

Reversal of the International Normalized Ratio with recombinant activated factor VII in central nervous system bleeding during warfarin thromboprophylaxis: clinical and biochemical aspects

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Major bleeding is a frequent and hazardous complication associated with thromboprophylaxis using vitamin-K antagonists (VKA). Suggested regimens for control of highly elevated International Normalized Ratio (INR) and hemorrhagic events during VKA treatment include administration of vitamin K, infusion of fresh frozen plasma (FFP) or a prothrombin complex concentrate (PCC). In contrast, this communication present the first report on the efficacious use of recombinant factor VIIa (rFVIIa) as additional therapy in seven patients presenting with central nervous system (CNS) bleeding emergencies. Pre-treatment INRs ranged from 1.7 to 6.6, and 10 min after a single dose of rFVIIa (10–40 µg/kg) all INRs were ≤ 1.5. Six patients underwent drainage of the CNS hematoma and all patients survived. No untoward biochemical signs of coagulation activation were detected and no incidence of thromboembolism was observed. In ex-vivo experimental studies, profiles of continuous whole blood clot formation were evaluated by thrombelastography in 25 patients on VKA treatment (INR 1.7–4.3), demonstrating a significantly prolonged initiation phase and diminished

propagation of clot formation. Ex-vivo supplementation with rFVIIa to blood of six patients returned a distinct reduction of the prolonged initiation but variable changes in the maximum velocity of clot formation. The ex-vivo experiments and our clinical data support recent suggestions that rFVIIa might substitute for infusion of FFP or PCC in acute reversal of VKA treatment. *Blood Coagulation and Fibrinolysis* 14:469–477 © 2003 Lippincott Williams & Wilkins.

Blood Coagulation and Fibrinolysis 2003, 14:469–477

Keywords: warfarin, recombinant factor VIIa, thrombelastography

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Received 24 September 2002 Revised 17 February 2003
Accepted 21 February 2003

Introduction

Vitamin-K antagonists (VKA) are increasingly used in long-term prophylaxis against thromboembolism in various cardiovascular pathologies including atrial fibrillation and mechanical heart valves, as well as in the shorter term following venous thromboembolic manifestations like deep vein thrombosis and pulmonary embolism [1]. Despite meticulous surveillance of the treatment by regular International Normalized Ratio (INR) monitoring, bleeding remains a frequent complication. Minor bleeding events occur in up to 25% of VKA-treated patients per year [2], while major and potentially fatal hemorrhagic events such as central nervous system (CNS) bleedings are seen in up to six patients per 100 treatment-years despite aimed therapeutic intensities within the preferred range of INR 2.0–3.5 [3]. Increased risk of bleeding is a frequent occurrence when the INR level is greater than 4 [4]. The modalities adopted to reverse the anticoagulant effect in bleedings during VKA treatment are mainly determined by the type of bleeding

and its associated clinical risk. Management includes temporary discontinuation of the VKA treatment together with administration of vitamin K by the oral or the intravenous route [5,6]. In contrast, major, potentially fatal bleedings caused by trauma or due to VKA overdosing may require a more aggressive treatment regimen. Recent studies have reported on the efficacious use of prothrombin complex concentrates (PCC) in any emergent situation requiring correction of higher levels of VKA intensities in order to manage major VKA-induced bleedings [7–9]. Reportedly, coagulation activation and thromboembolic events may follow administration of a PCC [8,9]; in particular, following its prolonged use. Thrombotic adverse events have been described in hemophiliacs with inhibitors as well as in patients with severe factor VII deficiency receiving PCC [10,11]. Alternatively, infusion of fresh frozen plasma (FFP) has been adopted in acute cases. However, the effect may be inadequate and systemic overload has been observed following larger amounts of FFP [7].

Recent observations have shown that a recombinant activated factor VII (rFVIIa) molecule has been effective in supporting hemostasis during bleeding episodes in hemophiliacs with inhibitors [12]. Additionally, speculations have been raised that rFVIIa might possess a more general hemostatic capacity [13], as supported by case stories on the efficacy of rFVIIa in various types of coagulopathies [14–18]. Studies in animals and healthy volunteers on the potential of rFVIIa for reversal of stable VKA treatment has illustrated that rFVIIa can reverse VKA treatment within the various INR values tested [19–21]. Sporadic reports exist on the effect of rFVIIa in reversal of high INR values and on its efficacy in VKA-induced bleeding [22,23].

This communication deals with a series of seven patients undergoing VKA treatment who had been admitted to our hospital due to a serious CNS bleeding episode. In these patients, rFVIIa was adopted additionally to vitamin K infusion in order to correct the abnormal INR value and to provide sufficient hemostasis for acute surgical drainage. Additionally, we attempted to assess the *in vitro* hemostatic effect of rFVIIa when added to blood from other patients on stable VKA treatment, investigating the changes in real-time whole blood coagulation induced by the presence of rFVIIa [24].

