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Activated human platelets induce factor XIIa-mediated contact activation

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ABSTRACT

Earlier studies have shown that isolated platelets in buffer systems can promote activation of FXII or amplify contact activation, in the presence of a negatively charge substance or material. Still proof is lacking that FXII is activated by platelets in a more physiological environment. In this study we investigate if activated platelets can induce FXII-mediated contact activation and whether this activation affects clot formation in human blood.

Human platelets were activated with a thrombin receptor-activating peptide, SFLLRN-amide, in platelet-rich plasma or in whole blood. FXIIa and FXIa in complex with preferentially antithrombin (AT) and to some extent C1-inhibitor (C1INH) were generated in response to TRAP stimulation. This contact activation was independent of surface-mediated contact activation, tissue factor pathway or thrombin. In clotting whole blood FXIIa-AT and FXIa-AT complexes were specifically formed, demonstrating that AT is a potent inhibitor of FXIIa and FXIa generated by platelet activation. Contact activation proteins were analyzed by flow cytometry and FXII, FXI, high-molecular weight kininogen, and prekallikrein were detected on activated platelets. Using chromogenic assays, enzymatic activity of platelet-associated FXIIa, FXIa, and kallikrein were demonstrated. Inhibition of FXIIa in non-anticoagulated blood also prolonged the clotting time.

We conclude that platelet activation triggers FXII-mediated contact activation on the surface and in the vicinity of activated platelets. This leads specifically to generation of FXIIa-AT and FXIa-AT complexes, and contributes to clot formation. Activated platelets may thereby constitute an intravascular locus for contact activation, which may explain the recently reported importance of FXII in thrombus formation.

Introduction

Coagulation FXII of the contact system, is a multifunctional protein that influences several cascade systems, including the coagulation, fibrinolysis, complement, and kallikrein-kinin systems. In the presence of negatively charged surfaces, FXII initiates blood coagulation via contact activation, involving high-molecular weight kininogen (HK), plasma prekallikrein, and coagulation FXI. Apart from situations in which incompatibility reactions are triggered by biomaterials [1], FXII-mediated contact activation has not been considered to contribute to blood coagulation, since patients with FXII, prekallikrein, or HK deficiencies do not exhibit abnormal bleeding. However, interpreting the physiological role of FXII in coagulation is complicated by the dual activity of FXII; as a direct or indirect trigger of both the antagonistic fibrinolytic pathway and the kallikrein–kinin system. Thus, if fibrinolysis was attenuated as the result of a deficiency, the contribution of FXII to amplification of the coagulation would also be lost.

In vivo studies have indicated that mice lacking FXII exhibit defective thrombus formation, which causes unstable and non-occlusive platelet-rich thrombi [2,3]. These mice, like those treated with a FXIIa inhibitor, are protected from induced arterial thrombosis and stroke [4]. The mechanism by which FXII is recruited into the clot formation process is not fully understood even though generation of FXIIa directly on the platelet surface has been proposed [5].

Already in the sixties the idea about contact activation in the plasmatic atmosphere of platelets was raised and data indicating the presence of activated FXII and FXI on the platelet surface was presented [6,7]. In the early eighties it was shown that isolated platelets in buffer systems containing purified proteins could promote the activation of FXII [8]. The latter study was performed *in vitro* with isolated platelets and proteins, and modern works in blood plasma have only showed that platelets are able to amplify contact activation induced by a negatively charged substance or material, such as high-molecular weight dextran sulfate [5]. Proof is still lacking that activated platelets under physiological conditions themselves initiate the activation of the contact proteins.

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We have now revisited the hypothesis that FXIIa is generated directly on the platelet surface; in the present study, we have shown that activated platelets under nearly physiological conditions can bind FXII and initiate contact activation. This activation was independent of surface-mediated contact activation, tissue factor pathway, and thrombin, and led to the specific generation of FXIIa-antithrombin (AT) and FXIa-AT complexes. Furthermore, we have demonstrated the influence of this activation on clot formation.

Materials and methods

Heparin coating

Heparin coated containers were furnished with the Corline heparin surface (CHS, Corline Systems AB, Uppsala, Sweden), according to the manufacturer's recommendations. This surface is coated with heparin conjugates which are prepared by covalent binding of approximately 70 mol heparin/mol of carrier chain [9]. This procedure produces a surface with a heparin surface concentration of 0.5 μg/cm^2 and an AT binding capacity of 2–4 pmol AT/cm^2. The CHS prevents contact system activation [10] and has been shown to be totally stable with no detectable heparin leakage when incubated with plasma for up to 1 h [11].

