Journal of Thrombosis and Haemostasis, **1**: 243–249

ORIGINAL ARTICLE

Batroxobin-induced clots exhibit delayed and reduced platelet contractile force in some patients with clotting factor deficiencies

M. E. CARR JR,*†‡§¶ S. L. CARR,*§ T. TILDON,§¶ L. MCA. FISHER§¶ and E. J. MARTIN*§
*Coagulation Special Studies Laboratory, and Departments of †Medicine and ‡Pathology, Medical College of Virginia, VA, USA; §Virginia Commonwealth University, VA, USA; and ¶Richmond Veterans Administration Medical Center [5], Richmond, VA, USA

Summary. Thrombin causes platelet activation via multiple pathways, and deficient thrombin generation reduces platelet contractile force (PCF) during clot retraction. We hypothesized that PCF in blood samples from clotting factor-deficient patients would be diminished due to delayed or deficient thrombin generation. Blood samples from patients with fibrinogen, and factor V, VII, VIII, IX, X, XI and XIII deficiencies were compared to samples from normal controls. PCF in patient blood clotted with thrombin (1 NIH UmL^{-1}) was compared to PCF in clots formed with batroxobin (0.25 $\mu\text{g mL}^{-1}$). PCF in the former should be normal, but PCF in the latter is dependent on thrombin generation within the sample and might be deficient. In factor VII-($n = 2$, $P < 0.05$), factor VIII-($n = 6$, $P < 0.005$) and factor XI-($n = 2$, $P < 0.05$) deficient platelet-rich plasmas, PCF in batroxobin-induced clots was significantly lower than in thrombin-induced clots. In factor IX deficiency ($n = 2$), one patient had a dramatic reduction in PCF while a second patient had increased PCF. PCF was insignificantly ($P = 0.346$) reduced in two patients with factor X deficiency, and was normal in one patient with factor V deficiency. The factor X result is consistent with work in model systems, which indicates that as little as 1–3% factor X activity is sufficient to restore thrombin generation to normal. The factor V result probably indicates that the deficiency is incomplete. PCF in thrombin-induced clots was normal in all of these patients. Low fibrinogen and factor XIII deficiency reduced PCF in both thrombin- and batroxobin-induced clots. These results indicate that PCF is reduced, probably due to delayed thrombin generation, in some factor-deficient platelet-rich plasma samples.

Keywords: clot elastic modulus, clotting factors, prothrombin conversion, von Willebrand's disease.

Introduction

Patients with deficiencies of plasma clotting factors are at increased risk of bleeding due to impaired clot formation. With the exception of von Willebrand's disease (VWD), platelet function is generally considered to be normal in such patients. There are, however, numerous reports of abnormal bleeding times in hemophilia [1–4].

Thrombin is a profound agonist of platelet function that leads to platelet activation via multiple pathways [5]. If thrombin generation or activity is inhibited by heparin, bleeding times are prolonged and clot retraction is inhibited [6,7].

It is now possible to measure the force generated by platelets during clot formation and clot retraction [8]. The forces generated are normally measured in thousands of dynes [9]. The importance of thrombin generation to the process of platelet force development can be illustrated by comparing samples clotted with thrombin to samples clotted with batroxobin. Batroxobin is a snake-venom enzyme that directly cleaves fibrinogen but is not inhibited by antithrombin III [10]. In normal blood anticoagulated with sodium citrate, the addition of thrombin or batroxobin plus calcium chloride leads to both clot formation and force development [11,12]. When clotted with batroxobin plus calcium, blood samples that contain heparin [7] or other antithrombin agents [13] demonstrate diminished (or even absent) platelet contractile force (PCF). Direct inhibition of thrombin or prevention of thrombin formation on the platelet surface, results in decreasedPCF. The degree of force suppression may correlate with bleeding risk given the striking correlation between inhibition of PCF and bleeding during cardiopulmonary bypass [14].

While it is possible to measure thrombin generation by simple recalcification of citrated whole blood, the kinetics may be significantly delayed and do not necessarily correlate with the initial development of PCF. This occurs because PCF cannot be measured in the absence of a fibrin network. When clotting is initiated solely by calcium addition, thrombin must be generated and the fibrin network must be formed before PCF can be detected. When clotting is initiated by batroxobin, the fibrin network is formed prior to the generation of thrombin. In this latter case, PCF is detected as soon as recalcification results in the generation of a small amount of thrombin.

