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Effect of GPIIb/IIIa Inhibition with Eptifibatide or Tirofiban on the Expression of Cellular Adhesion Molecules on Monocytes

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Abstract. *Aim: The aim of the present study was to investigate the effect of GPIIb/IIIa inhibition with eptifibatide and tirofiban on the expression of cellular adhesion molecules on monocytes at different temperatures. Materials and Methods: Circulation of blood from six volunteers was performed in an extracorporal circulation model at 36˚C and 18˚C for 30 min. The blood of each donor was prepared either with addition of eptifibatide or tirofiban, or was left untreated as control. CD54 and CD162 on monocytes was measured using flow cytometry. Results: Expression of CD11b was lower at 18˚C compared to 36˚C by 51% in the eptifibatide group (p=0.0043), by 29% in the tirofiban group (p=0.095) and by 34% in the control group (p=0.038). Expression of CD54 was not significantly different at 18˚C compared to 36˚C, neither with eptifibatide (p=0.29) nor tirofiban (p=0.48) nor in the control group (p=0.26). Expression of CD162 was lower at 18˚C compared to 36˚C by 40% using eptifibatide (p=0.0010), by 94% using tirofiban (p=0.0095) and by 34% in the control group (p=0.019). At 36˚C and 18˚C, no significant differences were found regarding the expression of CD11b, CD54 and CD162 between the eptifibatide-treated group, the tirofiban-treated group and the control group. Conclusion: GPIIb/IIIa inhibition with eptifibatide or tirofiban seems to have no effect on the expression of CD11b, CD54 and CD162 on monocytes during normothermia or hypothermia. Our results show that the beneficial effect induced by hypothermia on the extracorporal circulation-associated alteration of leukocyte function, with decreased expression of CD11b and CD162, seems not to be affected by additional treatment with eptifibatide or tirofiban.*

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The contact of blood with artificial biomaterials of an extracorporeal circulation (ECC) circuit (*e.g.* during heart surgery, hemodialysis, plasmapheresis) induces complex inflammatory responses, including activation of complement, the coagulation system, platelets and leukocytes, along with the expression of adhesion molecules and the release of inflammatory mediators (1-4). These ECC-associated alterations can lead to life-threatening complications, such as hemorrhage, thromboembolism, and inflammatory organ dysfunction, and can ultimately lead to multiple-organ failure (1, 3-5). Undoubtedly, better therapeutic strategies can be based only on a solid understanding of the complex interactions between the various components and the mechanisms involved in the inflammatory response (4). Since the inflammatory reactions are multi-factorial, combined therapies may be more efficient than a single intervention to improve outcome (4). Modification of techniques or mechanical devices, pharmacological therapies and strategies such as therapeutic hypothermia have improved outcome after coronary bypass surgery.

Temporary blockade of the main platelet receptor GPIIb/IIIa during ECC, also termed 'platelet anaesthesia', is an experimental pharmacological strategy to protect platelets during ECC (6). Additionally, a small number of patients undergo emergency coronary artery bypass grafting during ST-elevation myocardial infarction. In some cases, these patients were pre-treated with GPIIb/IIIa inhibitors (7). The main effect of GPIIb/IIIa inhibitors such as tirofiban and eptifibatide is their strong anti-platelet effect (8).

Nevertheless, experimental data suggest that GPIIb/IIIa receptor inhibitors also exercise anti-inflammatory effects besides inhibition of platelet aggregation (9). For example, the GPIIb/IIIa inhibitor abciximab demonstrates affinity to an activated conformation of the CD11b/CD18 (macrophage-1 antigen; MAC1) receptor, which is present on monocytes, granulocytes and natural killer cells, and modulates leukocyte-platelet interactions and the inflammatory response (10). Additionally, an effect of GPIIa/IIIb inhibition on factor X and monocyte adhesion to intercellular adhesion molecules (ICAM) has been demonstrated (11). These effects may probably have an influence on the inflammatory response during ECC.

Activated leukocytes are key mediators of inflammatory reactions elicited by extracorporeal therapies. The cell–cell interaction between leukocytes, endothelial cells and platelets is mediated by adhesion molecules which are expressed on the surface of these cells. Recently, we demonstrated an effect of hypothermia on the expression of cellular adhesion molecules on monocytes during extracorporeal circulation (12). Data regarding the effect of GPIIb/IIIa inhibition on the expression of adhesion molecules on monocytes are sparse.