Blood sampling

Ethical approval was obtained from the regional ethics committee. Fresh human blood, obtained from healthy volunteers who had received no medication for at least 10 days, was collected in three different ways: In vacutainer tubes containing a thrombin inhibitor, lepirudin (Schering AG, Saksa, Germany), at a final concentration of 50 μg/mL. In an open system without anticoagulant or in the presence of 0.13 M sodium citrate and where the materials that came into contact with blood were furnished with the CHS.

Preparation of PRP and PPP

Blood was centrifuged for 15 min at room temperature (RT) at 150*g* to obtain platelet-rich plasma (PRP) and twice at 2200*g* to obtain platelet-poor plasma (PPP). Platelets were counted in a Coulter® A^C·T diff™ Analyzer (Coulter Corporation, Miami, FL, USA).

Activation of platelets with thrombin receptor agonist peptide (TRAP)

To activate platelets, a thrombin receptor agonist peptide (TRAP, Phoenix Pharmaceuticals, Belmont, CA, USA), SFLLRN-amide, which selectively binds to PAR-1, was used. PRP obtained from lepirudin-treated blood, was incubated with TRAP for 15 min at 37 °C at a final concentration of 20 μM in polypropylene tubes (Safe-lock tubes 1,5 ml, Eppendorf AG, Hamburg, Germany); non-activated platelets incubated simultaneously served as controls. This activation was also performed in heparinized tubes (CHS) in the presence of a tissue factor inhibitory monoclonal antibody (no 4509, American Diagnostics, Stamford, CT, USA) at a final concentration of 20 μg/mL. To stop the reaction EDTA at a final concentration of 10 mM was added. PPP was obtained and ATP release from the platelets was measured with an ATP Kit (SL144–041, BioThema Luminescent Assays, Stockholm, Sweden). The remaining PPP was stored at −70 °C for further analysis.

Whole blood models

(A) Ten microliters of rabbit brain thromboplastin-S (Biopool International, Ventura, CA, USA) diluted 1:100 in saline buffer, was added to: whole blood (800 μL), PRP (400 μL, diluted with PPP to the same platelet count as in blood), and PPP (400 μL) from non-anticoagulated blood obtained by open system. The samples were incubated at 37 °C for 2.5, 15, and 30 min in heparinized tubes (CHS).

(B) Inhibition of glycoprotein IIb/IIIa with abciximab (Reopro, Eli Lilly, Sweden AB, Solna, Sweden) was evaluated using a loop model. Loops of polyvinyl chloride (PVC) tubing (inner diameter: 4 mm, length: 400 mm) were used. 3 mL fresh non-anticoagulated blood was added to two loops. To one of them abciximab (final concentration of 50 μg/mL), was added. The loops were rotated vertically at 33 rpm for 30 min in a 37 °C incubator.

To stop the reactions, EDTA was added to give a final plasma concentration of 10 mM. All samples were centrifuged at 2200*g* for 15 min at 4 °C, and the plasmas obtained were stored at −70 °C until analyzed. Immediately before the reaction was stopped (in A), 10 μL samples of activated PRP were removed to measure the thrombin activity (chromogenic assays).

Preparation of platelets for flow cytometry and chromogenic assays

Platelets were activated with TRAP as described above but no EDTA was added after the incubation. Instead the platelet pellets obtained after centrifugation at 1100*g* for 15 min at RT were washed twice and resuspended to 3×10^9 or 10×10^9 or 350×10^9 cells/L in 37 °C Tyrodes buffer (pH 6.5) containing 3.5 mg/mL BSA (Sigma–Aldrich, St. Louis, MO, USA), 1 μM PGE_1 (Sigma–Aldrich), and 2 IU/mL heparin (LØvens Kemiske Fabrik, Ballerup, Denmark).