We hypothesized that force development in blood samples clotted with batroxobin would be diminished in patients with clotting factor deficiencies. The present study was undertaken to test this hypothesis. Samples from patients with factor (F)V, FVII, FVIII, FIX, FX, FXI and von Willebrand factor deficiencies were studied. PCF and clot elastic modulus (CEM) in

Correspondence: Marcus E. Carr Jr., Department of Medicine, Box 980230 Virginia Commonwealth University, Richmond, Virginia 23298–0230, USA. Tel.: +1 804 828 9723; fax: +1 804 828 8079; e-mail: mcarr@hsc.vcu.edu

Received 27 February 2002, accepted 31 May 2002

© 2003 International Society on Thrombosis and Haemostasis

patient platelet-rich plasma clotted with thrombin were compared to PCF and CEM in clots formed with batroxobin. Platelet aggregation studies were also performed on all patient samples. Patient results were compared with results from normal controls.

Materials and methods

This study was approved by the Institutional Review Board for Human Studies of Virginia Commonwealth University and written consent was obtained from all patients and normal volunteers. Blood was obtained via aseptic venipuncture into evacuated tubes containing 3.8% sodium citrate. Platelet-rich plasma was prepared by centrifuging the blood for 10 min at 180 *g*. Platelet-poor plasma was prepared by additional centrifugation at 2000 g for 20 min. The fibrinogen concentration of plasma samples was determined by the modified method of Clauss [15].

Human thrombin, greater than 90% alpha, was purchased as a lyophilized powder from Sigma Chemical Co. (St. Louis, MO, USA). The material, with a specific activity of 4300 NIH $U\,mL^{-1}$ was dissolved in water, diluted with $0.10\,mol\,L^{-1}$ NaCl to a final concentration of 20 $units\,mL^{-1}$, divided into 1 mL lots, and frozen at −90 °C. Thrombin was free of plasmin and plasminogen. Batroxobin was purchased as Atroxin®, *Bothrops atrox* venom protein (Sigma Diagnostics), was dissolved in deionized water, and used without further purification. It was maintained on ice and used within 3 h of being prepared. Nanopure water was used in the preparation of all solutions.

Plasma clot formation was induced by the addition of $CaCl_2$ and either batroxobin ($0.25\,\mu g\,mL^{-1}$) or thrombin (1.0 NIH $U\,mL^{-1}$). PCF development and CEM were measured for 1200 s.

Measurement of platelet contractile force

The Haemodyne® hemostasis analyzer (Haemodyne Inc., Richmond, VA, USA) (Fig. 1) measures forces generated by platelets within a clot formed between a thermostated cup and parallel upper plate [8,16]. Before clotting, the upper plate is centered above the cup and lowered into the sample. As the clot forms, it attaches to the inner walls of the cup and the bottom of the upper plate. The entire sample volume is contained between the upper and lower surfaces. Once clotting is complete, platelets within the network pull fibrin strands inward, transmitting force through the network to the surfaces to which the clot is adherent. Force measurement is accomplished utilizing a displacement transducer coupled to the upper plate. As platelets contract, the transducer produces an electrical output proportional to the amount of force generated. The transducer output is continuously recorded.

Measurement of clot elastic modulus

CEM was measured by applying a downward force of known magnitude to the upper plate of the Haemodyne apparatus [17].

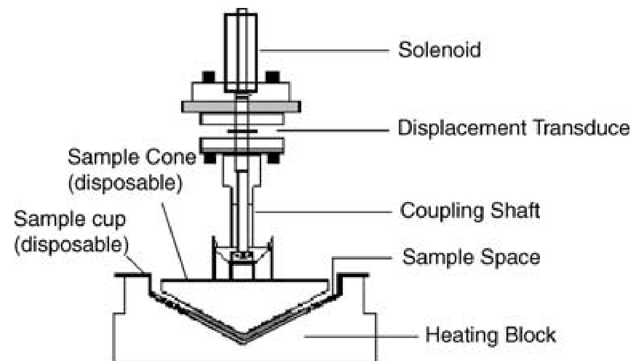

Fig. 1. Schematic diagram of the Haemodyne instrument used to measure force developed by platelets during clot formation. As the clot retracts a downward force is exerted on the plate attached to the transducer. The resultant downward deflection of the plate generates a voltage which is proportional to the downward force.

The amount of downward displacement caused by the force was measured. The ratio of downward force (stress) to downward displacement (strain) was used to calculate the elastic modulus. Where stress equals the force applied divided by the area of application and strain is the degree of shape change induced by the applied force.

Measurement of platelet aggregation in platelet-rich plasma

Platelet aggregation was measured utilizing a Biodata® (Biodata Corp, Horsham, PA, USA) model PAP-4 platelet aggregometer. Into an aggregometer cuvette equipped with a stirring bar, 450 µL of platelet-rich plasma was placed . Platelet concentration was between 200 000 and 400 000 $\mu^{-}U^{-}L^{-1}$. Platelet aggregation was monitored optically as a decrease in optical density. Agonists included $20\,\mu mol\,L^{-1}$ ADP, $11\,\mu mol\,L^{-1}$ epinephrine, $1.9\,\mu g\,mL^{-1}$ collagen, and 1.0 and $0.5\,mg\,mL^{-1}$ ristocetin.