Materials and methods

Patients with acute CNS bleeds

Patients acutely admitted with overt CNS bleedings ($n = 6$) or with an imminent risk of development of hematoma ($n = 1$) required immediate reversal of VKA treatment, as judged by University Clinic neurosurgeons. All but one patient had received vitamin-K

supplementation as first-line therapy and three patients also received different amounts of FFP, but the INR response was regarded insufficient at the time of presentation (see Table 1). Six out of the seven patients underwent neurosurgical drainage of hematomas shortly after a bolus administration of 10–40 µg/kg rFVIIa, giving an intended target value of INR at ≤ 1.5 . The hemostatic and clinical effect was assessed by subjective evaluation of hemostasis during and after the surgical procedure, and through recording of post-operative transfusion requirements. The long-term clinical outcome was evaluated in retrospect from clinical records and hospital discharge information.

Patients studied in vitro

Blood samples from 25 patients (17 males and eight females), 29–85 years of age (average 65 years) on life-long stable VKA treatment due to an implanted mechanical heart valve comprised the experimental study material. The average INR at the time of study was 2.80 (range 1.68–4.27). Pregnant females and patients with renal insufficiency, cases suspected of malignancy, patients with a previous arterial or venous thrombotic event, with chronic infections, as well as patients with verified congenital or acquired hemostatic deficiencies were not included. Additionally, in six patients with an unintended INR level ≥ 2.8 (range 2.8–4.4) the ex-vivo response to ex-vivo supplementation of rFVIIa was studied. Previously obtained whole blood coagulation recordings in 30 healthy males were used as reference material because healthy men had returned the least accelerated whole blood clotting profile [24].

Blood samples

Blood samples in all patients for the in-vitro studies

Table 1 Demographic and clinical data from the patients who underwent treatment with recombinant activated factor VII (rFVIIa)

Patient	Gender	Age (years)	Indication of VKA	Admission diagnosis	Admission INR	Dose of vitamin K ($t = 0$) (mg)	Treatment prior to rFVIIa	Time at rFVIIa administration (h)	Dose of rFVIIa (µg/kg)	INR	
										Pre-rFVIIa	Post-rFVIIa
A	Male	65	Valvular disease	Cerebellar hemorrhagia	3.6	*	–	–	30	3.6 [§]	1.2
B	Male	51	Mechanical mitral valve	Spinal subarachnoidal hemorrhagia	3.2	–	–	–	30 [‡]	2.8	1.3
C	Male	84	Atrial fibrillation	Subdural hemorrhagia	3.0	10 i.v.	7 × FFP + 2 × Plt + 2 × Eryt	+ 12	10 [†]	1.7	0.9
D	Female	66	Atrial fibrillation	Frontal intracranial hematoma	3.4	10 i.v.	–	+ 6	15	2	1
E	Female	53	Cardiomyopathia and atrial fibrillation	Intracerebral hemotoma	2.9	10 i.v.	2 × FFP	+ 4	15	2.2	1.5
F	Male	62	Atrial fibrillation	High-risk spinal stenosis due to trauma	> 7.0	1 i.v.	–	+ 1	40 [‡]	6.6	1.5
G	Male	76	Atrial fibrillation	Subdural hemorrhagia	4.1	10 i.v.	2 × FFP	+ 3	30	3.9	1.1

The surgical procedure was performed briefly after administration of rFVIIa. Eryt, red cell concentrate ~300 ml; FFP, fresh frozen plasma ~220 ml; Plt, platelet suspension ~300 ml. Normal ranges: prothrombin time (PT), 9–11 s; activated partial thromboplastine time (aPTT), 24–35 s; platelets, $(150–390) \times 10^9/l$; fibrinogen, 1.8–3.9 g/l; D-dimer, < 500 mg/l. *The patient has been abstinent from vitamin-K antagonist (VKA) for 9 days due to an International Normalized Ratio (INR) > 7. On the ninth and seventh day prior to admission, the patient received 10 mg vitamin K per-orally. †The patient received 10 µg/kg each 6 h for 36 h. ‡The patients received the bolus injection twice with a 6 h interval. Other patients received one bolus injection of rFVIIa. §Discrepancies in the pre-rFVIIa INR and the pre-rFVIIa PT are most likely due to an unknown delay in the time of blood sampling for measurement of INR and measurement of the PT. In addition, the INR and PT were performed employing different types of tissue factor compositions for activation (see Materials and methods).

and ex-vivo supplementation of rFVIIa were drawn into citrated Venoject tubes (Terumo Europe, Leuven, Belgium) (0.129 mol/l, 3.8 w/v%) mixing one part of citrate with nine parts of blood, employing minimum stasis and a 21-gauge butterfly needle, discarding the first tube aspirated. The blood sample rested for 30 min at room temperature prior to whole blood coagulation analysis with and without ex-vivo supplementation of rFVIIa. Remaining blood was centrifuged at 2800 g for 30 min at 4°C, and platelet poor plasma was obtained and frozen at -70°C in smaller aliquots for subsequent analysis of the clotting activity of vitamin-K-dependent coagulation factors.