Flow cytometry

Platelet samples (100 μL containing 10×10^9 cells/L) were incubated for 60 min at RT with the following antibodies: anti-human P-selectin-RPE (Serotec, Oxford, UK) or Alexa Fluor® 488-labeled (Molecular Probes/Invitrogen, Eugene, OR, USA) sheep anti-human FXII, sheep anti-human FXI, sheep anti-human prekallikrein, and sheep anti-human HK (all from The Binding Site, Birmingham, UK) or normal sheep IgG-FITC (Caltag Laboratories, Burlingame, CA, USA) as a negative control. All antibodies were used at a final concentration of 5 μg/mL, except for anti-human P-selectin-RPE, which was used according to the manufacturer's recommendation. The labeled platelets were then washed in Tyrodes buffer and fixed with 0.2% paraformaldehyde. Other samples (100 μL containing 3×10^9 cells/L) were incubated for 60 min at RT with 10 μL annexin V (Becton Dickinson, San Jose, CA, USA). The bound fluorochrome-labeled antibodies and annexin V staining were monitored with a FACSCalibur (Becton Dickinson) and BD CellQuest Pro software. For each sample, ~30,000 cells were analyzed.

Chromogenic assays for thrombin and platelet-associated FXIIa, FXIa, and kallikrein

The chromogenic substrate S-2238 (Haemochrom Diagnostica, Mölndal, Sweden) was used to detect thrombin activity. PRP and PPP samples (10μL) were diluted 1:10 in Tris buffer (50 mM Tris, 175 mM NaCl, 7.5 mM EDTA, pH 8.4), and 100 μL of 0.44 mM S-2238 was added and incubated for 5 min at 37 °C. The reaction was stopped by adding 100 μL of 20% citric acid, and the absorbance at 405 nm was measured in the supernatant after centrifugation at 2200*g* for 2 min.

Chromogenic substrate S-2302 (Haemochrom Diagnostica) at a concentration of 2.5 mM was used to measure platelet-associated FXIIa and kallikrein. For the FXIIa assay, prekallikrein (Enzyme Research Laboratories, South Bend, IN, USA) at a final concentration of 0.35 μM was added to amplify the reaction. FXIa was assayed

with chromogenic substrate S-2366 (Haemochrom Diagnostica) at a concentration of 2.5 mM. To confirm that the generated activity came from the enzyme being measured, specific inhibitors were used. To inhibit FXIIa, corn trypsin inhibitor, CTI (Enzyme Research Laboratories) was used at 3.5 μM. For kallikrein and FXIa assays, soybean trypsin inhibitor, SBTI (Sigma–Aldrich) was used at 4.7 μM. For all assays, 100 μL of washed platelets diluted to 350×10^9 cells/L were preincubated for 10 min in the presence and absence of an inhibitor. Thereafter, 100 μL substrate was added and incubated for up to 3 h at 37 °C. The reaction was stopped and the absorbance measured as previously described. The absorbance increased linearly and data from each blood donor are presented for the time point giving the highest absorbance at the linear slope for TRAP-activated platelets. In the FXII assay, the absorbance due to spontaneous hydrolysis of prekallikrein was subtracted.