Statistical analysis

Data are presented as mean ±SEM. Student's paired *t*-test was used to test for statistical significance of normally distributed values. Differences were considered statistically significant at $P < 0.05$.

Results

The effect of FVII deficiency on PCF is depicted in Fig. 2 and listed in Table 1. As seen in the lower panel, PCF in normal volunteer ($n = 5$) platelet-rich plasma clotted with batroxobin (closed circles) did not differ significantly ($P = 0.208$) from PCF in thrombin-induced clots (open circles). In FVII-deficient platelet-rich plasma, PCF in thrombin-induced clots is equivalent to that in normal clots, but PCF in batroxobin-induced clots is significantly lower ($n = 2, P < 0.05$). In the patient with severe FVII deficiency (< 1%) PCF was dramatically reduced (H-1, Table 1).

© 2003 International Society on Thrombosis and Haemostasis

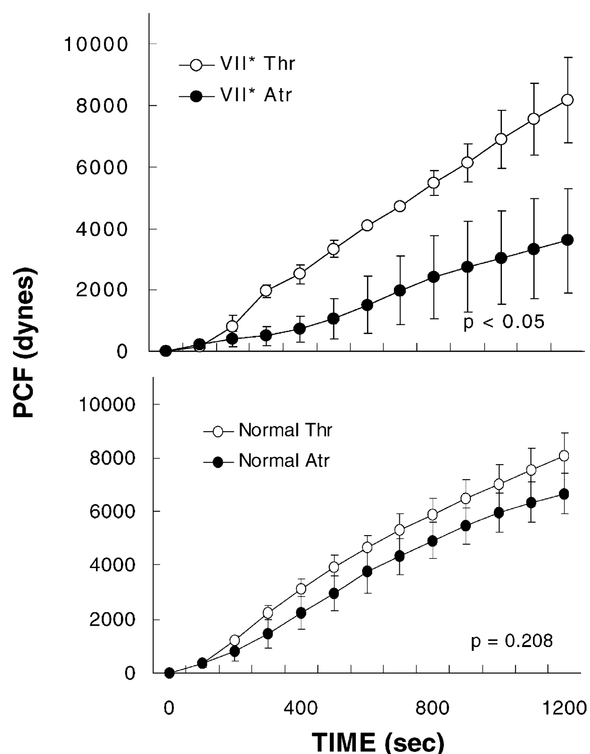

Fig. 2. Reduced platelet contractile force in batroxobin induced clots in patients ($n = 2$) with congenital factor VII deficiency. Open symbols are for clots formed with thrombin (1 NIH U mL^{-1}), while filled symbols are for clots induced with batroxobin (0.25 μg mL^{-1}). Patient data are plotted in the upper panel, while normal data are plotted in the lower panel. Thrombin or batroxobin was added at time zero. Other clotting conditions include: pH 7.4, ionic strength 0.15 mol L^{-1}, calcium 10 mmol L^{-1} and temperature 37 °C. Data from normal volunteers ($n = 5$) and patients are plotted as the mean ±the standard error of the mean (SEM).

The effect of FVIII deficiency on PCF is depicted in Fig. 3 and listed in Table 1. When FVIII-deficient platelet-rich plasma is clotted with thrombin (open circles), PCF is equivalent to that of normal platelet-rich plasma. However, PCF in batroxobin-induced clots is significantly ($n = 6$, $P < 0.005$) reduced. Although PCF in batroxobin-induced clots was reduced in all patients, FVIII level did not appear to correlate with the degree of force reduction (Table 1).

The effect of FXI deficiency on PCF is depicted in Fig. 4 and listed in Table 1. As with FVII- and FVIII-deficient plasmas, FXI-deficient platelet-rich plasma displayed normal PCF when clotted with thrombin but significantly ($n = 2$, $P < 0.05$) reduced force when clotted with batroxobin (closed circles, Fig. 4). The effect of FIX deficiency was variable. While one patient (A-1) had a dramatic reduction in PCF in the batroxobin-induced clot, a second patient (P-1) actually had increased PCF with batroxobin (Table 1).

The effects of common pathway factor deficiencies are depicted in Figs 5 and 6 and are detailed in Table 1. PCF was reduced in batroxobin clots from two patients with FX deficiency. Although the decrease appeared to be substantial (Fig. 5), the reduction was not statistically significant ($P = 0.346$) given the small ($n = 2$) sample size. The one patient with FV-deficient plasma did not demonstrate decreased force when batroxobin was used as the clotting agent (F-1, Table 1). The effect of afibrinogenemia is seen in Fig. 6. Platelet force is decreased in clots formed with either thrombin or batroxobin. The effect of FXIII deficiency is noted in Table 1. As with low fibrinogen, PCF is decreased in clots formed with thrombin. PCF in the FXIII batroxobin-mediated clot was slightly higher but remained lower than that in the normal controls.