The aim of the present study was to examine the effect of GPIIa/IIIb inhibition with eptifibatide and tirofiban on the expression of the cellular adhesion molecules CD11b (MAC1), CD54 (ICAM1) and CD162 (P-selectin glycoprotein ligand-1; PSGL1) on monocytes at different blood temperatures using an *in vitro* ECC model.

Materials and Methods

Inclusion of healthy volunteers was after informed consent was obtained and the study protocol conforms to the ethical guidelines of the 1975 Declaration of Helsinki and was approved by the local Ethics Committee (65/2002V).

Blood sampling. Blood from non-medicated healthy male volunteers (n=6) was collected by venipuncture with a 21-gauge needle from an antecubital vein. The first 5 ml of blood were discharged before additional blood samples were drawn for analysis. All blood samples were anti-coagulated with 3 U/ml heparin.

Modified Chandler loop and sample preparation. Experiments were performed using an *in vitro* model (modified Chandler loop) as described previously (6,12). For each experiment, six PVC tubings without additional coating (Jostra, Hechingen, Germany) were filled with 20 ml of blood from a single donor. Samples of two of the six PVC tubings were prepared by the addition of eptifibatide (Integrillin®; Essex Pharma GmbH, München, Germany), two tubings were prepared with the addition of tirofiban (Aggrastat®; MSD Sharp and Dome GmbH, Haar, Germany) and two tubings were left untreated as controls. Afterwards, the tubings were closed into circuits (tubing length: 50 cm, diameter 3/8×3/32 inch) with a piece of silicone tubing. One tubing containing eptifibatide, one tubing containing tirofiban and one control tubing were rotated vertically (30 rpm) during incubation in a water-bath at 36˚C. The other three tubings were rotated during incubation in a water bath at 18˚C. The respective temperatures were established by keeping the water bath at the desired temperature. For each experiment, all test tubings were filled with blood from a single donor and were run in parallel. Samples for analysis were taken after 30 minutes of circulation.

Sub-sample preparation for flow cytometry. The following incubation steps were performed immediately after 30 min of blood circulation in the ECC model using a previously described method (12). Expression of the following adhesion molecules on monocytes was measured by flow cytometry (EPICS XL-MCL; Coulter Electronics, Krefeld, Germany). Leukocytes were detected using fluorescein-isothiocyanate (FITC)-conjugated anti-CD45 (leukocyte common antigen) (Becton and Dickinson Biosciences, Heidelberg, Germany). The following phycoerythrine (PE)-conjugated monoclonal antibodies were used for cell detection in fluorescence-activated cell sorting (FACS): anti-CD11b, anti-CD54 (both from Becton and Dickinson Biosciences) and CD162 (Immunotech; Coulter, Krefeld, Germany). Whole blood (100 μl) was incubated with saturating concentrations of FITC-conjugated anti-CD45 and PE-conjugated monoclonal antibodies for 20 min at room temperature. Erythrocytes were lysed and leukocytes were fixed with a commercially-available solution (FACS Lysin Solution; Becton and Dickinson Biosciences). Samples were then incubated for 10 min in the dark. Thereafter, samples were centrifugated at 200 ×g for 10 min, the pellet washed with phosphate-buffered saline (Gibco, Karlsruhe, Germany), and recentrifuged. The pellet was then resuspended in phosphate-buffered saline and applied to the flow cytometer equipped with a 488-nm argon laser. Results are expressed as the mean fluorescence intensity (MFI) of CD11b, CD54 and CD162 on monocytes.

Statistical methods. Two groups of variables with Gaussian distributions were compared by unpaired Student t-test. The Mann–Whitney *U*-test was used as a non-parametric test. Deviations from a Gaussian distribution were tested by the Kolmogorov–Smirnov test. The Kruskal–Wallis test was used to analyze the significance of differences between the three groups (eptifibatide, tirofiban and control tubing) incubated at 18˚C or at 36˚C. Data are presented as the median with minimum and maximum. Two-tailed *p*-values of less than 0.05 were regarded as significant, *p*-values between 0.05 and 0.1 as significant by tendency. The calculations were performed with InStat (GraphPad Software, San Diego, USA) and IBM SPSS software (IBM SPSS Statistics, Germany).

Results

The volunteers (n=11) had no history of acute or chronic disease and were non-medicated. Their mean age was 26.5 years. All donors were male.