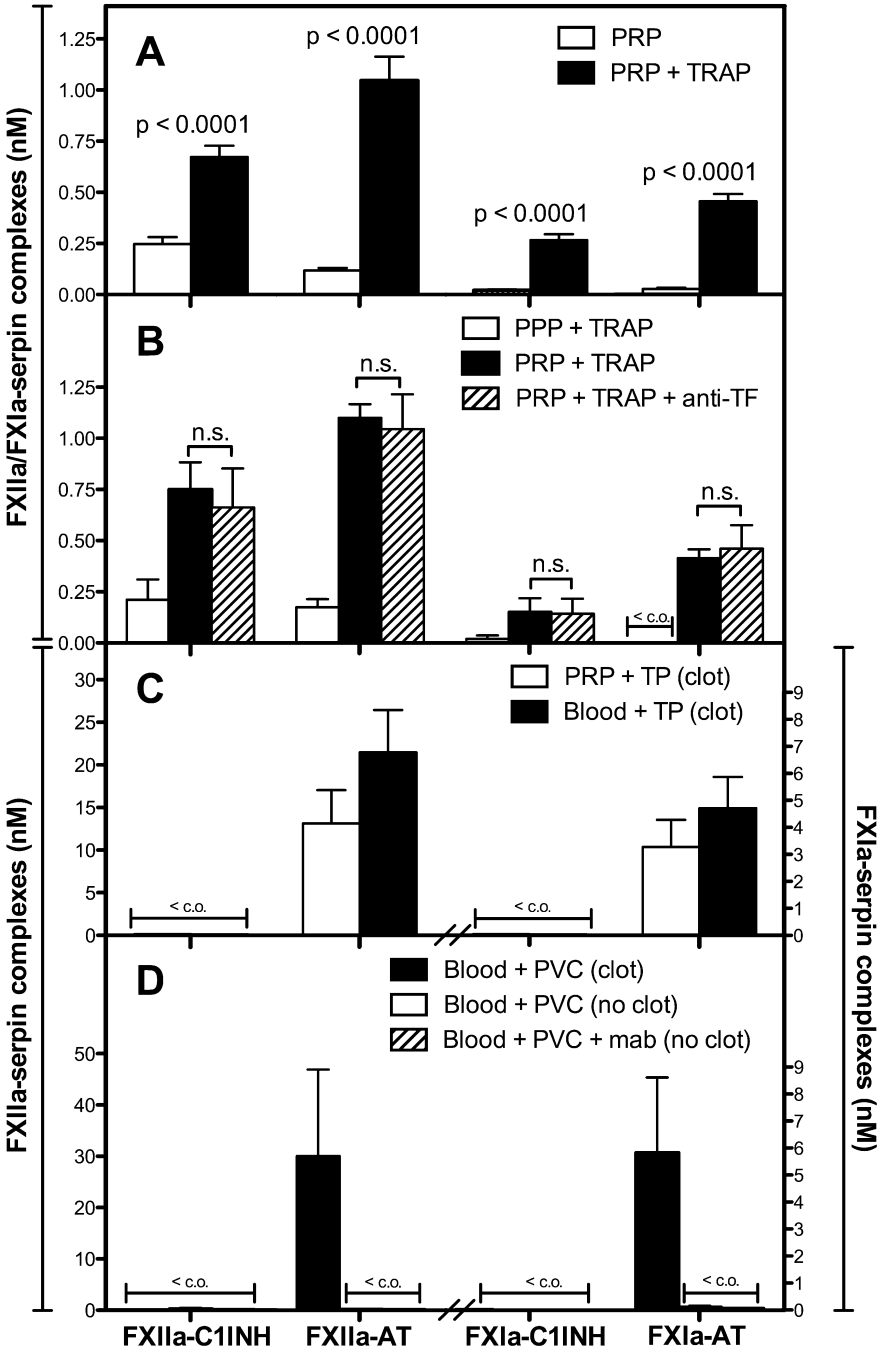

Fig. 1. Generation of platelet-mediated enzyme–serpin complexes. Generation of FXIIa-C1INH, FXIIa-AT, FXIa-C1INH, and FXIa-AT in plasma. Data represent the means ± SEM. (A,B) Complexes in lepirudin-treated PRP or PPP samples incubated for 15 min at 37 °C. (A) Non-activated (PRP) and TRAP-activated platelets (PRP + TRAP) incubated in plastic tubes ($n = 23–26$). (B) Control experiments, the activating-peptide added to plasma without platelets (PPP + TRAP) and TRAP-activated platelets in heparin-coated tubes (to avoid surface-mediated contact activation) in the absence (PRP + TRAP) and in the presence (PRP + TRAP + anti-TF) of an inhibitory tissue factor (TF) monoclonal antibody ($n = 5$). (C,D) Complexes in non-anticoagulated PRP and whole blood incubated for 30 min at 37 °C. (C) To initiate coagulation, non-anticoagulated PRP (PRP + TP) and whole blood (Blood + TP) were activated with thromboplastin (TP) in heparinized tubes ($n = 5$). (D) Control experiment, to initiate coagulation non-anticoagulated blood in the absence (Blood + PVC) and in the presence of abciximab (Blood + PVC + mab), which inhibits platelet aggregation by blocking GPIIb/IIIa, was rotated in PVC tubing (n = 8). Blood without abciximab was divided into bars without ($n = 3$) and with clots ($n = 5$). C.o., cut off.

Clotting times in PRP

All equipment that came into contact with PRP was coated with heparin (CHS). PRP obtained from citrated blood and a STart4 coagulometer (Diagnostica Stago, Asnières, France) were used. First 90 μL of PRP and 10 μL of Tris buffer (20 mM Tris, 30 mM NaCl, pH 8.2 [control]) or CTI (final concentration 3.5 μM) or an inhibitory tissue factor monoclonal antibody (No. 4509, American Diagnostics) were preincubated for 10 min. Thereafter, 75 μL of $CaCl_2$ (20 mM) was added to start the clotting reaction. To activate platelets, TRAP (20 μM) was added simultaneously with calcium.