The effects of various factor deficiencies on CEM are detailed in Table 2. With the exception of the afibrinogenemic patient, thrombin-induced plasma clots from factor-deficient patients demonstrated normal CEM. The elastic moduli of batroxobin-induced clots were significantly ($P < 0.05$) lower than those of their corresponding thrombin-induced clots. The CEM for the patient with afibrinogenemia was significantly lower than normal or than any other factor-deficient patient.

Platelet aggregations were normal in all patients and volunteers with the exception of the VWD patients who demonstrated decreased ristocetin-induced agglutination (data not shown).

Discussion

Extrinsic pathway factor deficiencies

The findings of this study are in basic agreement with the limited studies of platelet function in clotting factor-deficient patients. While normal when clotted with thrombin, FVII-deficient blood clotted with batroxobin displays delayed and reduced PCF. CEM is also reduced in batroxobin-induced clots. These findings are consistent with normal intrinsic platelet function but delayed or deficient prothrombin conversion in FVII-deficient plasma. These results agree with reports of a prolonged bleeding time (BT) in a patient with documented FVII deficiency [18] and with delayed fibrinopeptide production in shed blood from bleeding times done in patients with FVII deficiency [19]. However, it should be appreciated that prolonged bleeding times may not be uniformly associated with decreased FVII levels as demonstrated by the finding of normal BT in patients with warfarin-induced FVII deficiency (mean level 7%) [20].

Intrinsic pathway factor deficiencies

As with FVII deficiency, FVIII-deficient blood clotted with thrombin displays normal PCF development. Factor VIII-deficient clots formed by batroxobin demonstrate delayed and reduced PCF. This is true whether the reduction in FVIII is congenital or acquired. CEM is also reduced in batroxobin-induced clots. These results are consistent with prolongation of the bleeding time noted in several investigations [1–4]. In these reports, the percentage of hemophiliacs with prolonged bleeding times ranged from 20% [1] to 85% [4]. Comparisons are

© 2003 International Society on Thrombosis and Haemostasis

Table 1 Platelet contractile force in patients with congenital plasma clotting factor deficiencies

Patient	Factor deficiency	Factor level (%)	Platelet contractile force (dynes)		TB%D (T-B)/T (%)
			Thrombin	Batroxobin	
Extrinsic pathway deficiencies					
H-1	VII	<1	6815	1911	−71.9
G-1	VII	29	9592	5298	−44.7
Intrinsic pathway deficiencies					
L-2	XI	<6	9686	4875	−49.6
J-2	XI	<1	13 230	9.060	−31.5
P-1	IX	2	6118	8960	+46.5
A-1	IX	3	16 170	3795	−76.5
R-1	VIII	8	9555	4336	−54.6
B-2	VIII (acq)	1	9256	5587	−39.6
J-1	VIII	1	4480	1600	−64.2
S-1	VIII	<1	8400	3103	−63.1
D-1	VIII (acq)	<1	16 281	11 872	−27.1
L-1	VIII	2	6440	6196	−3.7
Common pathwvay deficiencies					
F-1	V	5	7634	9520	+24.7
B-1	X	<6	5130	4032	−21.4
J-4	X	<6	8096	3647	−54.9
A-2	I	undetectable	1820	1638	−10.0
M-1	XIII	(<1h)*	2897	4000	+38.1
von Willebrand disease					
C-1	VWF	24	9156	4713	−48.5
J-3	VWF	4	4832	6664	+37.9
S-2	VWF	37	14 214	9612	−32.4

*Patient clot dissolved in 5 mol L^{-1} urea in less than 1 hour. acq, Acquired.

complicated by varying definitions of ‘prolonged’ and by varying procedures for the BT (Simplate I, Simplate II, etc.). It is unclear from these reports whether the BT correlates with the degree of disease severity. Closure time measured by the PFA-100, a combined assessment of platelet adhesion and aggregation, has been shown to be normal in children with hemophilia [21]. Most recently, the hemostatic action of recombinant FVIIa in FVIII and FIX-deficient blood has been postulated to be through a tissue factor-independent mechanism involving enhanced thrombin generation on the platelet surface [22]. This explanation would be entirely consistent with the results found in FVIII and IX-deficient patients assessed in the present study.

