Increased generation of platelet-derived microparticles following percutaneous transluminal coronary angioplasty

Judy A. Craft and Neville A. Marsh

Platelet-derived microparticles that are produced during platelet activation bind to traumatized endothelium. Such endothelial injury occurs during percutaneous transluminal coronary angioplasty. Approximately 20% of these patients subsequently develop restenosis, although this is improved by treatment with the anti-platelet glycoprotein IIb/IIIa receptor drug abciximab. As platelet activation occurs during angioplasty, it is likely that platelet-derived microparticles may be produced and hence contribute to restenosis. This study population consisted of 113 angioplasty patients, of whom 38 received abciximab. Paired peripheral arterial blood samples were obtained following heparinization and subsequent to all vessel manipulation. Platelet-derived microparticles were identified using an anti-CD61 (glycoprotein IIIa) fluorescence-conjugated antibody and flow cytometry. Baseline clinical characteristics between patient groups were similar. The level of platelet-derived microparticles increased significantly following angioplasty in the group without abciximab (paired *t* test, $P = 0.019$). However, there was no significant change in the level of platelet-derived microparticles following angioplasty in patients who received abciximab, despite requiring more complex angioplasty procedures. In this study, we have demonstrated that the level of platelet-derived microparticles increased during percutaneous transluminal coronary angioplasty, with no such increase with abciximab treatment. The increased platelet-derived microparticles may adhere to traumatized endothelium, contributing to re-occlusion of the arteries, but this remains to be determined. *Blood Coagul Fibrinolysis* 14: 719–728 © 2003 Lippincott Williams & Wilkins.

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School of Life Sciences, Queensland University of Technology, Brisbane, Australia.

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Correspondence and requests for reprints to Judy Craft, Department of Biological and Physical Sciences, The University of Southern Queensland, Toowoomba 4350, Australia.
E-mail: craft@usq.edu.au

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Introduction

Platelet-derived microparticles (PMPs) are produced by a variety of in-vitro stimuli, such as thrombin, collagen [1,2], high shear stress [3,4] and artificial surfaces [5], while thromboxane A_2 and adenosine diphosphate (ADP) do not alter PMP numbers [6].

The PMP membrane is similar to that of platelets, as PMPs produced from normal human blood contain glycoprotein (GP) Ib [7,8] and the GPIIb/IIIa complex [8,9], indicating potential roles in adhesion and aggregation. P-selectin is present on PMPs following C5b-9 assembly [10] or activation by thrombin with collagen [2], suggesting that P-selectin is shed from the platelet surface during PMP formation, although PMPs can be generated without α-granule secretion [7,11]. PMPs also contain activated factor IX [12], factor VIII [13], activated factor X [14] and activated factor V [10], suggesting that PMPs possess tenase and prothrombinase activity and thus contribute to blood coagulation. As the activated factor IX was more concentrated on PMPs than platelets relative to surface area [12], PMPs may in fact be proportionally more procoagulant than platelets.

Elevated levels of PMPs occur in a number of cardiovascular disorders, including small and large vessel cerebrovascular accidents [15], unstable angina [16], and patients with a recent myocardial infarction [17]. In addition, idiopathic thrombocytopaenia purpura patients with the highest levels of PMPs were asymptomatic, indicating the PMP protective benefit from bleeding [18].

Percutaneous transluminal coronary angioplasty (PTCA) is a widely used treatment of coronary artery disease. However, the balloon inflation during PTCA is sufficient to cause endothelial damage [19], thus exposing the subendothelial components including the basement membrane and collagen. This can lead to platelet activation and restenosis. Although adverse coronary events occur in approximately 20% of patients [20,21], the use of antiplatelet medication such as abciximab (anti-platelet GPIIb/IIIa complex) decreases this to 16.5% [21].

It is possible that during PTCA the resultant platelet activation also causes PMP production. This may have clinical importance for PTCA patients, as it is known

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that PMPs are procoagulant and have roles in adhesion and aggregation, and thus PMPs may contribute to restenosis after PTCA. Accordingly, the present study was designed to determine whether the levels of PMPs were altered during PTCA, and to examine any influence of abciximab on this process.

Methods

Patient population

The study population consisted of 113 patients undergoing elective PTCA, and the patient characteristics are presented in Table 1. The antiplatelet agent abciximab (ReoPro) was administered to 38 of these patients. Subjects were not randomized to abciximab or placebo treatment in this observational study due to the ethical implications of preventing high-risk patients from receiving abciximab treatment. Therefore, abciximab was administered in accordance with the standard guidelines for administration (namely, high risk of abrupt vessel closure). A bolus dose of heparin was administered to all patients prior to PTCA, with non-abciximab patients receiving 110 U/kg heparin and abciximab patients receiving 100 U/kg heparin. The standard abciximab dosing regime of 0.25 mg/kg bolus and a 10 μg/min 12 h infusion was used, with the bolus dose being administered prior to completion of PTCA. All patients were receiving ongoing aspirin treatment in conjunction with clopidogrel used in the month following PTCA. Relevant patient medical history was obtained, while progress information was documented at 1 month ± 1 week, 6 months ± 2 weeks, and 12 months ± 4 weeks after PTCA for incidence of angina, PTCA, coronary artery bypass grafting (CABG) or death. Experience of angina was documented by means of a qualitative questionnaire recording incidence of chest pain. Informed consent was obtained from all participants, and research was performed with approval from The Prince Charles Hospital and Queensland University of Technology human research ethics committees.