ELISAs

Thrombin–antithrombin (TAT). Plasma levels of TAT were measured with a sandwich ELISA. TAT was captured in wells coated with anti-human thrombin diluted 1:120 and HRP-coupled anti-human antithrombin antibody diluted 1:120 was used for detection (both antibodies from Enzyme Research Laboratories). A standard prepared by diluting pooled human serum in normal citrate–phosphate-dextrose plasma was used. Values were expressed as μg/L.

FXIIa-C1-inhibitor (C1INH), FXIa-C1INH, FXIIa-AT, FXIa-AT. These complexes were measured by sandwich ELISA according to the method of Sanchez et al. [12]. In short, microtitre plates where coated with either anti-FXII or anti-FXI and the bound complexes detected with either anti-C1INH or anti-AT antibodies conjugated with HRP. The CV for all the assays is approximately 10%.

Statistical analyses

Non-parametric statistical analyses were performed (GraphPad Prism Ver4, GraphPad Software, Inc., San Diego, CA). The Mann–Whitney rank-sum test was used for comparisons between groups in the unpaired design (Figs. 1 and 3), whereas the Wilcoxons signed-rank test was used for paired comparisons (P-selectin expression, release of ATP, annexin V staining, Figs. 2 and 4). All tests were two-sided. Analysis of correlation was calculated with Spearman's rank-order correlation coefficient. In the Results section, means ± standard errors of the mean (SEM) are presented.

Results

Evaluation of platelet activation in PRP

The quality of non-activated and activated platelets used in the experiment below was validated by monitoring P-selectin expression, release of ATP, and changes in cell-surface charge. Flow cytometric analyses demonstrated low levels of P-selectin expression on non-activated platelets when compared to platelets activated with TRAP (mean fluorescence intensity [MFI], 250 ± 34 vs. 1070 ± 57; $n = 17$, $p = 0.0003$). TRAP-activated platelets released significantly higher levels of ATP (0.3 ± 0.1 vs. 4.8 ± 0.7 nM/(1×10^9 platelets); $n = 10$, $p = 0.0039$). Furthermore, staining with annexin V, confirmed that TRAP-activated platelets exposed a more negatively charged cell surface than did non-activated platelets (MFI, 30 ± 3 vs. 90 ± 16; $n = 15$, $p < 0.0003$).

Generation of platelet-mediated enzyme-serpin complexes

To determine whether TRAP-activated platelets in lepirudin-treated PRP are able to induce activation of the proenzyme factors XII and XI, we tested the supernatants by sandwich ELISA to detect complexes between enzymes and serpins. Levels close to the detection limit of the assays were found in all supernatants obtained from non-activated platelets. TRAP-mediated activation of platelets led to the formation of FXIIa-C1INH, FXIIa-AT, FXIa-C1INH, and FXIa-AT complexes (Fig. 1A).

In another experiment, platelet activation was triggered by TRAP in lepirudin-treated PRP in the presence and absence of a tissue factor blocking monoclonal antibody in heparinized tubes in order to avoid both tissue factor pathway and surface-mediated contact activation in the tubes (Fig. 1B) [10]. In PPP samples only levels close to the detection limit of the assays were found and TRAP-activated platelets generated the same amounts of complexes with or without inhibition of tissue factor. These data show that FXII and FXI are activated as a direct consequence of platelet activation in PRP.

Coagulation was triggered by thromboplastin in non-anticoagulated whole blood and PRP (Fig. 1C). Heparinized tubes were used