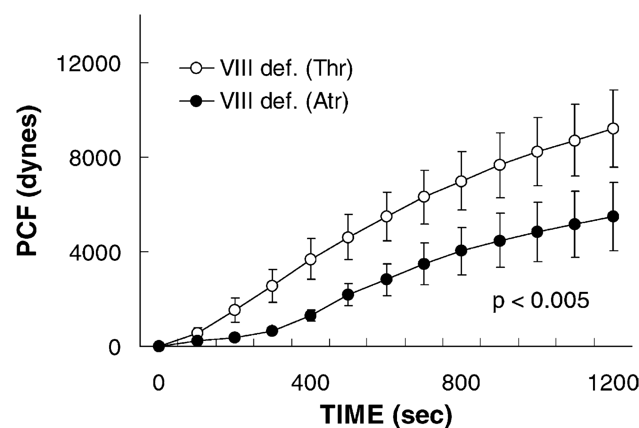

Fig. 3. Reduced platelet contractile force in batroxobin-induced clots in patients ($n = 6$) with factor VIII deficiency. Data from clots formed with thrombin are plotted as open circles, while data from clots formed with batroxobin are plotted as filled circles. Data are plotted as the mean ±SEM. Clotting conditions were as described in Fig. 2.

When clotted with thrombin, FXI-deficient blood displayed normal PCF development, but PCF was delayed and reduced when identical samples were exposed to batroxobin. Previous investigations of platelet function in FXI-deficient blood have produced varying results. In one report, the BT was found to be prolonged in one FXI-deficient patient [23]. In another study, platelet aggregation and adhesion were found to be normal in these patients [24]. Part of the variability in response may be due to the presence of a molecule in platelets that possesses FXI activity. The first indication of this possibility was the demonstration of increased FXI activity in platelet-rich plasma that had been frozen and then thawed [25]. The increased FXI activity was subsequently shown not to be due to increased FXI antigen [26]. Platelets apparently contain a high molecular weight molecule with FXI activity [27]. This molecular species can perhaps substitute for FXI activity in FXI-deficient blood. This could help explain the significant variability in clinical bleeding risk in this population. This platelet-derived FXI activity may also be responsible for the differences between FXI-deficient blood and FVIII-deficient blood when samples are exposed to subendothelium. Platelet and fibrin deposition are much more diminished in the FVIII-deficient samples [28].

Effects of FIX-deficient plasma were unclear in the limited number of samples tested in this study. In one patient, PCF was dramatically reduced in the batroxobin based assay, while in the

© 2003 International Society on Thrombosis and Haemostasis

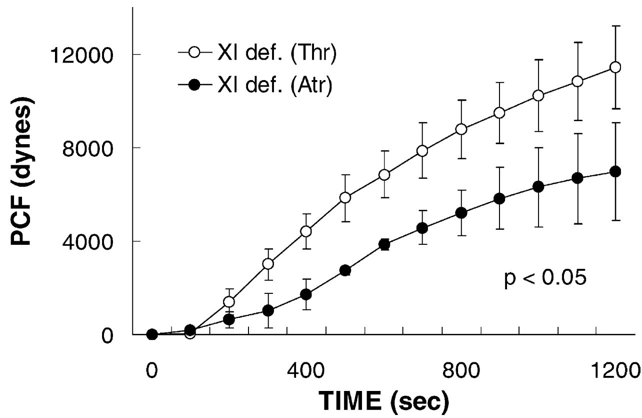

Fig. 4. Effect of factor XI deficiency on platelet contractile force in thrombin-(○) and batroxobin-(●) induced clots. Clotting conditions were as described in Fig. 2.

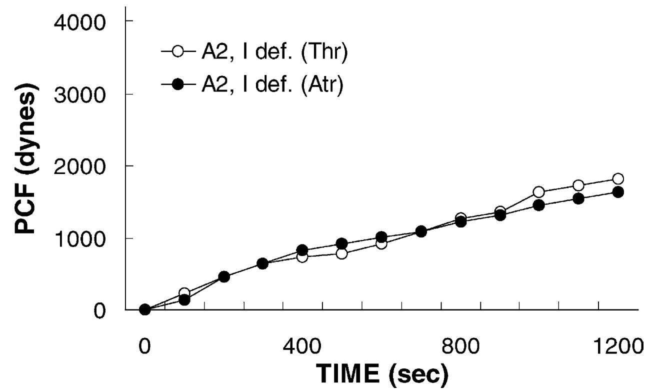

Fig. 6. Effect of afibrinogenemia in patient A2 on platelet contractile force in thrombin-(○) and batroxobin-(●) induced clots. Data are presented as the mean ±SEM for each measurement. Clotting conditions were as described in Fig. 2.

other patient no reduction was noted. This was true despite the fact that the FIX levels were virtually identical in these patients. The literature on platelet function in FIX-deficient patients is limited, but has demonstrated prolonged BT in some of these patients [1]. The hemostasis time, a modified bleeding time test, has also been shown to be prolonged in about 50% of hemophilia B patients [29].

