The GP IIb/IIIa inhibitor abciximab (ReoPro®) decreases activation and interaction of platelets and leukocytes during in vitro cardiopulmonary bypass simulation

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Abstract

Objective: Cardiopulmonary bypass (CPB) induces a systemic inflammatory response and increases expression of the platelet activation marker P-selectin which mediates binding of platelets to leukocytes. Inhibition of the platelet GP IIb/IIIa receptor during CPB has been shown to protect platelets without increasing bleeding complications and is assumed to reduce the inflammatory response. The aim of this study was to investigate the effect of the GP IIb/IIIa inhibitor abciximab (ReoPro®) on the function and interaction of platelets and leukocytes during experimental CPB.

Methods: Heparinized (3 U/ml) fresh whole blood of healthy volunteers was treated before continuous in vitro circulation in a well established CPB model with 3.2 μg/ml abciximab (n = 6) or left untreated as control (n = 6). Measurements were made before (baseline) and after 30 and 60 min of circulation and comprised: percentage of platelets expressing P-selectin and percentage of platelet-bound leukocytes (flow cytometry), release of the leukocyte activation marker PMN-elastase (ELISA), and platelet and leukocyte counts.

Results: Abciximab almost completely prevented a CPB-induced increase of platelet P-selectin and platelet–leukocyte binding after 30 and 60 min of circulation, and significantly inhibited release of PMN-elastase after 30 min of circulation. Furthermore, abciximab significantly inhibited a CPB-induced decrease of platelet and leukocyte counts. Conclusions: Abciximab inhibits CPB-induced activation, interaction and consumption of platelets and leukocytes in vitro. GP IIb/IIIa inhibition should be considered as a promising approach not only to conserve platelet function but also to inhibit pro-inflammatory events during CPB in vivo.

Keywords: Cardiopulmonary bypass; Inflammation; GP IIb/IIIa inhibition; Platelet anesthesia

1. Introduction

Contact of blood with the artificial surfaces of the cardiopulmonary bypass (CPB) components induces a patient-dependent systemic inflammatory response which includes alteration and activation of platelets and leukocytes [1]. In this context, CPB induces an upregulation of the platelet activation marker P-selectin, which mediates binding of platelets to leukocytes, leading to formation of platelet-leukocyte aggregates [2]. Both platelets and leukocytes can modulate each others function. Leukocytes enhance platelet-mediated aggregation via interaction of their P-selectin ligand with P-selectin [3]. Furthermore, binding of leukocytes to platelets promotes leukocyte activation [4].

The most effective therapy for platelet-inhibition is achieved by blocking the platelet fibrinogen receptor GP IIb/IIIa and is used routinely to reduce ischemic complications during angioplasty and acute coronary syndromes [5]. Three GP IIb/IIIa inhibitors are approved in many countries: abciximab (ReoPro®, the Fab fragment of a chimeric human-mouse antibody), eptifibatide (Integrelin®, a cyclic heptapeptide), and tirofiban (Aggrastat®, a tyrosine-derived nonpeptide molecule) [6]. While the recovery of platelet function after abciximab administration exceeds 12 h, the effects of tirofiban and eptifibatide disappear in less than 6 h.

With regard to cardiac surgery, there have been concerns that GP IIb/IIIa inhibition may result in severe bleeding complications. However, several clinical studies have shown that coronary artery bypass grafting (CABG) can be safely performed after GP IIb/IIIa inhibitor treatment. Vahl et al. reported that coronary artery bypass grafting (CABG) can be performed with similar results in abciximab-pretreated patients as in patients with other types of preoperative anticoagulation [7]. Moreover, Bizzarri et al. as well as Dyke et al. reported significant reductions of CPB-induced
platelet loss in patients undergoing CABG after prior tirofiban or epoetin administration [8,9]. The mechanism of this platelet sparing effect may be explained by conservation of platelets by GP IIb/IIIa inhibitors against activation through CPB and has been termed 'platelet anaesthesia' [10]. Another effect of GP IIb/IIIa inhibition has been proposed by Koster et al. who reported that tirofiban administration during CPB attenuated activation of inflammatory markers during CPB [11]. To evaluate the unclear mechanism of this phenomenon, the effect of abciximab on platelet-leukocyte function during experimental CPB was investigated in our study.

2. Materials and methods

2.1. Cardiopulmonary bypass model

The experiments were carried out using a well established in vitro heart-lung-machine model which has been described previously [12]. Fresh human whole blood (not older than 30 min) from healthy volunteers was recirculated in a closed-loop CPB system with oxygenation and simulation of arterial counter-pressure. For each CPB run 500 ml blood from one single donor was used. The length of the tubing was defined in a manner identical to surgical conditions. The test series consisted of 12 membrane oxygenators of the same construction (CPB). These 12 membrane oxygenators were divided into single donor was used. The length of the tubing was defined in a manner identical to surgical conditions. The test series included 12 membrane oxygenators of the same construction series without any surface treatment (i.e. heparin-coating, etc.). These 12 membrane oxygenators were divided into two groups: One group was treated with 3.2 μg/ml abciximab (n = 6), the other group was left untreated as control (n = 6).