Blood collection and preparation

Paired peripheral arterial blood samples from the PTCA sheath were collected into 0.105 mol/l sodium citrate anticoagulant tubes. The first sample was obtained following heparinization (baseline sample), while the second sample was taken subsequent to all balloon inflations and stent placement (post-PTCA sample), and this sample contained abciximab when administered. In order to avoid a possible artifactual increase in PMPs due to heparin, all blood samples in this study were obtained following patient heparinization. Only samples taken from the sheath during PTCA were obtained and tested, as the higher shear forces of sampling by venipuncture at other times would make such samples incomparable with those obtained through the wider lumen of the PTCA sheath. Whole blood was diluted (1/10) in Dulbecco's phosphate buffer, and then added in equal quantities to undiluted monoclonal antibodies and incubated in the dark for 3 min. A further dilution in 500 μl Dulbecco's phosphate buffer was followed by immediate flow cytometry. These minimal incubation times allowed flow cytometry of samples at approximately 5 min *ex vivo*.

Table 1 Comparison of patient characteristics between the patient groups without abciximab and with abciximab

Characteristic	Without abciximab ($n = 75$)	With abciximab ($n = 38$)	*P* value
Age (years)	62.9 ± 1.14	61.0 ± 1.96	NS
Male gender	62 (82.7%)	31 (81.6%)	NS
Coronary risk factors:			
Hypertension	46 (61.3%)	21 (55.3%)	NS
Hyperlipidaemia	64 (85.3%)	27 (71.1%)	NS
Diabetes			NS
Type I (IDDM)	1 (1.3%)	0 (0.0%)	
Type II (NIDDM)	5 (6.7%)	3 (7.9%)	
Smoking			NS
Never smoked	22 (29.3%)	12 (31.6%)	
Quit at least 1 month prior	43 (57.3%)	22 (57.9%)	
Smoking within 1 month	10 (13.3%)	4 (10.5%)	
Previous myocardial infarction	32 (42.7%)	17 (44.7%)	NS
Previous percutaneous transluminal coronary angioplasty	18 (24.0%)	3 (7.90%)	0.043
Previous coronary artery bypass graft	12 (16.0%)	5 (13.2%)	NS
Medications			
Lipid lowers	47 (62.7%)	21 (55.3%)	NS
β-blockers	41 (54.7%)	25 (65.8%)	NS
Ion channel modifiers	34 (45.3%)	20 (52.6%)	NS
Peripheral vasodilators	34 (45.3%)	27 (71.1%)	0.010

Age is expressed as mean ± standard error of mean. All other characteristics are expressed as number of patients and percentage of the patient group. *P* values from chi-squared tests for age, diabetes and smoking, and Fisher's exact tests for other variables. IDDM, insulin-dependent diabetes mellitus; NIDDM, non-insulin-dependent diabetes mellitus; ns, not significant.

As a positive control for the PMP levels, a sample of plasma was obtained by centrifugation at $3000 \times g$ for 10 min. This was then subjected to a twice freeze–thaw cycle, and diluted and stained as for the normal whole blood. The purpose of this procedure was to produce PMPs in significant numbers by freezing and thawing, thus providing biological material with which to validate the flow cytometry analysis of PMPs. A positive control used for the PMP levels and the expression of CD62P and PAC1 was by addition of 100 μmol/l ADP (Helena Laboratories, Beaumont, Texas, USA) during antibody incubation.

Monoclonal antibodies

Peridinin chlorophyll protein-conjugated anti-CD61, fluorescein isothiocyanate-conjugated anti-PAC1, and phycoerythrin-conjugated anti-CD62P were purchased from Becton Dickinson (San Jose, California, USA). These were used to identify the platelet membrane GPIIIa (anti-CD61), the platelet membrane GPIIb/IIIa complex on activated platelets (anti-PAC1), and the membrane protein P selectin (GMP-140, PADGEM) that is released upon α-granule secretion (anti-CD62P). Anti-CD61 was used to identify PMPs and platelets as it is known that PMPs express GPIIIa [7], and was therefore used in every tube. For measurement of CD62P and PAC1, triple staining in combination with CD61 was used.