After 30 min of blood circulation in the ECC model the expression levels of CD11b measured by flow cytometry as the MFI was lower at 18˚C compared to 36˚C by 51% in the eptifibatide group (p=0.0043), by 29% in the tirofiban group (p=0.095) and by 34% in the control group (p=0.038). Expression of CD162 was lower at 18˚C compared to 36˚C

by 40% using eptifibatide (p=0.0010), by 94% using tirofiban (p=0.0095) and by 34% in the control group (p=0.019). Expression of CD54 did not change at 18˚C compared to 36˚C neither with eptifibatide (p=0.29) or tirofiban (p=0.48), nor in the control group (p=0.26); (Table I). After 30 min of blood circulation in the ECC model at 36˚C no significant differences were found in the expression of CD11b, CD54 and CD162 on monocytes between the eptifibatide-treated group, the tirofiban-treated group and the control group. There were also no significant differences in the expression of CD11b, CD54 and CD162 on monocytes between the three groups at 18˚C. Medians with quartiles of the expression levels measured by flow cytometry are depicted in Figures 1-3.

Table I. *Expression levels of CD11b (macrophage-1 antigen; MAC1), CD54 (intercellular adhesion molecule-1; ICAM1) and CD162 (P-selectin glycoprotein ligand-1; PSGL1) on monocytes at normothermia and hypothermia in the eptifibatide-treated group, the tirofiban-treated group and the control group. Data are presented as median (minimum–maximum) values of mean fluorescence intensity (MFI).*

	MFI		
	36˚C	18˚C	p-Value
CD11b			
Eptifibatide	25.8 (15.6-48.1)	12.7 (7.2-17.4)	0.0043
Tirofiban	23.6 (12.8-35.6)	16.8 (12.7-18.0)	0.095
Control	22.4 (17.9-44.4)	14.9 (11.4-21.3)	0.038
CD54			
Eptifibatide	5.96 (4.17-13.4)	5.02 (4.07-6.66)	0.29
Tirofiban	5.24 (4.10-6.82)	4.80 (3.62-5.91)	0.48
Control	5.90 (4.19-7.21)	4.85 (3.72-5.85)	0.26
CD162			
Eptifibatide	33.8 (20.7-36.2)	20.3 (11.8-22.2)	0.0010
Tirofiban	31.2 (21.3-43.5)	1.95 (1.45-2.57)	0.0095
Control	31.0 (22.8-37.0)	20.4 (12.0-26.2)	0.019

Discussion

The present investigation examined the effect of GPIIb/IIIa inhibition with eptifibatide and tirofiban on the expression of the cellular adhesion molecules CD11b, CD54 and CD162 on monocytes using a well-established *in vitro* model simulating ECC. The experiments were performed during normothermia (36˚C) and hypothermia (18˚C). The results demonstrate that the expression of these adhesion molecules on monocytes is not affected by GPIIb/IIIa inhibition with eptifibatide or tirofiban, neither during normothermic conditions nor during hypothermia.

The contact of blood with artificial biomaterials of the ECC circuit induces complex inflammatory reactions which can lead to life-threatening complications. A key role triggering these inflammatory responses play activated leukocytes and their interaction with activated platelets and the endothelium. The cell–cell interaction of these cells is mediated by adhesion molecules which are expressed on the surface of the cells. Therapeutic strategies during ECC such as therapeutic hypothermia or platelet anaesthesia have an impact on the activation of platelets and leukocytes and may influence the inflammatory reactions during ECC. An effect of hypothermia on the expression of cellular adhesion molecules on monocytes during extracorporeal circulation was recently demonstrated by our group (12). Platelet anaesthesia is a pharmacological strategy to protect platelets during ECC by temporary blockade of the main platelet receptor GPIIb/IIIa (3, 6). The currently available GPIIb/IIIa inhibitors differ markedly in pharmacokinetics, pharmacodynamics, and specific receptor affinity (10, 11, 13). The molecular basis of these blocking agents are either monoclonal antibodies (*e.g.* abciximab), ligand mimetic peptides (*e.g.* epitifibatide) or synthetic ligand mimetics (*e.g.* tirofiban) (11). Separate and distinct binding sites on the GPIIb/IIIa receptor complex have been defined for abciximab and for the small-molecule GPIIb/IIIa inhibitors (eptifibatide, tirofiban). Abciximab has a high affinity, and the small-molecule inhibitors a relatively low affinity, for binding to the GPIIb/IIIa receptor. Similarly, the pharmacokinetic off-rates of these two types of agents are markedly different (10, 13, 14).