Medicine for ex-vivo supplementations

rFVIIa (NovoSeven; Novo Nordisk, Bagsvaerd, Denmark) was obtained from the manufacturer.

Coagulation analyses

The following tests were performed in the patients undergoing treatment with rFVIIa: INR, prothrombin time (PT), activated partial thromboplastin time (aPTT), factor II:C, factor VII:C, factor X:C, functional fibrinogen, D-dimer, and the platelet count. INR and coagulation factor activities were recorded as described in the following. The PT, aPTT, and the level of functional fibrinogen were analyzed employing recombinant human tissue factor (TF) (Innovin), aPTT test reagent (Platelet LS), and fibrinogen test reagents (Multifibren U), respectively. All reagents and the BCT Analyzer were from Dade Behring (Marburg, Germany). D-dimer was determined immunometrically employing monoclonal antibodies adhered on a commercial test-card system (Nycocard; AXIS-SHIELD

PoC A/S, Oslo, Norway). The whole blood platelet count was recorded using a counting chamber (Thoma, Assistant, Sondheim, Germany) and phase-contrast microscopy.

The tests performed in the patients studied *in vitro* comprised: factor II:C, factor VII:C, factor IX:C, factor X:C, and the INR value. The one-stage clotting activities were recorded on Thrombolyzer Chrom equipment (Benk Elektronik, Norderstedt, Germany) using a TF-dependent system employing Innovin (Dade Behring) as the TF source and human-deficiency plasma as the test base (factor II, Stago Deficient II; BioMérieux, Boulogne, France; FVII, prepared in house from plasma from a severe factor VII-deficient patient; factor X, Stago Deficient X; BioMérieux). In the case of factor IX, the one-stage method utilized Platelin LS (Organon Teknika, Turnhout, Belgium) as activator and a commercial factor IX deficiency plasma (CryoChech; Precision BioLogic Inc., Dartmouth, Canada) as test base. The PT for determination of the INR were recorded on a BCT Analyzer (Dade Behring) by incubating 7 µl citrated plasma with 42 µl citrated sodium barbital buffer (barbital sodium, 0.979 g; sodium chloride, 3.668 g; pH 7.35), following addition of 100 µl rabbit brain tissue factor (Nycotest PT reagent, International Sensitivity Index = 0.396; Medinor A/S, Copenhagen, Denmark) and photometric recording of the clotting time.

Real-time continuous whole blood coagulation based on thrombelastography

In all patients studied *in vitro*, a profile of real-time continuous whole blood clot formation was recorded on

PT (s)		aPTT (s)		Platelet (mia/l)		Fibrinogen (g/l)		D-dimer (µg/l)		Postoperative transfusion requirement
Pre-rFVIIa	Post-rFVIIa	Pre-rFVIIa	Post-rFVIIa	Pre-rFVIIa	Post-rFVIIa	Pre-rFVIIa	Post-rFVIIa	Pre-rFVIIa	Post-rFVIIa	
19 ^s	ND	36	ND	419	ND	8.8	ND	500	ND	-
30	11	37	34	138	119	3.2	3.5	500	500	3 × FFP + 3 × Eryt
15	9	33	31	102	103	5.2	5.2	1500	1500	-
13	8	27	27	200	198	2.6	2.7	500	500	2 × FFP + 1 × Eryt
20	7	29	27	244	173	3.1	3.4	2200	1700	-
63	15	37	36	147	146	2.9	2.7	500	500	-
25	9	34	32	136	135	2.4	2.7	1200	1800	-

a roTEG Thrombelastography Coagulation Analyzer (Pentapharm, Munich, Germany) as reported elsewhere [24]. In short, thrombelastographic profiles were produced by incubating 300 μ l of 30-min-rested, pre-warmed (37°C) whole blood with 20 μ l human recombinant tissue factor (Innovin) diluted in a sodium barbital buffer (sodium barbital, 28.5 mmol/l; pH 7.4). The final TF dilution was 1:17000, roughly corresponding to a final reaction concentration at \approx 0.35 pmol/l. Coagulation was initiated by addition of 20 μ l of 200 mmol/l CaCl₂ and all analyses were processed for at least 90 min. Traditional thrombelastographic parameters like clotting time (CT), clot formation time (CFT), and maximal clot formation (MCF) (see Fig. 1a) were recorded. The CT expresses a measure of the initiation of clot formation. Furthermore, the digital signal from the roTEG Analyzer was imported into a software program (DyCoDerivAn; AvordusoL, Risskov, Den-

mark) for calculation of dynamic coagulation parameters (Fig. 1b) such as the maximum velocity (MaxVel) of clot formation and the time until the occurrence of the maximum value (t_{MaxVel}), parameters that concur with the propagation of whole blood clot formation.