To ensure that abciximab did not interfere with the binding of CD61, in-vitro testing was performed using 12 of the blood samples from the PTCA patients consented to participate in this study. Heparinized blood samples were obtained and paired aliquots were treated simultaneously with or without abciximab added *in vitro* (at the same dose normally administered *in vivo*), followed by incubation with CD61. Samples were otherwise treated in accordance with other samples in this study. There was no significant difference in the level of PMPs between the CD61 tubes and the CD61 with abciximab tubes (mean ± standard error of mean: 4.86 ± 0.48, CD61 only; 4.47 ± 0.49, CD61 with abciximab; paired t test, $P =$ not significant). This indicated that abciximab did not limit the binding of CD61 to the PMPs.

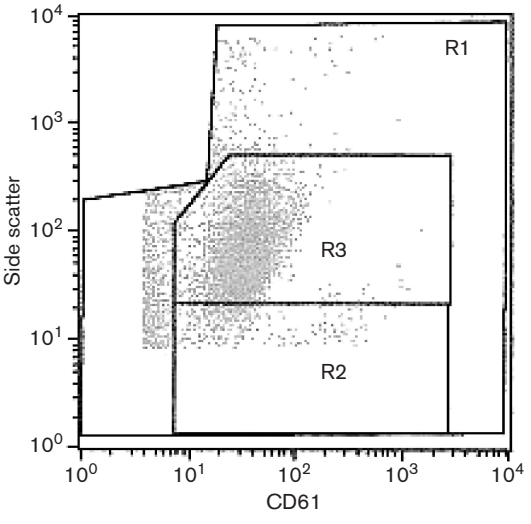
Flow cytometry

Prepared blood was tested on a FACScan flow cytometer (Becton Dickinson), which was calibrated daily using CaliBRITE three-colour beads and FACSComp software (version 4.0, © 1995–97 Becton Dickinson). Data acquisition and analysis was performed with CELLQuest software (version 3.1, © 1994–97 Becton Dickinson). The flow cytometer was set with the side scatter (SSC) and fluorescence voltages on a logarithmic (log) scale, and data acquired using a side scatter threshold on a dot plot of CD61-log against SSC-log. A region (R1) was set to acquire all data except that which was CD61-negative with a high SSC profile (leukocytes), with files containing 10 000 events. Analysis was performed using a histogram of CD61-log against counts to set a gate for the CD61-positive events. These events were collected into a CD61-log against SSC-log dot plot, where a region was set around the PMP population (R2), and platelets (R3) were also distinguished (Fig. 1). PMPs were identified as those particles smaller than the unstimulated platelets that were CD61-positive. The amount of PMPs was expressed as a percentage of all CD61-positive events (PMP%). For other indicators of platelet activation, dot plots of PAC1-log and CD62P-log against CD61-log were used (Fig. 2), and PAC1 and CD62P expressed as a percentage of all CD61-positive events.

Statistical analysis

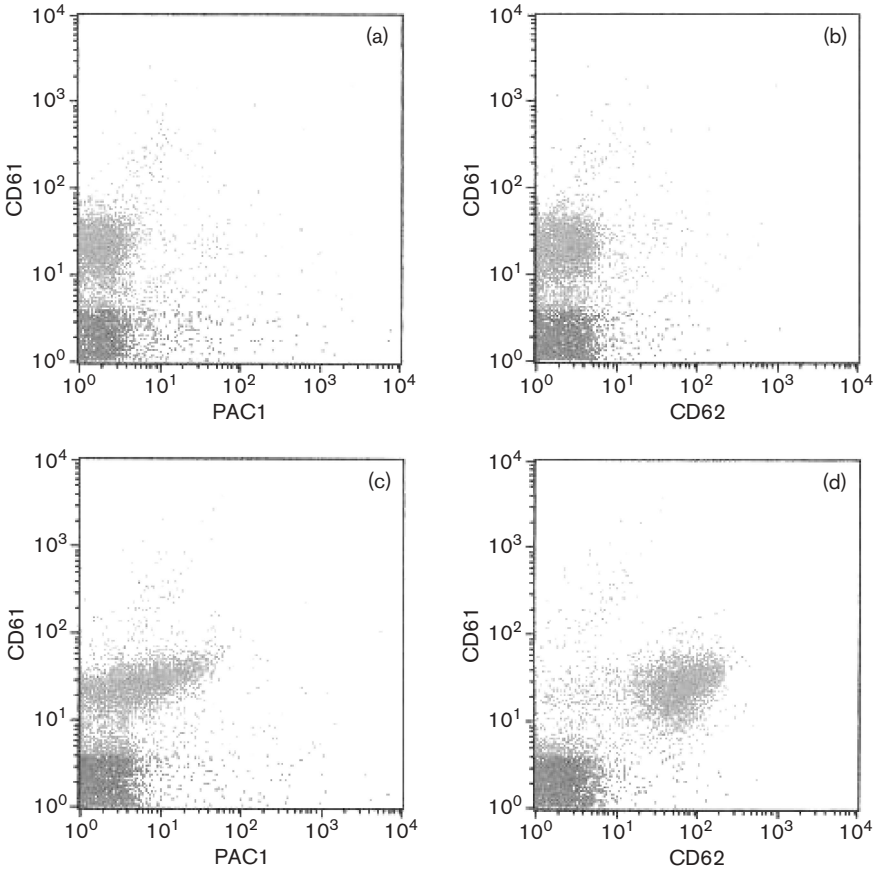
Statistical analysis of patient baseline clinical characteristics between the two groups was performed using Fisher's exact tests for gender, hypertension, hyperlipidaemia, medications, prior myocardial infarction, prior PTCA and previous CABG, while chi-squared testing was used for age, diabetes and smoking. Characteristics of the PTCA procedure were compared between the two patient groups, with arterial approach, number of arteries treated, rotablation and occlusion aspiration compared by chi-squared testing, while the number of stents, number of inflations, the maximum inflation