Common pathway factor deficiencies

PCF in clots formed with batroxobin was apparently reduced in both patients with FX deficiency and was substantially lower (< 50% of the PCF seen with thrombin) in one. However, due to the limited number (two) of patients studied, the differences did not reach statistical significance ($P = 0.346$). One group of investigators found abnormal platelet aggregations and BT in a patient with acquired FX deficiency [30], while another group found no effect of RPR 130737, a direct Xa inhibitor, on platelet aggregations [31]. The absence of an effect on aggregation is not inconsistent with the effects on BT and PCF since the former does not require the presence of thrombin. The FX result is consistent with work in model systems which indicates that as little as 1–3% FX activity is sufficient to restore thrombin generation to normal [32,33]. In our patients the FX levels were reported as less than 6%. Thus, one patient's FX level may well have been lower than 1% while the other was higher.

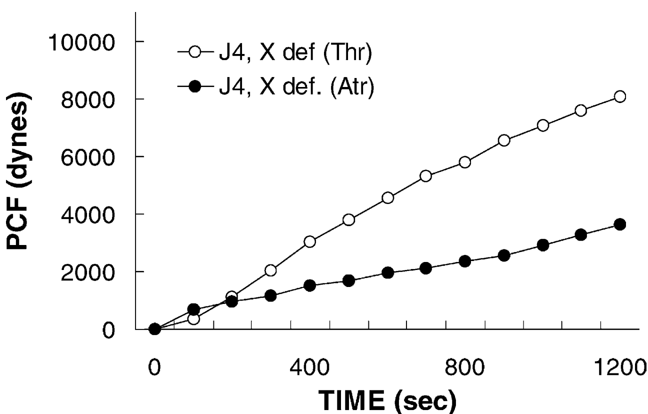

Fig. 5. Effect of factor X deficiency in patient J4 on platelet contractile force in thrombin-(○) and batroxobin-(●) induced clots. Clotting conditions were as described in Fig. 2.

The changes in PCF and CEM in the one FV-deficient patient who was tested were minimal. These results probably indicate that the deficiency is incomplete. Limited reports are available regarding platelet function in this disorder, but at least one patient with combined FV and FVIII deficiency was found to have normal platelet adhesion and aggregation [34]. One potential explanation for the lack of effect of the FV deficiency is the presence of some FV in platelets from patients with FV deficiency [35]. Recent work in artificial plasma systems suggests that the 20% of FV that is normally contained in platelets may be the critical component in platelet-centered prothrombin conversion [36]. In addition, work in transgenic mice indicates that complete absence of FV is incompatible with life, but trace levels allow the mice to be born and survive to adulthood [37]. These results may indicate the critical requirement for at least some FV activity, but the potential for relatively normal function in the setting of low FV levels.

PCF and CEM were dramatically reduced in both thrombin and batroxobin clots in the one patient with afibrinogenemia. Thus, these effects appear to be independent of prothrombin conversion. PCF is known to be sensitive to fibrinogen concentrations below 50 mg dL^{-1} [9], and CEM is directly dependent on fibrinogen concentration [38].

PCF and CEM were not lower in FXIII-deficient blood clotted by batroxobin indicating normal prothrombin conversion. Interestingly, however, PCF with thrombin was lower in this patient than in any other patient besides the patient with afibrinogenemia. PCF was less than 50% that seen in blood from normal controls. CEM was not significantly reduced, but the modulus measurement was made after only 1200 s of clotting. A significant amount of additional covalent

© 2003 International Society on Thrombosis and Haemostasis

Table 2 Clot elastic modulus in patients with congenital plasma clotting factor deficiencies

Patient	Factor deficiency	Factor level (%)	Platelet contractile force (dynes)		TB%D (T-B)/T (%)
			Thrombin	Batroxobin	
Extrinsic pathway deficiencies					
H-1	VII	<1	20 760	14 013	−32.5
G-1	VII	29	59 002	22 421	−62.1
Intrinsic pathway deficiencies					
L-2	XI	<6	23 355	17 516	−25.0
J-2	XI	<1	35 032	23 355	−33.3
P-1	IX	2	20 019	20 019	0.0
A-1	IX	3	24 370	17 516	−28.1
R-1	VIII	8	35 032	16 985	−51.5
B-2	VIII (acq)	1	25 478	20 760	−18.5
J-1	VIII	1	16 486	25 478	+54.5
S-1	VIII	<1	21 558	12 185	−43.5
D-1	VIII (acq)	<1	43 117	32 030	−25.7
L-1	VIII	2	20 019	18 684	−6.7
Common pathwvay deficiencies					
F-1	V	5	29 501	26 691	−10.9
B-1	X	<6	19 328	16 015	−17.1
J-4	X	<6	23 355	23 852	+2.1
A-2	I	undetectable	12 739	12 739	0.0
M-1	XIII	(<1h)*	19 328	19 328	0.0
von Willebrand disease					
C-1	VWF	24	32 972	21 558	−34.5
J-3	VWF	4	21 558	28 026	+30.0
S-2	VWF	37	29 501	24 912	−15.6

*Patient clot dissolved in 5 mol L^{-1} urea in less than 1 hour. acq, Acquired.

crosslinking would be expected to occur after this time period. This could explain the difference in effects noted in the present study vs. those seen in prior studies where measurements were made one to several hours after initiation of clotting [39].