Data analysis and statistics

Using the DyCoDerivAn software, the first derivative of the thrombelastographic clot formation signal was calculated in a discrete manner. Glitches originating from noise were removed by a low-pass filter and the maximum slope (MaxVel) of the elasticity profile and its time of occurrence (t_{MaxVel}) were calculated. Spearman rank correlation coefficients (CC) between INR, factor II:C, factor VII:C, factor IX:C, factor X:C, and the mean values of MaxVel, t_{MaxVel} , and MCF were calculated by Analyse-it version 1.62 (Analyse-it Software Ltd., Leeds, UK), a statistical add-in program for Excel (Microsoft, Phoenix, Arizona, USA). Furthermore, an unpaired *t* test was performed to investigate the difference of the mean value of MaxVel, t_{MaxVel} , and MCF between the reference group and the VKA group. $P < 0.05$ was considered statistically significant.

Ethics

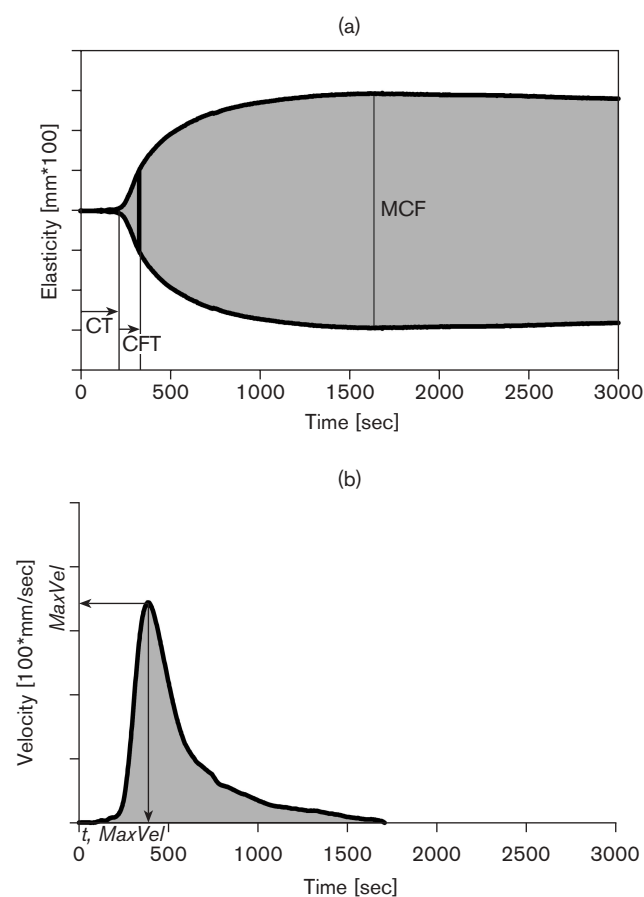
The study plan had been approved by the Biomedical Ethics Committee of Aarhus County, and all participants in the systematic part of the investigation consented their participation prior to enrollment.

Results

Clinical results of treatment with rFVIIa

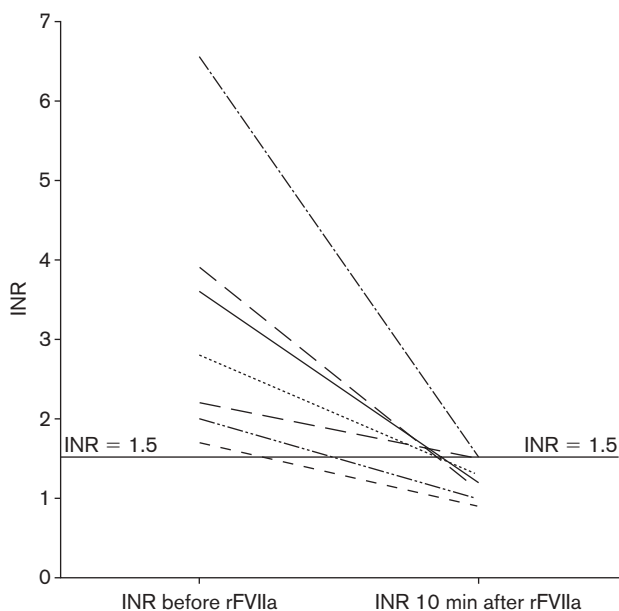
Seven patients (five males and two females), with an age from 51 to 84 years (average 65 years), were treated acutely with rFVIIa. Clinical and biochemical results from each patient are presented in Table 1, and INRs before and after administration of rFVIIa are outlined in Figure 2. Pre-treatment INRs as measured by the Department of Clinical Biochemistry ranged from 1.7 to 6.6, and post-dose INRs ranged from 0.9 to 1.5. Pre-dose PTs as measured by our local coagulation laboratory ranged from 13 to 60 s and post-dose PTs from 7 to 15 s (reference value, 9–11 s). Factor VII:C increased from an initial 0.03–0.49 U/ml to 4.9–11.4 U/ml ($P = 0.002$) 10 min after rFVIIa administration. Factor X:C values rose from 0.06–0.37 U/ml to 0.24–0.93 U/ml ($P = 0.045$), while factor II:C only was slightly elevated from the initial 0.13–0.33 U/ml to 0.24–0.38 U/ml ($P = 0.111$) 10 min after rFVIIa administration. No biochemical signs of gross coagulation activation were observed as assessed by the platelet count, the level of fibrinogen, and the D-dimers 10 min after administration of rFVIIa. In three of the seven patients, whole blood roTEG coagulation profiles before and after rFVIIa were recorded. The graphs of Figure 3 illustrate the profiles from patient F (see Table 1) before and after the first and the second bolus injection of rFVIIa