Fig. 1



Representative flow cytometry acquisition and analysis dot plot. R1 was set to acquire all data except that which was CD61-negative with high side scatter profile. R2 was set around the platelet-derived microparticles, and R3 was set around the platelets. The dot plot is taken from one patient; the baseline sample is without abciximab treatment.

Fig. 2



Acquisition dot plots of (a), (c) PAC1-log and (b), (d) CD62P-log against CD61-log. (a) and (b) unstimulated samples, (c) and (d) same samples after addition of in-vitro adenosine diphosphate. Dot plots are taken from one patient; the baseline sample is without abciximab treatment.

pressure and time, as well as total procedure time were compared using analysis of variance.

The PMPs were analysed using one-tailed paired *t* tests, and the results of these tests were confirmed using regression and observing the significance of the constant. The PAC1 and CD62P expression were also compared using one-tailed paired *t* tests, as were results of the frozen-thawed plasma and ADP in-vitro studies.

The paired *t* tests were calculated using Microsoft Excel software (version 2000 © 1985–99 Microsoft), while all other statistical analysis was made using Datadesk software (version 6.0.1; Datadesk, Ithaca, New York, USA).

Results

Patient clinical characteristics

A total of 75 patients without abciximab and 38 patients with abciximab treatment were studied, with most clinical characteristics being similar in both groups (Table 1). More patients had previously undergone PTCA in the group without abciximab than in the group receiving abciximab (24.0 and 7.9%, respectively), although a Fisher's exact test demonstrated that this difference was only marginally significant ($P = 0.043$). Both patient groups also had similar treatment with lipid lowers, β-blockers and ion channel modifiers, although significantly more patients were being treated with peripheral vasodilators in the abciximab group (Fisher's exact test, $P = 0.010$).

Details of the PTCA procedure characteristics are presented in Table 2. Patients who received abciximab had significantly more complex PTCA procedures, as they needed treatment of more coronary arteries ($P = 0.011$) and required more stents ($P = 0.039$), with 27% of patients without abciximab compared with 39% of abciximab patients having multiple stents. Also, these patients required significantly higher inflation pressures ($P = 0.022$) and maximum inflation times ($P < 0.0001$) than those who did not receive the drug. The total PTCA procedure time was longer for the abciximab patients, although this did not reach statistical signifi-

Table 2 Percutaneous transluminal coronary angioplasty procedure characteristics

Characteristic	Without abciximab ($n = 75$)	With abciximab ($n = 38$)	*P* value
Arterial approach			NS
Femoral artery	65 (86.7%)	34 (89.5%)	
Radial artery	10 (13.3%)	3 (7.9%)	
Brachial artery	0 (0.0%)	1 (1.3%)	
Number of coronary arteries treated			0.0107
1	63 (84.0%)	23 (60.5%)	
2	11 (14.7%)	15 (38.5%)	
3	1 (1.3%)	0 (0.0%)	
Number of stents used			0.0388
0	7 (9.3%)	5 (13.2%)	
1	48 (64.0%)	18 (47.4%)	
2	15 (20.0%)	6 (15.8%)	
3	4 (5.3%)	4 (10.5%)	
4	1 (1.3%)	4 (10.5%)	
5	0 (0.0%)	1 (2.6%)	
Rotablation	2 (2.7%)	1 (2.6%)	NS
Occlusion aspiration	1 (1.3%)	0 (0.0%)	NS
Number of inflations	4.89 ± 0.36	6.19 ± 0.71	NS
Maximum inflation time (s)	47.76 ± 1.87	74.30 ± 5.78	< 0.0001
Maximum inflation pressure (atm)	14.99 ± 0.45	16.71 ± 0.55	0.0219
Total procedure time (min)	38.38 ± 2.31	48.19 ± 4.87	0.0515

The arterial approach, number of coronary arteries treated, number of stents used, rotablation and occlusion aspiration are expressed as the number of patients and percentage of the patient group, while other characteristics are expressed as the mean ± standard error of mean. The total procedure time was measured from administration of heparin to withdrawal of the guidewire. *P* values are from chi-square tests for arterial approach, number of arteries treated, rotablation and occlusion aspiration, and from analysis of variance tests for other variables.

cance ($P = 0.051$). These differences prevented direct statistical comparisons between patient groups.