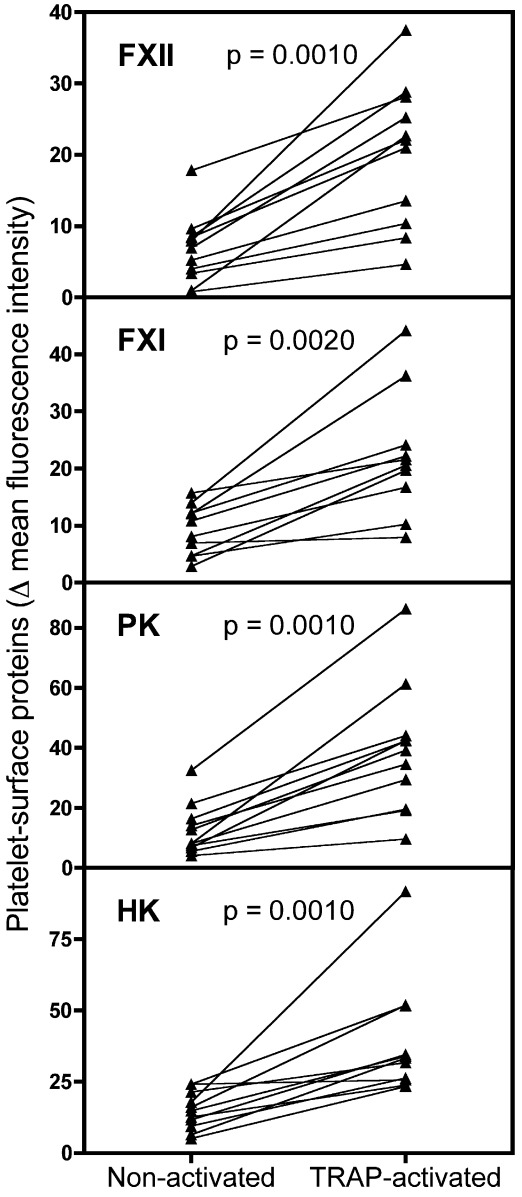

Fig. 2. Flow cytometric analyses of platelet-surface contact activation proteins. Platelets were TRAP-activated in lepirudin-treated PRP for 15 min, washed and stained for surface contact activation proteins. The mean fluorescence intensity (MFI) of non-activated and TRAP-activated platelets is shown, after subtracting the corresponding MFI of the negative control. Each panel shows the expression of one protein, with a line connecting the values from each individual ($n = 10–11$). PK, prekallikrein; HK, high-molecular weight kininogen.

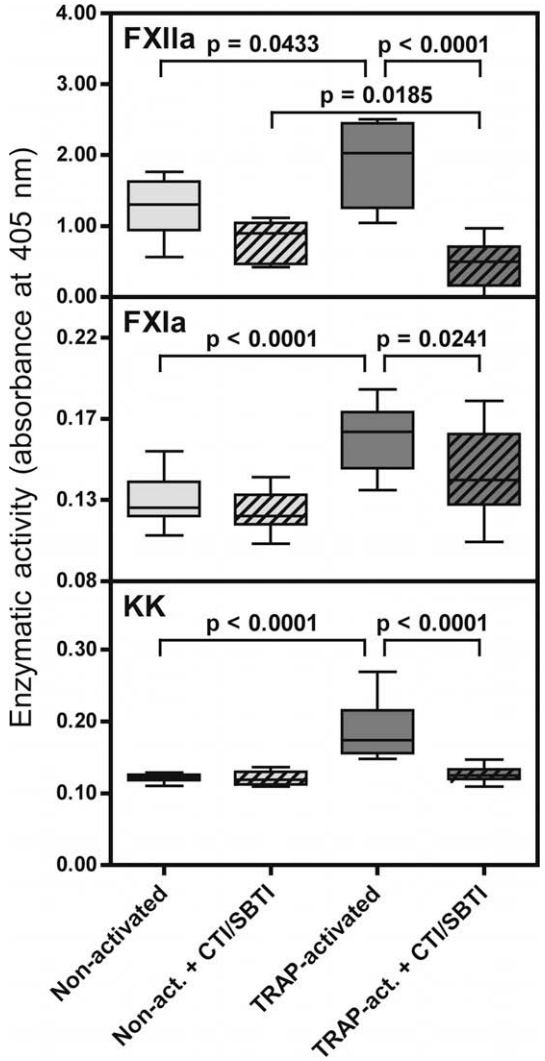

Fig. 3. Enzyme activity of FXIIa, FXIa, and kallikrein associated with platelets. Platelets were activated with TRAP in lepirudin-treated PRP for 15 min, washed and incubated with the appropriate chromogenic substrates as detailed in Materials and methods. The upper panel shows FXIIa activity, the middle panel FXIa activity, and the lower panel kallikrein (KK) activity. Corn trypsin inhibitor (CTI) was used in the FXIIa assay, and soybean trypsin inhibitor (SBTI) in the FXIa and kallikrein assays in order to inhibit the enzymatic activity (n = 10–15).

to avoid contact activation on the plastic surface. Thromboplastin activation caused an increase in FXIIa-AT and FXIa-AT in PRP and whole blood. Neither FXIIa-C1INH nor FXIa-C1INH was detected after thromboplastin activation demonstrating that C1INH did not primarily regulate these enzymes in clotting blood or PRP. The thrombin activity (r_s = 0.46, p = 0.0024) measured with chromogenic substrate S-2238 and the generation of TAT (r_s = 0.93, p < 0.0001) were correlated with the amount of FXIIa-AT.