Fig. 1



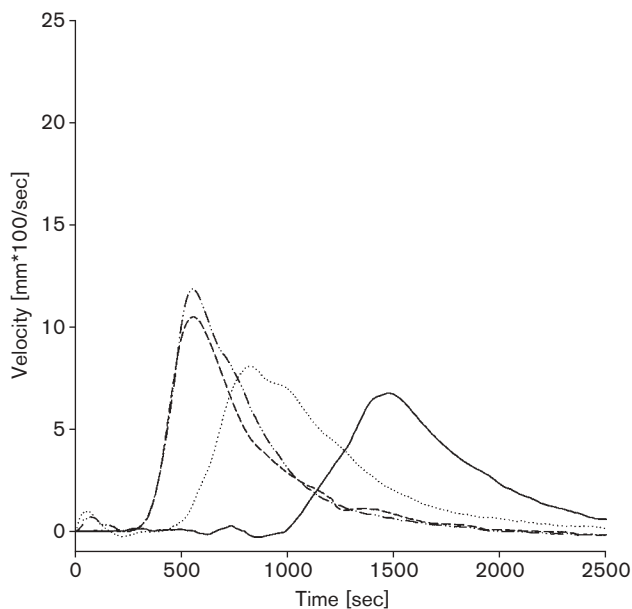
(a) Standard thrombelastographic tracking. CT, clotting time; CFT, clot formation time; MCF, maximum clot formation. (b) velocity profile, the first derivative of the roTEG thrombelastographic course. Maximum velocity (MaxVel) marked with a horizontal arrow. Time to maximum velocity (t_{MaxVel}), marked with a perpendicular arrow, is the time until maximum velocity.

Fig. 2



International Normalized Ratio (INR) values before and 10 min after a single dose of recombinant activated factor VII (rFVIIa) in seven patients with neurological hemorrhagic emergency.

Fig. 3



Real-time continuous whole blood coagulation profiles of patient F on vitamin-K antagonist (VKA) (International Normalized Ratio = 6.6). (—) Before first bolus injection of 40 µg/kg recombinant activated factor VII (rFVIIa). (···) After first bolus injection of 40 µg/kg rFVIIa. (---) Before second bolus injection of 40 µg/kg rFVIIa. (- · - ·) After second bolus injection of 40 µg/kg rFVIIa.

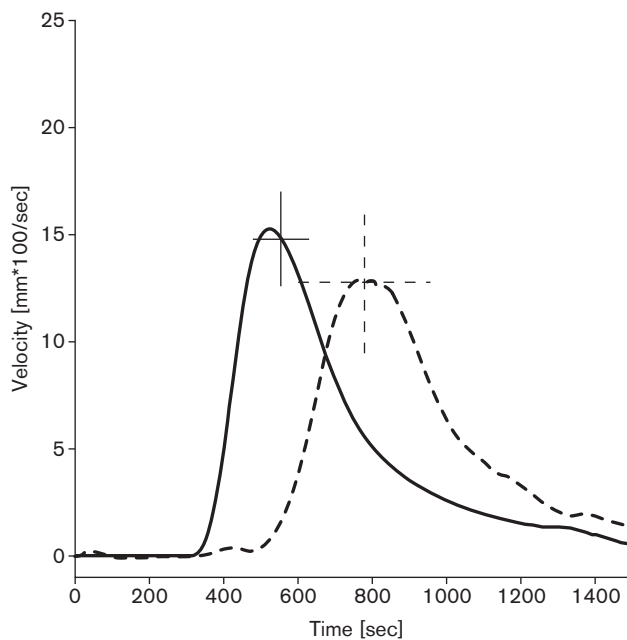
(40 µg/kg), respectively. As illustrated, the first bolus injection of rFVIIa returned a pronounced effect on the whole blood coagulation with a slightly increased MaxVel and a distinctly shortened t_{MaxVel} , similarly to the findings from our ex-vivo supplementation of rFVIIa. The whole blood coagulation profile before the second bolus injection of rFVIIa (40 µg/kg) appeared almost normal and the effect of rFVIIa was modest, probably due to a delayed effect of vitamin-K supplementation.