In the PTCA follow-up period, angina was reported in approximately 29–39% of patients at all time points (Table 3). Small numbers of patients required further PTCA in both patient groups (5.3% without abciximab and 7.9% with abciximab), while none of the patients receiving abciximab during their PTCA required CABG. Of those not treated with abciximab, five patients required subsequent revascularization as follows: patient 1 required PTCA within 6 months to a native artery; patient 2 had PTCA to instent restenosis of the right coronary artery at both 6 and 12 months; patient 3 required a single CABG to instent restenosis of the left anterior descending artery within 6 months, with the graft site then treated by PTCA within 12 months; patient 4 required a triple CABG within 6 months to treat severe instent restenosis of the left circumflex artery; and patient 5 had widespread arterial occlusions requiring a sextuple CABG within 6 months. The three patients treated with abciximab during their PTCA who required subsequent PTCA included one at 6 months to the site of the original PTCA, and individuals at both 6 and 12 months who had different arteries treated. The only incidence of death was in the abciximab group. This patient received chemotherapy on the day prior to death, and then deterioration commenced with chest pain and shortness of breath, leading into electromechanical dissociation, and death at termination of mechanical ventilation. A total of 10 patients were not contactable at 1 month, which increased to 19 patients uncontactable by 1 year.

PMP positive control using a twice freeze–thaw cycle

As a positive control to validate the flow cytometric analysis of PMPs, corresponding samples of plasma were subject to a twice freeze–thaw cycle to induce PMP production. The frozen–thawed plasma contained significantly more PMPs than the fresh whole blood,

Table 3 Patient outcomes following percutaneous transluminal coronary angioplasty (PTCA)

	Without abciximab ($n = 75$)				With abciximab ($n = 38$)			
Time of patient contact	Angina	PTCA	CABG	Death	Angina	PTCA	CABG	Death
1 month ± 1 week	23 (30.7%)	0 (0.0%)	1 (1.3%)	0 (0.0%)	14 (36.8%)	0 (0.0%)	0 (0.0%)	0 (0.0%)
6 months ± 2 weeks	28 (37.3%)	1 (1.3%)	3 (4.0%)	0 (0.0%)	15 (39.5%)	2 (5.3%)	0 (0.0%)	0 (0.0%)
12 months ± 4 weeks	22 (29.3%)	3 (4.0%)	0 (0.0%)	0 (0.0%)	12 (31.6%)	1 (2.6%)	0 (0.0%)	1 (2.6%)

Patients were contacted up to 1 year following their PTCA for incidence of angina, PTCA, coronary artery bypass graft (CABG) or death. Results are expressed as the number of patients and the percentage of the patient group.

regardless of treatment with abciximab (Table 4). There were significant increases of approximately 60–64% in the baseline samples in both patient groups ($P < 0.001$ without abciximab, $P = 0.030$ with abciximab), and significant increases of approximately 105–148% in the post-PTCA samples in both patient groups ($P < 0.001$ for both groups).

Positive control using in-vitro ADP

The addition of in-vitro ADP affected the proportion of CD61-positive PMPs. In both patient groups, the percentage of PMPs was decreased in the presence of ADP (Table 5). For non-abciximab patients, PMPs decreased by approximately 25% in the baseline sample and by approximately 20% in the post-PTCA sample ($P < 0.001$ for both). In the abciximab patient group, decreases of approximately 28% were also found in the baseline sample, and in the post-PTCA sample a decrease of approximately 44% occurred ($P < 0.001$).

The use of ADP produced significant increases in expression of PAC1 in both the non-abciximab-treated and abciximab-treated groups (Table 6). Substantial increases of approximately five-fold were found in both the baseline and post-PTCA sample for non-abciximab patients ($P < 0.001$ for both groups). A significant increase of approximately six-fold also occurred with exogenous ADP in the abciximab-treated patients in the baseline sample ($P < 0.001$). However, the ADP-induced increase following administration of abciximab during PTCA appeared to be limited with only a two-fold increase ($P = 0.029$).

In vitro ADP also significantly increased the expression of CD62P in both patient groups (Table 6). In non-abciximab patients, there were approximately seven-fold to nine-fold increases in CD62P expression, with the baseline and post-PTCA samples ($P < 0.001$ for both). Similarly, for patients treated with abciximab, increases were in the range of approximately 10-fold to 12-fold, for the baseline and post-PTCA samples ($P < 0.001$ for both). It is interesting to note that the apparent limitation of abciximab on ADP-induced PAC1 expression (described earlier) was not evident for the ADP-induced CD62P expression.