Coagulation was also initiated in non-anticoagulated whole blood upon contact with the polymer and air surface in plastic (PVC) tubing. Macroscopic clotting was induced in 5 of 8 tubing. In 8 other tubing, clotting was abrogated by the addition of abciximab blocking GPIIb/IIIa. An increase in FXIIa-AT and FXIa-AT was observed in clotting blood but not in those without clotting (n = 3) or in the abciximab treated tubing (n = 8), (Fig. 1D). Negligible levels of FXIIa-C1INH and FXIa-C1INH were detected in all tubing. These data show that activated platelets are required for the formation of FXIIa-AT and FXIa-AT.

Contact activation proteins exposed on the platelet surface

The exposure of FXII, FXI, PK, and HK on non-activated and TRAP-activated platelets was measured by flow cytometry. For non-activated platelets low increases of the MFI were detected demonstrating minor exposures of the contact system proteins. The MFI, thus the protein exposure, increased significantly for all four contact system proteins when the platelets were TRAP-activated (Fig. 2). The increased MFI values were yet rather low for several individuals and only a small proportion of the platelet populations exposed the contact activation proteins. Similar results were obtained if the platelets were activated in whole blood instead of PRP (not shown).

Enzymatic activity of FXIIa, FXIa, and kallikrein associated with platelets

In order to determine whether bound FXII, FXI, and prekallikrein were activated in response to platelet activation, we assessed the enzymatic activity of their activation fragments in the presence of lepirudin, which inhibits thrombin. Washed non-activated and TRAP-activated platelets obtained from PRP were incubated with chromogenic substrates: either S-2302, for kallikrein but also to detect FXIIa (in the presence of prekallikrein) or S-2366, for FXIa. The FXIIa assay, which was amplified by the addition of prekallikrein, gave a higher response than the other assays and background absorbance due to spontaneous hydrolysis of prekallikrein had to be subtracted. Otherwise, no absorbance was detected when the chromogenic substrates were incubated in only buffer. Purified enzymes elicited a linear increase in the absorbance (not shown). Higher levels of enzyme activity were detected for FXIIa, FXIa, and kallikrein in the fractions containing TRAP-activated platelets compared to non-activated ones (Fig. 3). The enzyme activity was reduced or completely obliterated by CTI or SBTI. Thus, active enzymes of the contact system can be demonstrated on platelets activated in PRP.

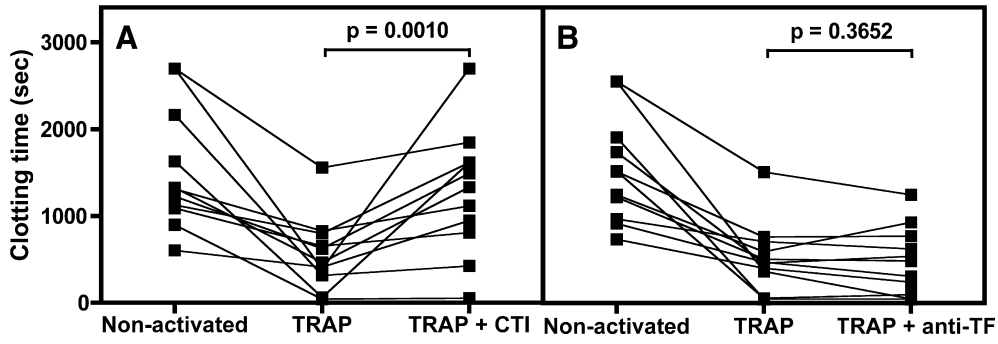

Fig. 4. Clotting time in PRP. Clotting times for non-activated and TRAP-activated platelets in recalcified citrate-treated PRP in heparin-coated tubes (to avoid surface-mediated contact activation). (A) FXIIa inhibition with corn trypsin inhibitor (CTI). (B) Tissue factor inhibition with an inhibitory antibody (anti-TF). Each line connects data from one individual (n = 11).