Abciximab has cross-affinity for the additional integrin receptors vitronectin ($\alpha V\beta 3$) and MAC1 (CD11b/CD18), which modulate multiple functions distinct from platelet aggregation (13). Interest in these abciximab non-platelet receptor effects has been heightened by the observation that platelet inhibition alone may not fully explain the magnitude of clinical benefit attributable to abciximab therapy (13). On the other hand, cross-reaction with the activated form of CD11b/CD18 was reported for abciximab but not shown for tirofiban and eptifibatide (9, 13, 15, 16). The CD11b/CD18 is a heterodimer in the $\beta 2$ integrin family that promotes adhesion of neutrophils and monocytes to CD54 on endothelial cells, extracellular matrix, components of the complement system and of the coagulation cascade (*e.g.* Factor X and fibrinogen) (14, 17). It is stored in secretory granules in these leukocytes and can be rapidly mobilized to the cell surface following cell activation (14). Simon *et al.* demonstrated that abciximab directed against platelet IIb/IIIa also directly binds to MAC1-expressing monocytic and transfected cells and thereby inhibits MAC1-dependent adhesion to fibrin(ogen) and ICAM1 (17). Additionally, abciximab inhibits fibrinogen and factor X binding to MAC1, MAC1-mediated conversion of factor X to factor Xa and fibrinogen-mediated monocyte aggregation and monocyte adhesion as well as MAC1-mediated monocyte adhesion on ICAM1 (11). Experimental data *in vivo* and *in*

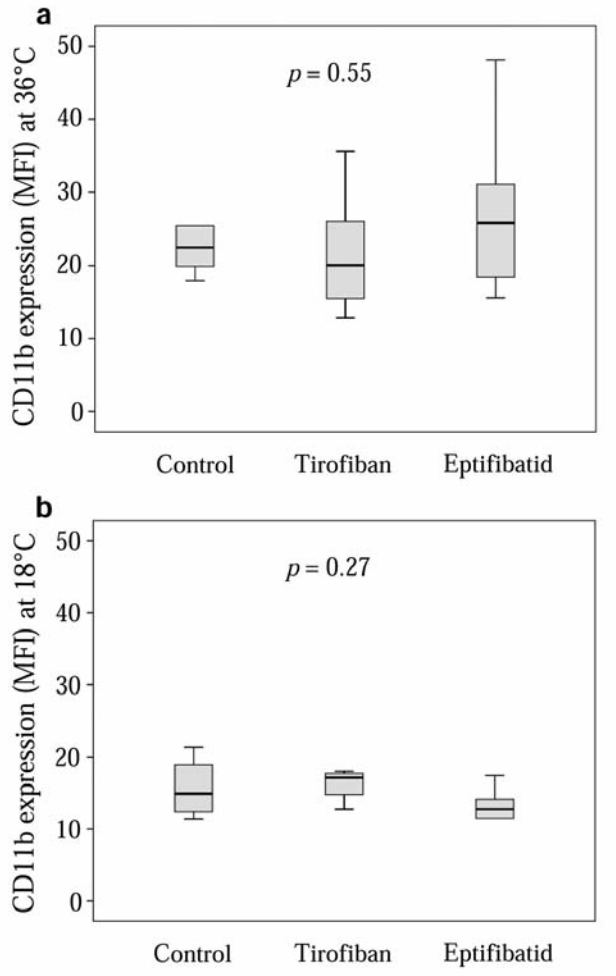

Figure 1. *Expression of CD11b (macrophage-1 antigen; MAC1) on monocytes at normothermia (a) and hypothermia (b) in the eptifibatide-treated group, the tirofiban-treated group and the control group. Data are presented as medians of mean fluorescence intensity (MFI) with 25th and 75th percentiles (boxes) and 10th and 90th percentiles (whiskers).*