Total PAC1 and CD62P expression following PTCA

A slight non-significant decrease of 3.3% occurred in

Table 4 Increased platelet-derived microparticles (PMPs) in frozen–thawed plasma compared with fresh whole blood from percutaneous transluminal coronary angioplasty (PTCA) patients

	PMPs (% of CD61-positive events)			
	Baseline		Post-PTCA	
Patient group	Fresh whole blood	Frozen–thawed plasma	Fresh whole blood	Frozen–thawed plasma
Without abciximab ($n = 75$)	3.70 ± 0.29	5.92 ± 0.56 ($P < 0.001$)	4.05 ± 0.24	8.32 ± 0.67 ($P < 0.001$)
With abciximab ($n = 38$)	3.74 ± 0.42	6.12 ± 1.24 ($P = 0.030$)	3.96 ± 0.57	9.82 ± 1.66 ($P < 0.001$)

Frozen–thawed plasma samples were incubated with anti-CD61-peridinin chlorophyll protein antibody and tested by flow cytometry in the same manner as the samples tested at the time of PTCA. Values are expressed as the mean ± standard error of mean. *P* values are from a paired *t* test comparing the frozen–thawed sample with the corresponding unfrozen whole blood sample.

Table 5 The effect of in-vitro adenosine diphosphate (ADP) on platelet-derived microparticles (PMPs) in whole blood samples from percutaneous transluminal coronary angioplasty (PTCA) patients

	PMPs (% of CD61-positive events)			
	Baseline		Post-PTCA	
Patient group	Without ADP	With ADP	Without ADP	With ADP
Without abciximab ($n = 75$)	4.23 ± 0.21	3.21 ± 0.18 ($P < 0.001$)	3.99 ± 0.22	3.17 ± 0.25 ($P < 0.001$)
With abciximab ($n = 38$)	4.04 ± 0.42	2.90 ± 0.34 ($P < 0.001$)	4.41 ± 0.72	2.48 ± 0.24 ($P < 0.001$)

Whole blood samples were prepared with and without the addition of 100 μmol/l ADP *in vitro*. Values are expressed as the mean ± standard error of the mean. *P* values are from a paired *t* test comparing samples with and without the addition of ADP *in vitro*.

Table 6 The effect of in-vitro adenosine diphosphate (ADP) on PAC1 and CD62P in whole blood samples from percutaneous transluminal coronary angioplasty (PTCA) patients

	PAC1 (% of CD61-positive events)				CD62P (% of CD61-positive events)			
	Baseline		Post-PTCA		Baseline		Post-PTCA	
Patient group	Without ADP	With ADP	Without ADP	With ADP	Without ADP	With ADP	Without ADP	With ADP
Without abciximab ($n = 75$)	4.89 ± 0.69	24.37 ± 1.59 ($P < 0.001$)	4.73 ± 0.49	23.37 ± 1.89 ($P < 0.001$)	3.99 ± 0.69	36.44 ± 2.40 ($P < 0.001$)	4.09 ± 0.80	36.56 ± 2.65 ($P < 0.001$)
With abciximab ($n = 38$)	3.73 ± 0.27	22.17 ± 2.83 ($P < 0.001$)	3.09 ± 0.25	6.50 ± 1.76 ($P = 0.029$)	2.76 ± 0.21	34.57 ± 3.84 ($P < 0.001$)	2.91 ± 0.48	30.64 ± 3.87 ($P < 0.001$)

Whole blood samples were incubated with anti-CD61-peridinin chlorophyll protein, anti-PAC1-fluorescein isothiocyanate and phycoerythrin-conjugated anti-CD62P, prepared with or without the addition of in-vitro ADP, and tested by flow cytometry. Values are expressed as the mean ± standard error of mean. *P* values are from a paired *t* test comparing with and without ADP.

Table 7 The effect of percutaneous transluminal coronary angioplasty (PTCA) on the number of platelet-derived microparticles (PMPs) and expression of CD62P and PAC1 in whole blood samples

	PMPs (% of CD61-positive events)		PAC1 (% of all events)		CD62P (% of all events)	
Patient group	Baseline	Post-PTCA	Baseline	Post-PTCA	Baseline	Post-PTCA
Without abciximab ($n = 75$)	3.75 ± 0.25	4.14 ± 0.22 ($P = 0.019$)	4.89 ± 0.69	4.73 ± 0.49 ($P =$ NS)	3.99 ± 0.69	4.09 ± 0.80 ($P =$ NS)
With abciximab ($n = 38$)	3.91 ± 0.44	4.29 ± 0.63 ($P =$ NS)	3.73 ± 0.28	3.09 ± 0.24 ($P = 0.011$)	2.76 ± 0.21	2.91 ± 0.48 ($P =$ NS)

Whole blood samples were incubated with anti-CD61-peridinin chlorophyll protein antibody for the PMP results, or incubated with anti-CD61-peridinin chlorophyll protein, anti-PAC1-fluorescein isothiocyanate and phycoerythrin-conjugated anti-CD62P for the PAC1 and CD62P results, and tested by flow cytometry. Values are expressed as the mean ± standard error of mean. *P* values are from a paired *t* test comparing baseline with post-PTCA. NS, not significant.

total expression of PAC1 after PTCA in non-abciximab patients (Table 7). However, there was a significant decrease of 17.2% in PAC1 expression in patients treated with abciximab ($P = 0.011$). There was no significant change in the total expression of CD62P in all of the CD61-positive events from baseline to post-PTCA, regardless of treatment with abciximab. Only a slight increase of 2.5% occurred in non-abciximab patients and of 5.4% in abciximab patients.