Von Willebrand's disease

Blood samples from two patients with VWD had decreased PCF when exposed to batroxobin, but a third patient with even lower von Willebrand factor activity demonstrated the opposite effect on PCF. vWF, at the levels present in these patients, does not appear to have a consistent effect on prothrombin conversion or PCF development. This may not be the case in Type III vWD where VIII activity may be significantly reduced.

Conclusion

In patients with FVII, VIII, X or XI deficiency, PCF was reduced in platelet-rich plasma clotted with batroxobin. PCF was not reduced in a single patient with FV deficiency and divergent results were seen in two patients with IX deficiency. Even when clotted with thrombin, PCF was reduced in fibrinogen and FXIII-deficient patients. Due to variable results found in this study, the definition of von Willebrand factor's effect on prothrombin conversion will require additional investigation. The batroxobin PCF assay may offer a simple way to monitor prothrombin conversion on the platelet surface in patient blood samples.

References

1 Buchanan GR, Holtkamp CA. Prolonged bleeding time in children and young adults with hemophilia. *Pediatrics* 1980; **66**: 951–5.

2 Eyster ME, Gordon RA, Ballard JO. The bleeding time is longer than normal in hemophilia. *Blood* 1981; **58**: 719–23.

3 Smith BS, Baglini R, Meissner GF. The prolonged bleeding time in hemophilia A. Comparison of two measuring technics and clinical associations. *Am J Clin Path* 1985; **83**: 211–5.

4 Stuart MJ, Walenga RW, Sadowitz PD, Maltby A, Kelton JG, Gauldie J. Bleeding time in hemophilia A. Potential mechanisms for prolongation. *J Pediatr* 1986; **108**: 215–8.

5 Yamamoto N, Greco NJ, Barnard MR *et al.* Glycoprotein Ib (GPIb)-dependent and GPIb-independent pathways of thrombin-induced platelet activation. *Blood* 1991; **77**: 1740–8.

6 Heiden D, Mielk CH, Jr RodvienR. Impairment by heparin of primary haemostasis and platelet [14C]5-hydroxytryptamine release. *Br J Haematol* 1977; **36**: 427–36.

7 Carr ME, Carr SL, Greilich PE. Heparin ablates force development during platelet mediated clot retraction. *Thromb Haemost* 1996; **75**: 674–8.

8 Carr ME, Zekert SL. Measurement of platelet mediated force development during plasma clot formation. *Am J Med Sci* 1991; **302**: 13–8.

9 Carr ME, Carr SL. Fibrin structure and concentration alter clot elastic modulus but do not alter platelet mediated force development. *Blood Coagul Fibrinolysis* 1995; **6**: 79–86.

10 Bonilla CA. Defibrinating enzyme from timber rattlesnake [*Crotalus. J. horridus*] venom: a potential agent for therapeutic defibrination. *I Purification Properties Thromb Res* 1975; **6**: 151.

11 de Gaetano G, Bottecchia D, Vermylen J. Retraction of reptilase-clots in the presence of agents inducing or inhibiting the platelet adhesion-aggregation reaction. *Thromb Res* 1973; **2**: 71–84.

12 Carr ME, Carr SL. At high heparin concentrations, protamine concentrations which reverse anticoagulant effects are insufficient to reverse heparin anti-platelet effects. *Thromb Res* 1994; **75**: 617–30.

© 2003 International Society on Thrombosis and Haemostasis

13 Carr ME, Zekert SL. Effect of non-heparin thrombin antagonists on platelet force development during clot retraction. *Thromb Haemost* 1993; **69**: 1241.

14 Greilich PE, Carr ME, Carr SL, Chang AS. Reductions in platelet force development by cardiopulmonary bypass are associated with hemorrhage. *Anesth Analg* 1995; **80**: 459–65.

15 Clauss A. A rapid physiological coagulation method for determination of fibrinogen. *Acta Haematol* 1957; **17**: 237–46.

16 Carr ME. Measurement of platelet force: the Hemodyne Hemostasis analyzer. *Clin Lab Manage Rev* 1995; **9**: 312–20.

17 Carr ME, Carr SL. Abnormal clot retraction, altered fibrin structure and normal platelet function in multiple myeloma. *Am J Physiol* 1994; **266**: H1195–201.

18 Sikka M, Gomber S, Madan N, Rusia U, Sharma S. Congenital deficiency of factor VII. *Indian J Pediatr* 1996; **63**: 571–3.

