent thrombosis. It would therefore seem reasonable to avoid the administration of drugs known to have adverse effects on platelets in this setting. Even in patients scheduled to receive antiplatelet therapy after stenting, heparin is still administered at the time of the procedure. Further reductions in the thrombotic complications of angioplasty and stenting might therefore accrue from the use of alternative antithrombin agents such as hirudin[40], or if heparin continues to be used, from the use of low molecular weight rather than unfractionated heparins.

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