Before priming, the oxygenators and tubing were rinsed with 1000 ml ringer lactate for 30 min. The priming volume consisted of 78.3 ml 5% glucose solution (Delta-Pharma GmbH, Pfullingen, Germany), 206 ml ringer lactate (Fresenius, Bad Homburg, Germany) and 15.7 ml 8.4% NaHCO3 (Braun Melsungen AG, Melsungen, Germany). The priming volume was not discarded before addition of blood according to clinical procedures. 500 ml volume of fresh human blood was next circulated in this closed system, using a roller pump (Sarns Inc., Ann Arbor, MI, USA), for 60 min. A precisely calculated amount of heparin (Liquemin®, Hoffmann-La Roche, Basel, Switzerland) was added to achieve a final heparin concentration in the whole machine filling volume of 3 U/ml.

A constant blood flow of 3 L/min and a mean arterial side tubing pressure of 60 mm Hg were maintained, while a hypothermic regulator (type Q 102, Haake, Berlin, Germany) was added to achieve a final heparin concentration in the whole machine filling volume of 3 U/ml.

A constant blood flow of 3 L/min and a mean arterial side tubing pressure of 60 mm Hg were maintained, while a hypothermic regulator (type Q 102, Haake, Berlin, Germany) held the temperature at 28 °C at the arterial exit of the oxygenator.

2.2. Sample measurements

Blood was taken before (baseline) and after 30 and 60 min of circulation from the arterial exit of the oxygenator into tubes containing various anticoagulants required for the different assays. The first 2 ml of blood at each sampling point were discarded to avoid artificial activation. All plasma samples were shock frozen in liquid nitrogen and stored at −80 °C.

For each flow cytometric test, 50 μl of whole blood was used to evaluate P-selectin expression ('P-selectin-sample') and platelet-leukocyte binding ('platelet-leukocyte-sample'). For each ELISA test 3 ml of citrated blood and for determination of platelet and leukocyte counts 3 ml of EDTA blood were taken.

2.3. Flow cytometry

Five microlitres of a fluorescein isothiocyanate (FITC) labeled antibody (SZ 22, Beckman-Coultier GmbH, Krefeld, Germany) against the platelet molecule GP IIb (CD 41) were added to all whole blood samples (50 μl). Additional 5 μl of a phycoerythrine (PE)-labeled antibody against P-selectin (BD Biosciences, Heidelberg, Germany) were added to each 'P-selectin-sample'. For evaluation of platelet-bound leukocytes, additional 5 μl of a PE-labeled antibody against the panleukocyte marker CD 45 (BD Biosciences) were added to each 'platelet-leukocyte-sample'. After an incubation-period of 30 min at 37 °C, samples were fixed with CellFix® (BD Biosciences).

Flow cytometric measurements were performed within 6 h after fixation on a FACScan® cytometer (Becton Dickinson, Heidelberg, Germany). A total of 10,000 events were counted in each measurement. Samples were analyzed by triggering on a preset threshold of SZ 22-FITC fluorescence. Objects positive for SZ 22-binding were distinguished regarding their forward scatter (size) properties into a region depicting platelets bound in aggregates and another region with single platelets. Fluorescence of P-selectin- and CD45-antibodies were analyzed on aggregate-bound platelets and leukocytes. Antibody fluorescence was evaluated in histograms and is given as the percentage of aggregate-bound platelets positive for antibody-binding of P-selectin and CD45.

2.4. ELISA

Blood samples for PMN-Elastase-α1-PI complexes were determined using ELISA kits supplied by Merck AG (Darmstadt, Germany). All assays were performed in duplicate according to the manufacturer’s instructions.

2.5. Cell counts

EDTA blood samples were assayed on a blood cell counter.

2.6. Correction for hemodilution

The cell counts as well as the values from analyses of the soluble activation markers measured by ELISA techniques were corrected for hematocrit. Hemodilution, caused by addition of the priming volume to the CPB model prior to blood application, was corrected for each single experiment by multiplication of the respective dilution factor. The dilution factors were calculated by comparing the hematocrit from the donor blood with the hematocrit of each blood sample from the different sampling times.

For the flow cytometric analyses this correction procedure was not performed, because each test was carried out until 10,000 events were counted.
2.7. Statistical analysis

Analysis of normality using the Kolmogorov-Smirnov test and approximation by Dallal and Wilkinson excluded normal distribution of data. Results are given as median and interquartile range. Comparison of untreated with abciximab-treated samples and P-value calculation at each time-point were performed using the nonparametric Mann-Whitney test. Analyses were performed using GraphPad Prism version 4.01 for Windows (GraphPad Software, San Diego, CA, USA).

