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Platelet–monocyte aggregates predict troponin rise after percutaneous coronary intervention and are inhibited by Abciximab

M.J. Ray[a], D.L. Walters[b,*], J.N.H. Bett[b], J. Cameron[b], P. Wood[a], C.N. Aroney[b]

[a] *Department of Haematology, The Prince Charles Hospital, Brisbane, Australia*
[b] *Department of Cardiology, The Prince Charles Hospital, Brisbane, Australia*

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Abstract

Background: Platelet–monocyte aggregates and other markers of platelet activation were investigated before and after percutaneous coronary intervention (PCI) with abciximab therapy. The study sought to assess the relationship between the level of platelet–monocyte aggregation and increases in cardiac troponin I post coronary intervention. *Methods:* Blood samples were collected from 40 patients before PCI and 10 min after abciximab administration. These were tested for platelet activation markers by flow cytometry. Cardiac troponin I levels were assayed at baseline and at 24 h post PCI. *Results:* Compared to healthy controls, patients with coronary artery disease had elevated markers of platelet activation including platelet–monocyte aggregates, P-selectin and PAC-1 (a marker specific for activated glycoprotein IIb/IIIa) prior to PCI. Increased levels of platelet–monocyte aggregates before PCI were associated with increased expression of P-selectin on the platelet surface. Abciximab therapy reduced platelet–monocyte aggregate levels but had no effect on P-selectin expression. The high levels of expression of activated glycoprotein IIb/IIIa (PAC-1) on platelets prior to PCI was reduced with abciximab therapy. Patients with higher levels of platelet–monocyte aggregates prior to PCI were more likely to develop an elevation of cardiac troponin I during the 24 h after PCI. *Conclusions:* Increased levels of platelet–monocyte aggregates may predict patients at risk for troponin elevation following PCI and identify those most likely to benefit from abciximab.

Keywords: Angioplasty; Inhibitors; Platelets

1. Introduction

An invasive strategy has become the preferred mode of treatment for patients with high-risk acute coronary syndrome [1,2]. Adjunctive pharmacotherapeutic agents inhibiting platelet dependent thrombosis have improved the safety and efficacy of percutaneous coronary intervention (PCI) in these patients [3]. The glycoprotein IIb/IIIa receptor is considered the 'final common pathway' for platelet activation and this receptor is blocked by abciximab. This drug has been shown to reduce the morbidity and mortality of patients undergoing high risk PCI [3]. It is recognised that in addition to platelet–platelet interactions (homotypic aggregates), platelet–leukocyte (heterotypic) aggregation may play an important role in atherothrombogenesis [4].

Inflammation and thrombosis are intimately linked to the evolution of an acute coronary syndrome [5]. Inflammation, as indicated by elevated levels of C-reactive protein, is a marker of increased risk for cardiovascular events [6]. This increased state of inflammation is paralleled by activation of platelets with phosphorylation of the membrane protein P-selectin in the platelet alpha granule membrane [7]. The granule moves to the platelet surface exposing P-selectin and tethering the platelet to the monocyte via the monocyte receptor P-selectin glycoprotein ligand-1 (PSGL-1) to form a platelet–monocyte aggregate. P-selectin in the Weibel-Palade bodies of the vascular endothelium also moves to the surface on activation, binding to the PSGL-1 of monocytes and neutrophils causing cell rolling and recruitment [7]. Tissue factor on the monocyte is up regulated by P-selectin [8] to enhance fibrin formation [9]. Increased expression of tissue factor on monocytes has been shown to be associated with a prothrombotic state [10]. P-selectin together with platelet chemokines RANTES (regulated upon activation, normal T cell expressed and secreted) acts to regulate monocyte chemokine synthesis [11,12]. Plate-

* Corresponding author. Cardiology Department, Level 1, The Prince Charles Hospital, Chermside 4032, Queensland, Australia. Tel.: +61-73350-8111; fax: +61-7350-8715.
E-mail address: Darren_walters@health.qld.gov.au (D.L. Walters).

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let–leukocyte aggregates have also been demonstrated at increased levels following balloon angioplasty [13].

In this study, levels of platelet–monocyte aggregates as well as expression of both P-selectin and activated glycoprotein IIb/IIIa (as measured by PAC-1) on platelets of patients prior to PCI, were compared to healthy controls. Then the levels of circulating platelet–monocyte aggregates pre PCI were correlated to post procedure cardiac troponin I elevation. The effect of abciximab on circulating platelet–monocyte aggregates, platelet expression of P-selectin and PAC-1 was examined.