None of the patients died and there were no signs of thrombosis. In all cases the neurosurgeon assessed the hemostasis better than expected. Two of seven patients received blood components in the post-operative period (see Table 1).

In-vitro results in patients on VKA treatment

Twenty-five patients on VKA treatment were studied. None of the patients had overt bleeding at the time of analysis. Figure 4 depicts the velocity profiles of clot formation during whole blood coagulation. A significantly suppressed propagation of the clot formation was found as assessed by the significantly delayed t_{MaxVel}

Fig. 4



(—) Characteristic real-time continuous whole blood coagulation profile in a normal male. Vertical and horizontal lines indicate maximum velocity (MaxVel) and time to maximum velocity (t_{MaxVel}) (mean ± standard deviation), respectively, in 30 normal males. (---) Characteristic real-time continuous whole blood coagulation profile from patients on vitamin-K antagonist (VKA) treatment (International Normalized Ratio = 2.5). Vertical and horizontal lines indicate MaxVel and t_{MaxVel} (mean ± standard deviation), respectively in 25 patients on VKA.

[VKA group, mean = 777 s, standard deviation (SD) = 176 versus the reference group, mean = 552 s, SD = 75; $P < 0.01$] and decreased MaxVel (VKA group, mean = 12.8 mm 100/s, SD = 3.3 versus the reference group, mean = 14.8 mm 100/s, SD = 2.2; $P < 0.01$) in the series receiving VKA treatment. The prolonged t_{MaxVel} showed the following correlations with the INR (CC = 0.47, $P < 0.05$), factor II:C (CC = -0.37, $P = 0.07$), factor VII:C (CC = -0.47, $P < 0.05$), factor IX:C (-0.38, $P = 0.07$), and factor X:C (CC = -0.50, $P < 0.05$). Although the MaxVel declined with an increased VKA intensity, there was only a weak correlation between MaxVel and INR (CC = -0.27, $P > 0.05$). Also, there were no significant correlations within the single coagulation factors and the decline of MaxVel. The MCF in the persons on VKA treatment was significantly higher in comparison with the reference group (VKA group, mean = 62, SD = 5 versus reference group, mean = 60 mm, SD = 3.73; $P < 0.05$). No significant correlation was found between the MCF and INR or the single coagulation factor activity.

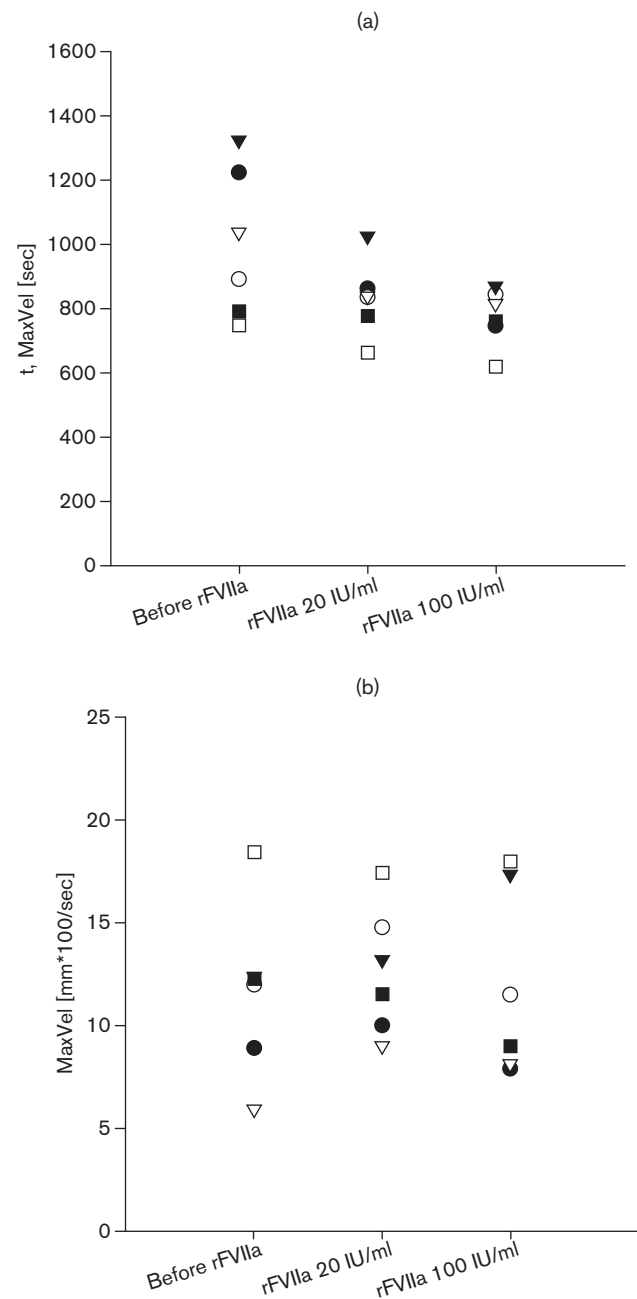
The initiation of whole blood clot formation, as evaluated by the CT in the series of patients receiving VKA treatment, was significantly prolonged as compared with the reference group (VKA, mean = 495.5, SD = 124.1 versus reference group, mean = 354.2, SD = 57.6; $P < 0.01$). The prolonged CT revealed the following correlations with the INR (CC = 0.42, $P < 0.05$), factor II:C (CC = -0.37, $P = 0.07$), factor VII:C (CC = -0.46, $P < 0.05$), factor IX:C (CC = -0.41, $P < 0.05$), and factor X:C (CC = -0.46, $P < 0.05$). Furthermore, the CFT in the VKA group was significantly prolonged compared with the reference group (VKA group, mean = 198.9, SD = 60.1 versus reference group, mean = 141.2, SD = 26.2; $P < 0.01$).