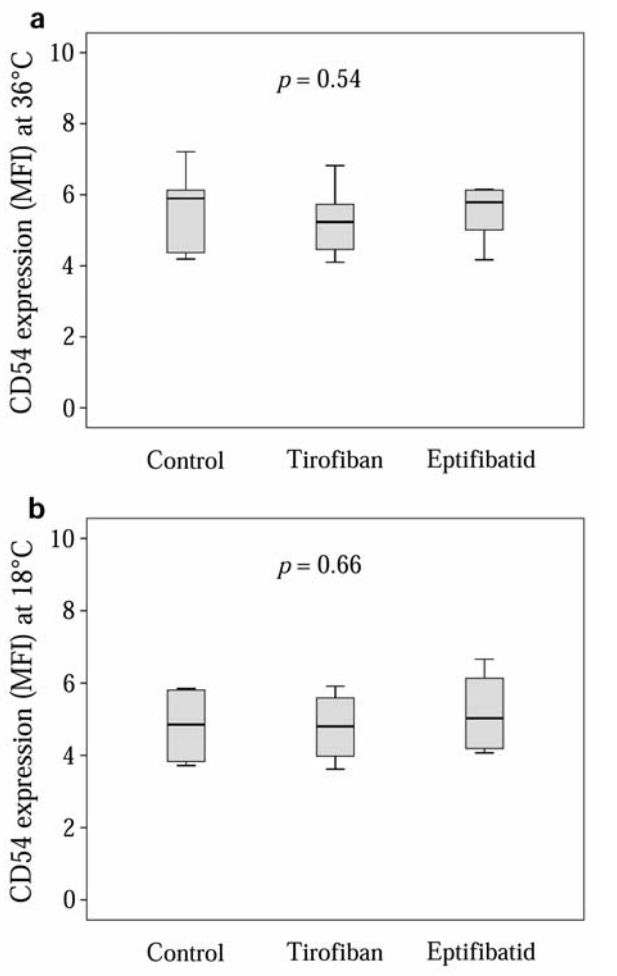

Figure 2. *Expression of CD54 (intercellular adhesion molecule-1; ICAM1) on monocytes at normothermia (a) and hypothermia (b) in the eptifibatide-treated group, the tirofiban-treated group and the control group. Data are presented as medians of mean fluorescence intensity (MFI) with 25th and 75th percentiles (boxes) and 10th and 90th percentiles (whiskers).*

vitro have shown that leukocyte CD11b expression, especially on neutrophils, diminished and remained low for some time after GPIIbIIIa blockade using abciximab (14). In the same study, changes in CD11b expression, either on isolated neutrophils or neutrophils or monocytes in whole blood, did not appear to involve direct interactions of abciximab with CD11b (14). In contrast, for the small molecule GPIIb/IIIa inhibitors eptifibatide and tirofiban, in the present investigation we did not find significant differences in the expression of the cellular adhesion molecules CD11b and CD54 on monocytes compared to the control group. Thus, the influence of GPIIb/IIIa inhibitors on the expression of adhesion molecules on leukocytes is probably not a class effect but is different for the available GPIIb/IIIa inhibitors due to differences in the molecular basis, specific receptor affinity and different binding sites to the GPIIb/IIIa receptor complex. On the other hand, anti-inflammatory properties of GPIIb/IIIa inhibitors may not be necessarily accompanied with a decrease in expression of cellular adhesion molecules, since the avidity of integrins may be rapidly and reversibly altered from a latent (inactive) state to a high-affinity (active) state without changes in receptor number (17). The MAC1 receptor (CD11b) undergoes a conformational change when the leukocyte is stimulated and contributes to the process of neutrophil adhesion, trans-migration across endothelium, neutrophil