2. Materials and methods

Informed consent was obtained from each patient. The study protocol conformed to the ethical guidelines of the 1975 Declaration of Helsinki and prior approval was obtained from the Prince Charles Hospital Human Ethics and Research Committee.

2.1. Percutaneous intervention procedure

In all patients PCI was performed via the femoral artery using a 6 French sheath and guiding catheter. A non-ionic contrast agent was used. All patients received between 100 and 300 mg of aspirin for a minimum of 1 week prior to the PCI. A single bolus of weight adjusted heparin (70 U/kg) was administered at the start of the procedure and further heparin was administered as required to attain an activated clotting time ≥ 250 s. After the lesion was crossed with the guidewire, all patients received a bolus followed by infusion of abciximab at the recommended weight-adjusted regimen [14]. All patients received clopidogrel (a thienopyridine) after the bolus of abciximab if it was ascertained that immediate coronary artery bypass grafting would not be required [15]. PCI was performed according to standard techniques and at the discretion of the operator. In all cases, an intracoronary stent was placed either directly or following initial balloon pre-dilatation. All procedures were considered successful and there were no major complications in any patient.

2.2. Patient population and sample collection

Twenty-six healthy volunteers and 40 PCI patients with coronary artery disease referred for PCI were included in the study. The healthy volunteers were male [6] with a mean age of 42 years (range 24–58 years). They had no history or symptoms of cardiovascular disease, hypertension, diabetes, dyslipidaemia, or smoking and were taking no medications. Hypertension was defined as a history of a systolic blood pressure exceeding 140 mm Hg or a diastolic greater than 90 mm Hg [16], such that medical therapy was deemed indicated by the treating physician. Diabetes was defined according to World Health Organisation criteria [17]. Dyslipidaemia was defined in accordance with the Australian Lipid Management Guidelines [18]. A history of cigarette smoking was based on patient self reports and the medical records of the treating physician. The clinical indication for patients undergoing PCI was classified as chronic angina, unstable angina or recent myocardial infarction in accordance with the definitions outlined in the Australian Management of Unstable Angina Guidelines [19]. Blood was collected from the healthy volunteers via slow withdrawal of blood from the antecubital vein.

Consecutive patients treated with abciximab were enrolled. This treatment was implemented when the operator decided the patient was at increased risk of morbidity related to the PCI. Blood samples were collected from these subjects by slow withdrawal from the femoral venous sheath: (a) immediately after heparinisation before PCI (pre-PCI), and (b) 10 min after the abciximab bolus and before thienopyridine administration (post-PCI). This ensured the thienopyridine would have no effect on the measures of platelet activation. Cardiac troponin I was tested at baseline and 24 h after PCI in all patients.

2.3. Flow cytometry

The validated methodology of Shattil [20] was used to detect activation of platelets in whole blood samples. Nine parts of venous blood was anticoagulated with one part of 0.105 M trisodium citrate (BD Biosciences, San Jose, CA, USA) (BD). In vitro changes occur rapidly after sampling of blood [20] so the cells were counted immediately as recommended by the European Working Group on Clinical Cell Analysis [21]. Standard methods for the performance of flow cytometry were used and our methods have been published previously [20–22]. All blood samples were incubated with BD monoclonal antibodies, which bind to their specific sites on the platelets or monocytes. CD42a Peridinin Chlorophyll Protein (PerCP) targets the glycoprotein Ib-IX-V present on all platelets. Platelet–monocyte aggregates were indicated by binding of CD42a as well as a second antibody, CD14 phycoerythrin (PE) that is specific for peripheral blood monocytes and 5000 cells were counted. Platelets in the whole blood were differentiated from other cells by CD42a binding as well as their laser deflecting characteristics of logarithmic forward scatter and side scatter and 3000 cells counted. P-selectin and activated glycoprotein IIb/IIIa expression on these platelets was indicated by binding of CD62P PE and PAC-1 fluorescein isiothyocyanate (FITC) antibodies, respectively. The monoclonal antibody PAC-1 recognizes activation-specific conformational changes in the glycoprotein IIb/IIIa complex that exposes the fibrinogen binding site and hence can be used to measure the activated glycoprotein IIb/IIIa complex. The degree of expression of each marker was measured against appropriate isotype negative controls. Patients' post-PCI blood samples were stimulated with ADP in vitro to provide positive controls.

A BD FACScan flow cytometer with a 488-nm argon-ion laser performed three-colour analysis. Data was analysed