Summarizing patients on VKA treatment, the activity of factor X:C (mean = 0.13 IU/ml, SD = 0.05) revealed the most pronounced decline, accompanied by a considerable reduction in factor II:C (mean = 0.22 IU/ml, SD = 0.07) while there was only a modest decrease in factor VII:C (mean = 0.28, SD = 0.13). A mild reduction only, was observed for factor IX:C (mean = 0.56, SD = 0.14). Pairwise, all mean values except factor II versus factor VII were significantly different ($P < 0.05$, one-way analysis of variance).

Ex-vivo supplementation of rFVIIa to blood from patients on VKA treatment

Figure 5 illustrates the effect before and after ex-vivo supplementation of rFVIIa corresponding to a final concentration of 20 IU/ml and 100 IU/ml. At both concentrations, rFVIIa caused an accelerated whole blood clot formation as registered by a pronounced shortening of the prolonged CT and t_{MaxVel} . However, there was no obvious difference between rFVIIa at 20 IU/ml

Fig. 5



(a) and (b) Data of time to maximum velocity (t_{MaxVel}) and maximum velocity (MaxVel) of clot formation, respectively, before and after ex-vivo addition of recombinant activated factor VII (rFVIIa) at final concentrations corresponding to 20 IU/ml and 100 IU/ml to six patients with the following International Normalized Ratio (INR) levels: ● INR = 4.4, ○ INR = 3.0, ■ INR = 2.8, □ INR = 3.4, ▼ INR = 3.3, ▽ INR = 3.2.

versus 100 IU/ml. Those with the most delayed clotting profiles returned the most distinct effect of ex-vivo supplementation of rFVIIa. Furthermore, an increased MaxVel of whole blood clot formation was registered in four of the six cases supplemented with 20 IU/ml rFVIIa

but in only two out of the six cases supplemented with 100 IU/ml.

Discussion

Bleeding appears the most frequent complication in VKA treatment [25] but only few studies have focused on the treatment options available for acute reversal of anticoagulation in case of a major and potentially hazardous bleeding. Recently, the Scientific Subcommittee on Control of Anticoagulants of the International Society on Thrombosis and Haemostasis (ISTH) has recommended the use of PCCs simultaneously with vitamin-K supplementation as first-line intervention in major bleedings owing to VKA treatment (Subcommittee Report, year 2001, available on the ISTH website (www.med.unc.edu/isth/). Since no PCC has been approved by our authorities, we used rFVIIa as an alternative treatment modality for major and potentially fatal bleedings occurring during VKA treatment.

In the experimental part of our investigations, real-time continuous clotting profiles in VKA blood by roTEG thrombelastography demonstrated a significantly suppressed propagation and prolonged initiation of whole blood clot formation as evaluated by a significantly reduced MaxVel and a delayed t_{MaxVel} as well as a significantly prolonged CT. Using TF-activated whole blood coagulation, others have observed that VKA treatment induces a prolonged initiation and a suppressed propagation of thrombin generation as determined by continuous measurement of the total amount of thrombin-antithrombin complexes generated [26]. The delayed t_{MaxVel} correlated with increasing INR and with the gradual reduction of factor II:C, factor IX:C, factor VII:C, and factor X:C, the latter of which returned the strongest correlation. Similar correlations were not found in the case of the maximum rate of clot development (MaxVel). A differentiated activity loss of the vitamin-K-dependent coagulation factors was identified with factor X:C expressing the most seriously reduced activity followed by factor II:C, factor VII:C, and factor IX:C, similar to previously published data [27]. This distribution apparently is unique for warfarin. In acenocoumarol, treatment factor VII:C returns the mostly suppressed activity [20] among the vitamin-K-dependent procoagulant factors. Among patients on VKA treatment we detected an enhanced MCF indicating increased clot strength, a phenomenon we are unable to explain at this time. In summary, our results demonstrate that VKAs prolong the initiation of clot formation and suppress the propagation of whole blood clot formation, although the final clot strength remains normal or more stable than normal.