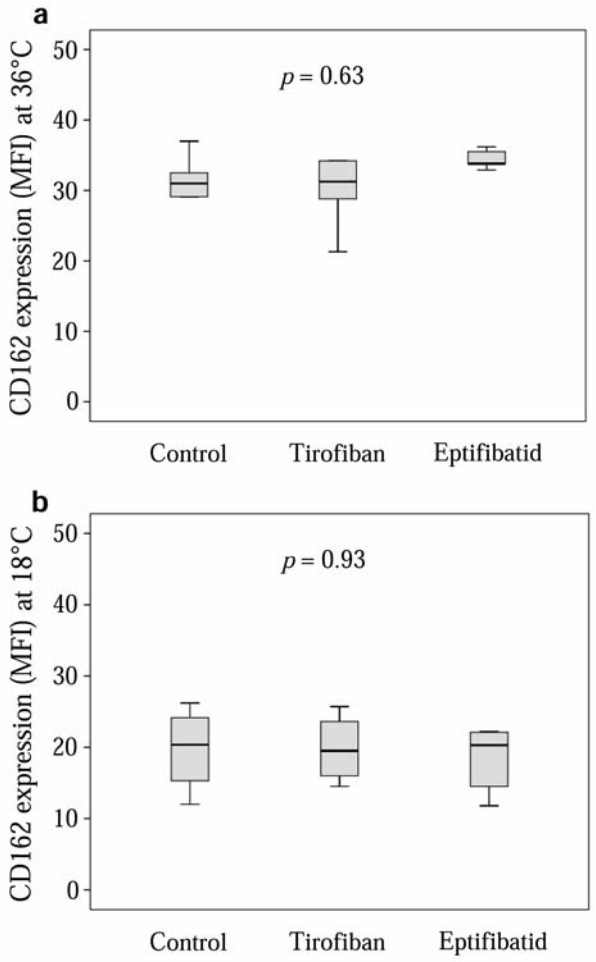

Figure 3. Expression of CD162 (P-selectin glycoprotein ligand-1; PSGL1) on monocytes at normothermia (a) and hypothermia (b) in the eptifibatide-treated group, the tirofiban-treated group and the control group. Data are presented as medians of mean fluorescence intensity (MFI) with 25th and 75th percentiles (boxes) and 10th and 90th percentiles (whiskers).

aggregation, chemotaxis, and phagocytosis of opsonised particles (10). Schwarz *et al*. demonstrated that abciximab binds specifically to MAC1 on activated monocytes, while unstimulated monocytes did not bind abciximab (11).

ICAM1/CD54 is a member of the immunoglobulin superfamiliy. It is expressed on the cell surface of a wide variety of cell types including endothelial cells and leukocytes functioning as a key receptor in the cell–cell interaction (18, 19). The nuclear factor kappaB (NFκB) signaling cascade is pivotal in ICAM1 activation and is activated by the proinflammatory cytokines tumor necrosis factor-α and interleukin-1β, the major inducers of ICAM1 expression in most cell types (19). Experimental data demonstrated that the GPIIb/IIIa inhibitors abciximab, eptifibatide and tirofiban completely prevent NF-κB activation in granulocyte macrophage colony-stimulating factor-treated neutrophils on fibronectin that had acquired GPIIb/IIIa receptors (9). These acquired receptors are functional, allowing for NF-κB activation (9). Clinical investigations have shown that tirofiban infusion suppresses the elevation of soluble ICAM1 in patients with unstable angina pectoris (8). In the present study, no effect of tirofiban and eptifibatide on the expression of cellular ICAM1 (CD54) on monocytes was found compared to the control group. One reason might be that NFκB-independent pathways may also participate in the induction of the ICAM1 expression (18).

Thrombin-activated platelets adhere to leukocytes *via* P-selectin. The interaction of platelets with leukocytes involves PSGL1 (CD162), and possibly MAC1 (CD11b), which interacts with fibrinogen bound to platelets *via* GPIIbIIIa or possibly tethering on platelet ICAM2 (14). Even though GPIIb/IIIa antagonists can completely block platelet aggregation, they do not prevent platelet activation, including surface expression of adhesion molecules such as CD62P. Platelet activation results in the formation of platelet–monocyte conjugates, mainly *via* CD62P on activated platelets binding PSGL1 on activated leukocytes. Some evidence was already provided that the GPIIb/IIIa antagonist eptifibatide may increase the formation of platelet-monocyte conjugates (20). Additionally, *in vitro* experiments have shown that tirofiban and epitfibatide but not abciximab enhance leukocyte-platelet aggregation in whole blood (10). Because leukocyte-platelet interactions mediate, in part, inflammatory reactions, Kereiakes *et al*. hypothesized that small-molecule GPIIb/IIIa antagonists such as tirofiban and eptifibtide (but not abciximab) may probably elicit a potentially deleterious cellular response despite their ability to inhibit platelet aggregation (10). In contrast Frelinger *et al*. found a GPIIb/IIIa antagonist-induced decrease in leukocyte–monocyte aggregates by using abciximab, eptifibatide or tirofiban (21). In the present study eptifibatide or tirofiban had no effect on the expression of PSGL1 (CD162) and MAC1 (CD11b) on monocytes.