PRP clotting times

The clotting time in non-activated and TRAP-activated PRP (in the absence of lepirudin) with or without inhibition of either tissue factor or FXIIa was measured with a coagulometer (Fig. 4). In order to minimize material-induced contact activation, all equipment that came into contact with blood and PRP was coated with heparin. TRAP-activated platelets triggered shorter clotting times than did non-activated controls. Inhibition of tissue factor, by blocking the active site with a monoclonal antibody, did not affect the clotting time. Inhibition of FXIIa with CTI consistently prolonged the clotting time. In the case of one donor with a normal clotting time for TRAP-activated platelets, no clotting was achieved in the presence of CTI. We made the interesting visual observation that when the formed clots were shaken, they were consistently less stable in the presence of CTI than were those in control samples and when tissue factor was inhibited.

Discussion

Previous *in vitro* studies have demonstrated that platelets trigger FXII-mediated coagulation activation in buffer systems containing purified proteins [8] or amplify contact activation, in the presence of a negatively charge substance or material, such as high-molecular weight dextran sulfate, in plasma [5]. However, they have not provided evidence that activated platelets under physiological conditions themselves trigger the activation of the contact proteins.

In a human *in vitro* model in which platelets were activated by TRAP in PRP or clotting induced in whole blood we showed that platelet activation triggers contact activation. This activation was independent of surface-mediated contact activation, tissue factor pathway or thrombin and led to the specific generation of FXIIa-AT and FXIa-AT complexes. Using lepirudin made it possible to activate platelets under physiological conditions in the absence of clotting and any amplification mediated by thrombin. Tissue factor was blocked by a active-site specific antibody and the heparin coating has previously been shown not to promote any FXII activation and to be stable with no leakage of heparin [10,11,13], thereby abolishing the influence of material-induced contact activation. The formation of complexes containing activated forms of FXII and FXI (FXIIa-C1INH, FXIIa-AT, FXIa-C1INH, FXIa-AT) in supernatants obtained from TRAP-activated platelets confirmed that activation of these proteins had occurred as a direct consequence of platelet activation.

Interestingly, formation of FXIIa-AT and FXIa-AT complexes seemed to be specific for platelet-triggered FXII and FXI activation since no significant C1INH complexes were formed in clotting blood in the whole blood models. The notion that activated platelets was responsible for the generation of FXIIa-AT and FXIa-AT was additionally supported by the abrogation through blocking of the fibrinogen receptor GPIIb/IIIa. FXIIa apparently mediated the activation of FXI, since thrombin in the presence of lepirudin cannot contribute to this activation in our model system. This is in line with the withdrawal of reports, which supported the paradigm claiming that thrombin-mediated feedback activation of FXI on the activated platelet surface is preferred over contact activation by FXIIa [14].

Flow cytometric and chromogenic substrates analyses supported the concept that proteins of the plasma contact activation system were exposed on the surface of activated platelets. The exposure was not pronounced but was consistently observed. Our experiments demonstrated that FXIIa, FXIa, and kallikrein were enzymatically active on the activated platelet surface and confirmed the flow cytometric data. FXIIa-specific enzymatic activity was inhibited by CTI, a specific FXIIa inhibitor.

The levels of TAT, an indirect measure of thrombin generation, and the directly determined thrombin activity in clotting blood, were both strongly correlated with the levels of FXIIa-AT indicating that the platelet-mediated activation of FXII and FXI enhances the thrombin generation. Studies on clotting time confirmed that FXIIa, together with platelets, contributes to clot formation, since inhibition of FXIIa by CTI clearly prolonged the clotting time in an experimental setup where the material-triggered contact activation and the tissue factor pathway were inhibited by heparin-coatings and anti-TF antibodies, respectively.

Conclusion

This study has demonstrated that FXII-mediated contact activation is elicited by activated platelets contributing to clot formation. Activated platelets constitute a nidus for contact activation inside the blood vessels and are able to recruit proteins of the contact system into the clot formation process. These observations together with the fact that mice lacking FXII exhibit defective thrombus formation supports the notion that platelet-induced contact activation is important for the stability of a blood clot [2,3]. In the current cell-based coagulation model, coagulation is initiated on tissue factor-bearing cells and further propagated on the surface of activated platelets [15]. In this propagation step, the platelet-mediated contact activation represents an extra amplification mechanism. One can hypothesize that in FXII-deficient patients circulating clot fragments, due to decreased clot stability, may become lodged in smaller vessels, and together with an assumed decreased fibrinolysis because of lacking the contact activation mechanism [16,17], contribute to vessel occlusion. This could potentially explain the thrombotic events observed in FXII-deficient patients [18–20].

Acknowledgments

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