Our in-vitro experiments with ex-vivo supplementation of rFVIIa to blood from patients on VKA treatment appear to show that rFVIIa improves the whole blood

coagulation primarily by shortening the delayed development of the maximum rate of clot formation (t_{MaxVel}). However, the maximum rate of clot formation was only minimally altered. No dose-response relationship was detected and the maximally obtainable effect of rFVIIa might be accomplished with small doses of rFVIIa.

The clinical data on the use of rFVIIa in seven patients with neurological bleeding emergencies due to VKA treatment illustrate that a small dose of rFVIIa may reverse elevated INRs to a level below 1.5, for subsequent safe surgery. Furthermore, the effect of rFVIIa on the whole blood clotting profile in patients was similar to those from our *in vitro* experiments. Drainage was successfully performed with no early or major bleeding complications. Some of this effect may be due to the pre-operative administration of vitamin K. One patient (patient F) did not receive vitamin K pre-operatively; however, he was transfused post-operatively with three bags of FFP to maintain an INR < 2.0. His total blood loss post-operatively was 300 ml, which required two bags of erythrocytes. Furthermore, no patient displayed signs of systemic coagulation activation or clinical adverse events of thrombotic nature. Muleo *et al.* [23] have proposed to use small doses of rFVIIa for reversal of VKA treatment, while others have suggested higher doses (at 80 µg/kg) twice the highest dose used in our study [22]. The small doses of rFVIIa in the current study were selected individually based on the level of INR before intervention and were partly based on the *in vitro* investigations showing that even quite small amounts of rFVIIa were effective in correcting the hemostatic deficit induced by VKA treatment. Moreover, previous pharmacokinetic investigations have demonstrated that therapeutic levels of INR induced by acenocoumarol were efficiently reversed by low doses of rFVIIa [20]. Additionally, in our study the effect of rFVIIa was evaluated *in vitro* by a thrombelastographic principle employing minute amounts of TF. In the light of our results, thrombelastography may be feasible for ex-vivo assessment of the effect of rFVIIa for reversal of the VKA-induced coagulopathy before in-vivo administration. The thrombelastographic analysis is easy to perform and data for interpretation were available within 30 min, which is acceptable in most acute clinical situations. Furthermore, using the first derivative of the raw signal and adequate filtering of glitches, the accuracy in interpretation is significantly facilitated.

rFVIIa has been recognized as an efficient hemostatic tool in several types of coagulopathies. The mechanisms responsible for this dramatic function of rFVIIa in the various clinical situations are still the subject of some controversy. The current results verified that rFVIIa significantly intensified the activity of factor X:C [28].

The increased activity of factor X:C in plasma is thought to be caused by a relatively accelerated activation of factor X induced by an increase in the amount and activity of the TF-FVIIa complex. Furthermore, the distinct effects of rFVIIa, as indicated by thrombelastographic monitoring of continuous whole blood coagulation, might point to the existence of other possible mechanisms involved in the enhanced thrombin generation promoted by activated platelets and blood cells [29].

The currently recommended procedures for the acute reversal of VKA, such as infusion of FFP and PCC, have several shortcomings. First, infusion of high volumes of FFP constitute a potential threat to the elderly patient due to risk of systemic overloading. Second, the delayed response to vitamin K administration or infusion of FFP prolongs the period of increased intra-cranial pressure and potentiates the risk of incarceration. Finally, the extended half-lives of factor II (60 h) and factor X (30 h) [30] as well as prolonged infusion of products containing factor II and factor X may contribute to an unwarranted accumulation of potentially pro-coagulant factors with an associated risk of coagulation activation and thrombosis. In contrast, rFVIIa has a short half-life of less than 3.0 h [21,31], whereby a chronic thrombogenic effect is less likely. The safety profile has been assessed as satisfactory [32].

In conclusion, accounting for the small number of patients in our study, the clinical results show that rFVIIa may be quite useful as adjunctive therapy in patients on VKA treatment who develop a serious central nervous system bleeding, spontaneously or following trauma. In patients on VKA treatment with no bleeding, the effects of addition of rFVIIa *ex vivo* were identical to those observed in patients with an acute CNS bleeding. The modified thrombelastographic procedure for study of whole blood coagulation on a bedside basis appears to be a useful tool for direct monitoring of the effects of various prescriptions known to improve hemostasis. However, further studies are required to optimize dosing as well as the method for monitoring of the effect of rFVIIa.

Acknowledgements

The authors are grateful to the Department of Clinical Biochemistry for performing the INR measurements and to all patients volunteering for the studies. Furthermore, we acknowledge the Department of Cardiothoracic and Vascular Surgery for assisting with organizing the project and recruitment of patients with mechanical heart valves on long-term VKA. Special thanks go to Niels Trolle Andersen, Department of Biostatistics, Aarhus University for statistical assistance.

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