Therapeutic hypothermia has been used clinically for many years to preserve the heart during surgery (*e.g*. coronary artery bypass) and to preserve organs before transplantation (22). Since CD11b, CD54 and CD162 play an important role in leukocyte-platelet interactions mediating *e.g*. ECC-induced inflammatory responses, we examined the effect of eptifibatide and tirofiban in combination with hypothermia on the expression of these adhesion molecules on monocytes in an *in vitro* ECC-model. The effect of hypothermia on the expression of these adhesion molecules in the absence of GPIIb/IIIa antagonists was reported elsewhere (12). Experimental data demonstrated that moderate hypothermia delays the production of NF-κB activation (23) and that the GPIIb/IIIa inhibitors abciximab,

eptifibatide and tirofiban completely prevent NF-κB activation in granulocyte macrophage colony-stimulating factor-treated neutrophils on fibronectin that had acquired GPIIb/IIIa receptors (9). Thus, GPIIb/IIIa inhibition during hypothermia may probably have an impact on the expression of adhesion molcules on monocytes. In a study of Frelinger *et al*. mild hypothermia augments eptifibatide- and tirofiban-induced inhibition of platelet aggregation and diminishes the GPIIb/IIIa antagonist-induced decrease in leukocyte-platelet aggregates (21). In the present investigation the expression of CD11b and CD162 was significantly reduced in the eptifibatide-treated, tirofiban-treated and the control group during hypothermia compared to normothermic conditions while hypothermia had no effect on the expression of CD54 on monocytes. In the case of CD11b using tirofiban, the reduction of expression from 36˚C to 18˚C was quite strong (29%) but only significant for tendency. Again no significant differences were found between the three groups during hypothermia. Thus, our data demonstrate that additional GPIIb/IIIa inhibition during hypothermia neither augments nor diminishes the beneficial effect of hypothermia during ECC regarding the decrease in expression of CD11b and CD162 on monocytes.

Conclusion

Our data demonstrate that GPIIb/IIIa inhibition with eptifibatide or tirofiban has no effect on the expression of the cellular adhesion molecules CD11b, CD54 and CD162 on monocytes during normothermia, as well as during hypothermic conditions in an ECC model. Additionally, GPIIb/IIIa inhibition seems to have no impact on the hypothermia induced decrease in the expression of CD11b and CD162 on monocytes. Thus, the hypothermia-induced beneficial effect on the ECC-associated alteration of leukocyte function is not affected by additional treatment with the GPIIb/IIIa inhibitors eptifibatide or tirofiban.

References

1 Tarnok A, Bocsi J, Rossler H, Schlykow V, Schneider P and Hambsch J: Low degree of activation of circulating neutrophils determined by flow cytometry during cardiac surgery with cardiopulmonary bypass. Cytometry *46(1)*: 41-49, 2001.

2 Rimmele T, Bishop J, Simon P, Carter M, Kong L, Lee M, Singbartl K and Kellum JA: What blood temperature for an ex vivo extracorporeal circuit? Artif Organs *35(6)*: 593-601, 2011.

3 Straub A, Azevedo R, Beierlein W, Wendel HP, Dietz K and Ziemer G: Tirofiban (Aggrastat) protects platelets and decreases platelet-granulocyte binding in an extracorporeal circulation model. Thorac Cardiovasc Surg *54(3)*: 162-167, 2006.

4 Wan S, Le Clerc JL and Vincent JL: Inflammatory response to cardiopulmonary bypass: Mechanisms involved and possible therapeutic strategies. Chest *112(3)*: 676-692, 1997.

5 Asimakopoulos G, Kohn A, Stefanou DC, Haskard DO, Landis RC and Taylor KM: Leukocyte integrin expression in patients undergoing cardiopulmonary bypass. Ann Thorac Surg *69(4)*: 1192-1197, 2000.

6 Straub A, Schiebold D, Wendel HP, Azevedo R, Dietz K and Ziemer G: Platelet anaesthesia during extracorporeal circulation: Differential effects of GP IIb/IIIa blockers on platelet activation marker P-selectin expression at hypothermia. Thromb Res *122(3)*: 383-389, 2008.

7 De Carlo M, Maselli D, Cortese B, Ciabatti N, Gistri R, Levantino M, Balbarini A, De Caterina R and Petronio AS: Emergency coronary artery bypass grafting in patients with acute myocardial infarction treated with glycoprotein IIb/IIIa receptor inhibitors. Int J Cardiol *123(3)*: 229-233, 2008.