Increase in PMPs following PTCA

There was a significant increase of 10.4% ($P = 0.019$) in the PMPs following PTCA in the patient group without abciximab (Table 7). Although the numerical increase is modest, results were confirmed by non-robust regression analysis, which demonstrated significance of the constant ($P < 0.01$). In contrast, the PMPs in patients who received abciximab did not show a significant change, with an increase of only 4%.

Discussion

In this study we have examined the level of PMPs in 113 PTCA patients, of whom 38 received abciximab. The patient groups were similar in baseline characteristics such as age and coronary risk factors, although significantly more patients not receiving abciximab had previous PTCA therapy. This difference prevented direct statistical comparisons between groups, and thus parameters were compared before and after PTCA in the same groups. The decision to treat with abciximab is based on severity of cardiovascular disease, and clearly those patients with more involved PTCA treatment were ultimately treated with abciximab. A combination of factors including number of stents and more vigorous inflations place this group of patients at higher risk of restenosis, thus requiring the abciximab protection to limit platelet aggregation. Within the year following PTCA, angina was relatively consistent between groups, with a tendency to decreased reporting of angina by 1 year, although this effect may be partly due to the inability to retain patient contact. While a total of eight incidents of revascularization were required in the year following PTCA in patients not treated with abciximab, five of these (6.7% of the patient group) were due to direct restenosis. Of the three incidents of revascularization needed for abciximab-treated patients, only one (2.6% of patient group) was due to direct restenosis. It is interesting to note that all of those requiring revascularization from the abciximab group could be treated by PTCA rather than CABG, indicating that the occlusions were of a lesser severity and demonstrating the ability of abciximab to limit subsequent restenosis.

As freeze–thaw cycles increase PMP production [22,23], this technique was employed to generate higher PMP numbers and thus verify the measurement of PMPs by flow cytometry. There was a significant increase in PMPs when plasma was subjected to a twice freeze–thaw cycle for all samples (regardless of abciximab treatment), demonstrating appropriate PMP measurement techniques. In the post-PTCA samples, the twice freeze–thaw resulted in a much greater PMP increase, which may be a reflection of the prothrombo-

tic state induced by PTCA. While there was a 105% increase PMPs in non-abciximab patients, an even greater 148% increase occurred in abciximab patients. Therefore, abciximab treatment has not limited the ability of platelets to produce PMPs by freeze–thaw cycles, and indicates that the GPIIb/IIIa site is not essential for PMP production under these conditions. This is supported by studies indicating that PMPs can be produced either by GPIIb/IIIa-dependent or GPIIb/IIIa-independent mechanisms [24,25].

These results demonstrate that the increase induced by the freeze–thaw method is not merely a non-specific response, as the increase ranged from 60 to 148%, with higher increases seen in the post-PTCA samples. Although comparisons were made between the fresh whole blood and frozen–thawed plasma samples, these results cannot be explained by a haematocrit effect as the PMPs are measured by expression of CD61, which is not affected by presence of other non-CD61-positive cells such as erythrocytes. The increase in PMPs may have been caused by increased budding, or by increased platelet lysis, although this was not addressed in this study. It is also possible that the larger PMPs may be releasing smaller sized PMPs, as a variety of PMP sizes have been reported and PMPs appear to be heterogeneous [26]. Therefore, it may be possible that PMPs actually vesiculate into smaller PMP fragments under certain conditions, producing PMPs of a variety of sizes.

There was a significant decrease in PMPs at all times with the in-vitro addition of ADP. As ADP causes platelet adhesion, it is probable that platelets and PMPs have adhered to the flow cytometry tubes, thus preventing them from being sampled and measured. It is also probable that ADP may cause PMP aggregation into larger particles, and thus they would no longer be detected and counted in the PMP analysis region. It has previously been demonstrated that ADP did not cause an increase in PMPs [6]. It is interesting to note that decreases of 20–28% were observed in most samples, with a large decrease of 44% in the post-PTCA sample when abciximab was used. Thus, the largest decrease in PMPs with in-vitro ADP was observed in the presence of abciximab. It is expected that blood from post-PTCA samples in patients from the abciximab group would be most prothrombotic due to the complexity of their PTCA. Therefore, it is possible that the addition of ADP to these blood samples caused extensive adhesion and/or aggregation via a mechanism not dependent on the GPIIb/IIIa complex, as it is known that the GPIIb/IIIa complex is not essential for vesiculation under particular conditions [24,25].