19 Weiss HJ, Lages B. Evidence for tissue factor-dependent activation of the classic extrinsic coagulation mechanism in blood obtained form bleeding time wounds. *Blood* 1988; **71**: 629–35.

20 Green D, Ts'ao CH. Warfarin-induced factor VII deficiency and the bleeding time. *Thromb Res* 1983; **29**: 403–6.

21 Rand ML, Carcao MD, Blanchette VS. Use of the PFA-100 in the assessment of primary, platelet-related hemostasis in a pediatric setting. *Semin Thromb Hemost* 1998; **24**: 523–9.

22 Hoffman M, Monroe DM 3rd, Roberts HR. Activated factor VII activates factors IX and X on the surface of activated platelets: thoughts on the mechanism of action of high-dose activated factor VII. *Blood Coagul Fibrinolysis* 1998; **9** (Suppl. 1): S61–5.

23 Brody JI. Prolonged bleeding times with factor IX and XI deficiency von Willebrand's syndromes. *Am J Med Sci* 1975; **269**: 19–24.

24 Girolami A, Casonato A, Randi M, de Marco L, Molaro G. Normal platelet adhesiveness and aggregation in congenital PTA or Hageman factor deficiency. *Folia Haematol Int Mag Klin Morph Blutforsch* 1980; **107**: 487–91.

25 Connellan JM, Castaldi PA, Muntz RH. The role of factor XI in the coagulant activity of platelets. *Haemostasis* 1977; **6**: 41–52.

26 Schiffman S, Rimon A, Rapaport SI. FactorXI and platelets: Evidence that platelets contain only minimal factor XI activity and antigen. *Br J Haematol* 1977; **35**: 429–36.

27 Walsh PN, Tuszynski GP, Greengard JS, Griffin JH. The possible role of platelets in bypassing the contact phase of blood coagulation. *Haematologia (Budap)* 1984; **17**: 169–78.

28 Weiss HJ, Turitto VT, Vicic WJ, Baumgartner HR. Fibrin formation, fibrinopeptide A release, and platelet thrombus dimensions on subendothelium exposed to flowing native blood: greater in factor XII and XI than in factor VIII and IX deficiency. *Blood* 1984; **63**: 1004–14.

29 Janzarik H, Heinrich D, Bodeker RH, Lasch HG. 'Haemostasis time', a modified bleeding time test and its comparison with the Duke and Ivy/template bleeding times. II: Application in bleeding disorders. *Blut* 1988; **57**: 111–6.

30 Edgin RA, Metz EN, Fromkes JJ, Beman FM. Acquired factor X deficiency with associated defects in platelet aggregation. A response to corticosteroid therapy. *Am J Med* 1980; **69**: 137–9.

31 Chu V, Brown K, Colussi D *et al. In vitro* characterization of a novel Xa inhibitor, RPR 130737. *Thromb Res* 2000; **99**: 71–82.

32 Allen GA, Wolberg AS, Oliver JA, Hoffman M, Roberts HR. Effect of varied procoagulant concentration on thrombin generation in a model system. *Thromb Haemost Supplement* 1999; **1**: 997.

33 Allen GA, Monroe DM, Roberts HR, Hoffman M. The effect of factor X level on thrombin geneartion and the procoagulant effect of activated factor VII in a cell-based model of coagulation. *Blood Coagul Fibrinolysis Supplement* 2000; **1**: S3–7.

34 Girolami A, de Marco L, Fabris F, Casonato A. Platelet adhesiveness and aggregation in combined factor V and factor VIII deficiency and in combined factor VII and factor VIII deficiency. *Folia Haematol Int Mag Klin Morph Bultforsch* 1977; **104**: 663–9.

35 Breederveld K, Giddings JC, ten Cate JW, Bloom AL. The localization of factor V within normal human platelets and the demonstration of a platelet-factor V antigen in congenital factor V deficiency. *Br J Haematol* 1975; **29**: 405–12.

36 Tracy PB, Mann KG. Abnormal formation of the prothrombinase complex: factor V deficiency and related disorders. *Hum Pathol* 1987; **18**: 162–9.

37 Yang TL, Cui J, Taylor JM, Yang A, Gruber SB, Ginsburg D. Rescue of fatal neonatal hemorrhage in factor V deficient mice by low level transgene expression. *Thromb Haemost* 2000; **83**: 70–7.

38 Carr ME, Shen LL, Hermans JA. Physical standard of fibrinogen: measurement of the elastic modulus of dilute fibrin gels with a new elastometer. *Anal Biochem* 1976; **72**: 202–11.

39 Shen LL, Hermans J, McDonagh J, McDonagh RP, Carr ME. Effects of calcium ion and covalent crosslinking on formation and elasticity of fibrin gels. *Thromb Res* 1975; **6**: 255–65.

© 2003 International Society on Thrombosis and Haemostasis