8 Ercan E, Bozdemir H, Tengiz I, Sekuri C, Aliyev E, Akilli A and Akin M: Decreased soluble cell adhesion molecules after tirofiban infusion in patients with unstable angina pectoris. Thromb J *2(1)*: 4, 2004.

9 Salanova B, Choi M, Rolle S, Wellner M, Luft FC and Kettritz R: Beta2-integrins and acquired glycoprotein IIb/IIIa (GPIIb/IIIa) receptors cooperate in NFκB activation of human neutrophils. J Biol Chem *282(38)*: 27960-27969, 2007.

10 Kereiakes DJ, Runyon JP, Broderick TM and Shimshak TM: IIb's are not IIb's. Am J Cardiol *85(8A)*: 23C-31C, 2000.

11 Schwarz M, Nordt T, Bode C and Peter K: The GP IIb/IIIa inhibitor abciximab (c7E3) inhibits the binding of various ligands to the leukocyte integrin Mac-1 (CD11b/CD18, αMβ2). Thromb Res *107(3-4)*: 121-128, 2002.

12 Swoboda S, Gruettner J, Lang S, Wendel HP, Beyer ME, Griesel E, Hoffmeister HM and Walter T: Hypothermia inhibits expression of CD11b (MAC-1) and CD162 (PSGL-1) on monocytes during extracorporeal circulation. In Vivo *27(4)*: 459-464, 2013.

13 Kereiakes DJ: Effects of GP IIb/IIIa inhibitors on vascular inflammation, coronary microcirculation, and platelet function. Rev Cardiovasc Med *7(Suppl 4)*: S3-11, 2006.

14 Mickelson JK, Ali MN, Kleiman NS, Lakkis NM, Chow TW, Hughes BJ and Smith CW: Chimeric 7E3 Fab (ReoPro) decreases detectable CD11b on neutrophils from patients undergoing coronary angioplasty. J Am Coll Cardiol *33(1)*: 97-106, 1999.

15 Seshiah PN, Kereiakes DJ, Vasudevan SS, Lopes N, Su BY, Flavahan NA and Goldschmidt-Clermont PJ: Activated monocytes induce smooth muscle cell death: Role of macrophage colony-stimulating factor and cell contact. Circulation *105(2)*: 174-180, 2002.

16 Coller BS: Potential non-glycoprotein IIb/IIIa effects of abciximab. Am Heart J *138(1 Pt 2)*: S1-S5, 1999.

17 Simon DI, Xu H, Ortlepp S, Rogers C and Rao NK: 7E3 Monoclonal antibody directed against the platelet glycoprotein IIb/IIIa cross-reacts with the leukocyte integrin MAC-1 and blocks adhesion to fibrinogen and ICAM-1. Arterioscler Thromb Vasc Biol *17(3)*: 528-535, 1997.

18 Gorgoulis VG, Zacharatos P, Kotsinas A, Kletsas D, Mariatos G, Zoumpourlis V, Ryan KM, Kittas C and Papavassiliou AG: P53 activates ICAM-1 (CD54) expression in an NF-κB-independent manner. EMBO J *22(7)*: 1567-1578, 2003.

19 Roebuck KA and Finnegan A: Regulation of intercellular adhesion molecule-1 (CD54) gene expression. J Leukoc Biol *66(6)*: 876-888, 1999.

20 Scholz T, Zhao L, Temmler U, Bath P, Heptinstall S and Losche W: The GPIIb/IIIa antagonist eptifibatide markedly potentiates platelet-leukocyte interaction and tissue factor expression following platelet activation in whole blood *in vitro*. Platelets *13(7)*: 401-406, 2002.

21 Frelinger AL, III, Furman MI, Barnard MR, Krueger LA, Dae MW and Michelson AD: Combined effects of mild hypothermia and glycoprotein IIb/IIIa antagonists on platelet-platelet and leukocyte-platelet aggregation. Am J Cardiol *92(9)*: 1099-1101, 2003.

22 Schwartz BG, Kloner RA, Thomas JL, Bui Q, Mayeda GS, Burstein S, Hale SL, Economides C and French WJ: Therapeutic hypothermia for acute myocardial infarction and cardiac arrest. Am J Cardiol *110(3)*: 461-466, 2012.

23 Kimura A, Sakurada S, Ohkuni H, Todome Y and Kurata K: Moderate hypothermia delays proinflammatory cytokine production of human peripheral blood mononuclear cells. Crit Care Med *30(7)*: 1499-1502, 2002.

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