There were significant increases in PAC1 expression in the presence of in-vitro ADP. However, the increase in PAC1 expression was limited in the presence of abciximab, as seen in the post-PTCA sample. As abciximab blocks the GPIIb/IIIa site, it is probable that it would limit the PAC1 binding. There was also a significant increase in CD62P with ADP, with no limitation of abciximab. As abciximab interacts with the GPIIb/IIIa complex, it would not be expected to alter the P-selectin expression. Together, these results indicate that in-vitro ADP has resulted in platelet activation.

The expression of PAC1 did not alter significantly in patients without abciximab treatment from baseline to post-PTCA in this study. However, there was a significant decrease in the level of PAC1 in abciximab patients following PTCA, as abciximab is an antagonist at the GPIIb/IIIa receptor and thus would limit PAC1 binding. In this study, peripheral arterial samples were obtained, although within the coronary sinus a significant increase in PAC1 expression has occurred following PTCA [27], but there was no significant change in PAC1 in peripheral venous blood [28]. In our study, there was no significant change in CD62P following PTCA regardless of abciximab use. Similar results with no significant change in P-selectin have been obtained in other work [27–29], and this is supported by in-vitro work showing that α-degranulation may not be directly related to PMP formation [30].

In the present study, we have clearly demonstrated that the level of CD61-positive PMPs increased significantly during PTCA in the group of 75 patients who were not treated with abciximab. It is expected that factors including the physical trauma due to balloon rupture of the plaque, and exposed surfaces provided by subendothelial structures and stents have contributed to the prothrombotic state. This may possibly be related to the increase in PMP formation. It is possible that microparticles from other sources such as endothelial cells were also altered with PTCA, although this was not determined in the present study. This trend towards increased PMPs has been noted in earlier studies, but low sample sizes prevented statistical significance being attained [27,29]. Our work demonstrates a significant increase in PMPs that is supported by statistical analysis ($P = 0.019$). While we have examined peripheral arterial blood, recently published work has also demonstrated a significant increase in PMPs in peripheral venous blood following PTCA [28]. Our results taken together with these results [28] conclusively demonstrate that there is an increase in the levels of PMPs with PTCA, as detected in both arterial and venous blood samples.

It is possible that the increase in PMPs that we have reported may be involved in restenosis after PTCA, although our sample size limits significant incidence of

restenosis events. It is probable that the PMPs may adhere to the traumatized site because, in an animal model of arterial endothelial injury, labelled PMPs were infused and there was a significantly higher increase in PMP adhesion at the injured site compared with at an uninjured site [31]. This significant PMP adhesion could initiate thrombus formation and lead to future ischaemic events. In earlier research, patients with activated coagulation and fibrinolysis who died due to acute myocardial infarction had fresh thrombi in their coronary arteries. Further examination demonstrated that those patients who died had the highest levels of PMP-bound GPIIbα in blood samples taken prior to death [32], which may have accelerated thrombi formation in the coronary vessels and contributed to the adverse patient outcomes. These studies demonstrate the possibility for PMPs to be involved in adhesion and aggregation, and potentially contribute to restenosis after PTCA.

In our study, the group of 38 patients treated with abciximab did not have a significant alteration in the level of PMPs. Therefore, it appears that the GPIIb/IIIa complex may be essential in PMP vesiculation during PTCA, as blocking this receptor with abciximab has prevented platelet vesiculation. Blockage of the GPIIb/IIIa Arg-Gly-Asp-Ser (RGDS) amino acid sequence has prevented PMP vesiculation [11], with the common Arg-Gly-Asp (RGD) site being blocked by abciximab in the present study. This clearly demonstrates the effectiveness of abciximab because, despite the more complex PTCA procedure, the level of PMPs did not significantly alter. However, as patients with Glanzmann's thrombasthenia [24] and those with the GPIIb/IIIa site blocked with other antibodies [25] are still able to produce PMPs, it is probable that, under certain conditions, an additional mechanism(s) of platelet vesiculation is involved.

Study limitations

As this study was non-randomized, it is acknowledged that there may be other important differences between the abciximab-treated and non-treated groups. Furthermore, the significant differences in the PTCA characteristics discussed contribute to differences in the study populations, while the differences in sample sizes have prevented direct comparisons between groups. Additional studies on abciximab-treated and non-abciximab-treated patients are needed to confirm the PMP changes during PTCA.

In conclusion, we have demonstrated that PTCA results in an increase in PMPs in peripheral arterial blood, a phenomenon that was prevented with the use of abciximab. As PMPs appear to be involved in adhesion, aggregation and blood coagulation, it is probable that these PMPs may contribute to restenosis events following PTCA, although the significance of this remains undetermined.

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