3. Results

3.1. Platelet activation and platelet-leukocyte binding

At baseline no significant differences of platelet P-selectin expression and platelet-leukocyte binding between control and abciximab-treated samples were observed. Circulation of blood in the CPB model induced increases of P-selectin expression and platelet-leukocyte binding. After 30 as well as after 60 min of circulation P-selectin expression and platelet-leukocyte binding were significantly lower in the abciximab-treated group compared to the untreated group (Figs. 1 and 2).

3.2. Leukocyte activation

Circulation of blood in the CPB model induced activation of neutrophils and consecutive release of PMN-elastase. After 30 min of circulation PMN-elastase concentration was significantly lower in abciximab-treated compared to untreated samples (Fig. 3).

3.3. Platelet and leukocyte counts

At baseline no significant differences of platelet and leukocyte counts between control and abciximab-treated samples were observed. Circulation of blood in the CPB model decreased platelet and leukocyte counts in untreated samples. After 30 as well as after 60 min of circulation in the CPB model platelet and leukocyte counts were significantly higher in abciximab-treated compared to untreated samples (Figs. 4 and 5).
4. Discussion

Our experiments show that abciximab prevents CPB-induced platelet and leukocyte alteration in vitro and elucidate some of the underlying mechanisms: Blockade of GP IIb/IIIa by abciximab decreases platelet-platelet interaction and binding and thereby activation of platelets by each other. The resulting inhibition of platelet P-selectin expression decreases the binding capacity of platelets for leukocytes, which may explain the abciximab-related decrease of platelet-leukocyte aggregates. Similar results have been reported by Steiner et al. who showed that abciximab suppresses platelet P-selectin expression and monocYTE-platelet cross-talk in vitro [13]. Furthermore, the reduction of platelet-leukocyte interaction by abciximab may decrease platelet-induced leukocyte activation and explain the observed abciximab-related inhibition of PMN-elastase release. However, the diminished release of PMN-elastase by abciximab was detected only after 30 min of circulation, which suggests that after longer periods of CPB, leukocytes become, in addition to platelet-mediated effects, activated by other factors.

Our experiments may also explain how abciximab inhibits CPB-induced decreases of platelet and leukocyte counts. Adsorbed fibrinogen at oxygenator membranes works as a strong adhesive matrix or ligand to the fibrinogen receptor GPIIb/IIIa [14]. Being a GP IIb/IIIa inhibitor, abciximab has the potential to decrease the number of cell-aggregates and single platelets bound to the oxygenator and tubing system of CPB, and to decrease the number of single platelets and leukocytes lost in aggregates.

In contrast to tirofiban and eptifibatide, which are specific for GP IIb/IIIa [6], abciximab has been reported to cross-react with the αMβ2 ‘Mac-1’ receptor and with the αVβ3 ‘vitronectin’ receptor. Both receptors are present on leukocytes and are involved in a number of different physiological and pathophysiological processes. Therefore, it remains unclear whether the effects of abciximab on leukocytes observed in our study may partially be caused by its cross-reactivity. Nonetheless, tirofiban, an agent specific for GP IIb/IIIa, has been reported to attenuate inflammatory markers during CPB in vivo. This observation shows that GP IIb/IIIa plays a major role in the pathogenesis of platelet and leukocyte-related inflammation and shows that GP IIb/IIIa inhibition alone may have the potential to reduce the development of the inflammatory response [11].

To evaluate the general effect of GP IIb/IIIa inhibition on platelet and leukocyte function and interaction in vitro, we used abciximab which has a dissociation half-life from GP IIb/IIIa of approximately 4 h [15]. For the clinical setting of cardiac surgery a short-acting or reversible GP IIb/IIIa inhibitor with a half-life of only a few minutes would be ideal, but is not available at the moment. Such a drug could be administered as ‘platelet anesthetic’ only during the period of CPB to protect platelets temporarily. Despite promising reports on the use of the short-acting GP IIb/IIIa inhibitors eptifibatide (half-life: approximately 1.5 h [15]) and tirofiban (half-life: 1.5-2 h [15]) before cardiac surgery [8,9], these do not also long-acting to allow platelet inhibition only during CPB and may increase post-bypass bleeding. Therefore, a very short-acting GP IIb/IIIa inhibitor should be developed to protect platelets only during CPB. In this way full platelet function may be provided immediately after CPB and possible bleeding complications may be avoided.

Despite limitations for the in vivo use of the GP IIb/IIIa inhibitor abciximab, our in vitro experiments show that the pharmacological principle of GP IIb/IIIa inhibition may be suitable for platelet protection during CPB in vivo. Furthermore, the inhibition of platelet-leukocyte interaction and leukocyte activation as observed in our experiments may be an additional positive aspect of GP IIb/IIIa inhibition that may influence the inflammatory response in vivo.

5. Conclusions

Abciximab inhibits activation, interaction, and consumption of platelets and leukocytes during continuous in vitro circulation of blood in a CPB model. A short-acting or reversible GP IIb/IIIa inhibitor should be considered not only to conserve platelet function but also to inhibit pro-inflammatory platelet-leukocyte interaction during CPB in vivo.

References


