

# Effects of recombinant activated factor VII on coagulation measured by thromboelastography in liver transplantation

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Besides the conventional laboratory tests, thromboelastography (TEG) is used to monitor hemostasis during liver transplantation. A previous pilot study suggested a beneficial effect of recombinant activated factor VII (rFVIIa) on transfusion requirements in liver transplantation. In the present study, we assess the effects of rFVIIa on coagulation variables and TEG. In six study patients, the prothrombin time (PT), the activated partial thromboplastin time (aPTT) and TEG variables [reaction time ( $r$ ), kinetic time ( $k$ ), or clot formation time,  $\alpha$  angle ( $\alpha$ ), and maximal amplitude (MA)] were recorded before and after the administration of a bolus of 80  $\mu\text{g}/\text{kg}$  rFVIIa. These patients were compared with six controls who did not receive rFVIIa. In contrast with the control group, a significant shortening of PT ( $P=0.028$ ) and aPTT ( $P=0.028$ ),  $r$  ( $P=0.046$ ) and  $k$  ( $P=0.043$ ) values, and a significant incline of the  $\alpha$  angle ( $P=0.028$ ) were noticed after injection of rFVIIa, whereas MA increased not significantly ( $P=0.075$ ). rFVIIa rapidly improved coagulation variables in liver transplant patients including PT and aPTT. Of the TEG variables,  $r$ ,  $k$  and  $\alpha$  angle significantly improved, and MA showed a trend to increase. These data suggest that rFVIIa not only influences the speed of clot formation, but also the physical properties of the clot, which cannot be detected by routine coagulation tests. *Blood Coagul Fibrinolysis* 13:309–313  
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## Introduction

In patients undergoing orthotopic liver transplantation (OLT), complex perioperative hemostatic disorders occur, including impaired production of coagulation factors, hyperfibrinolysis, thrombocytopenia and thrombocytopenia [1]. To improve these hemostatic disorders and to reduce blood loss and transfusion requirements, hemostatic variables are monitored during liver transplantation. Besides conventional laboratory tests, like the prothrombin time (PT), the activated partial thromboplastin time

(aPTT), fibrinogen plasma levels and platelet counts, thromboelastography (TEG) is used to assess hemostasis during liver transplantation [2]. TEG enables evaluation of the whole clotting process from its initiation, and the structural characteristics and stability of the formed clot. Routine laboratory tests are, in contrast with TEG, performed on plasma only and hence provide no information about interactions of blood cells, procoagulants and anticoagulants, and profibrotic and antifibrinolytic factors, essential in the clotting process [3].

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Recombinant activated factor VII (rFVIIa) (NovoSeven<sup>®</sup>; Novo Nordisk, Copenhagen, Denmark) has been demonstrated to correct the prolonged PT in patients with liver cirrhosis [4]. The effects of rFVIIa on coagulation demonstrated by TEG have been described in hemophilia A patients [5] and in a patient with liver cirrhosis requiring an invasive procedure [6], but thus far not in patients during liver transplantation.

In the present paper, we describe the effects of rFVIIa on coagulation demonstrated by TEG in liver transplant patients.

## Materials and methods

Six adult patients who were scheduled for elective OLT were enrolled in the non-randomized study. Transplantation was performed with preservation of the retrohepatic inferior vena cava (piggy-back technique). Red blood cells were transfused to maintain hematocrit levels above 0.25 platelet concentrates if the platelet count fell below  $50 \times 10^9/l$  and hemostasis was insufficient. Fresh frozen plasma (FFP) was administered if hemostasis was clinically insufficient and PT and/or aPTT values were 1.5 times the upper limit of their normal ranges or fibrinogen plasma levels were below 1 g/l. Fibrinogen concentrate was administered when fibrinogen levels fell below 0.7 g/l despite the administration of FFP. An intravenous bolus injection of 80  $\mu\text{g}$  rFVIIa/kg was administered within 10 min before the start of surgery, to be repeated if blood loss after the previous dose exceeded the patient's estimated circulating volume. An interval of at least 2 h was required between doses. rFVIIa was not to be given after the completion of the bile duct anastomosis. This dose regimen was to be adapted if, in two patients, after rFVIIa administration, clinically significant bleeding still occurred or thrombosis was observed. Concomitant hemostatic drugs were not allowed.

The PT, aPTT, fibrinogen, platelet count, and TEG were recorded 30–60 min before and 30–60 min after administration of rFVIIa. All blood samples for coagulation studies were drawn from a (not heparinized) arterial line, after discarding the first 5 ml. TEG analysis was performed on a Thrombelastograph D (Haemoscope Corp. & Launch Diagnostics, Niles, Illinois, USA). Whole blood (0.36 ml) was placed in a pre-warmed cup (37°C) and two drops of mineral oil were spread over the blood surface to prevent evaporation. TEG was performed without addition of celite. TEG recording started 4 min after blood sampling (chart

speed, 2 mm/min: the time in minutes is equal to the distance in millimeters divided by 2). The measured TEG variables were reaction time ( $r$ ), kinetic time ( $k$ ), or clot formation time,  $\alpha$  angle ( $\alpha$ ), maximal amplitude (MA) and the extension of fibrinolysis after 60 min ( $A_{60}$ ).

The effects of rFVIIa on coagulation variables and TEG in six study patients were compared with six consecutive controls who underwent OLT in the preceding year with the same transfusion policy and similar sampling procedure, but who did not receive rFVIIa. Study patients and controls were matched for TEG variables. The medical ethical board approved the study. Written informed consent was obtained from all study patients and controls.

### Statistical analysis

Statistics to test baseline differences between the study group and controls was performed with the Mann–Whitney U test. To test the effect of rFVIIa on coagulation variables, the Wilcoxon matched-pairs signed ranks test was used.  $P < 0.05$  (two-tailed) were considered statistically significant.

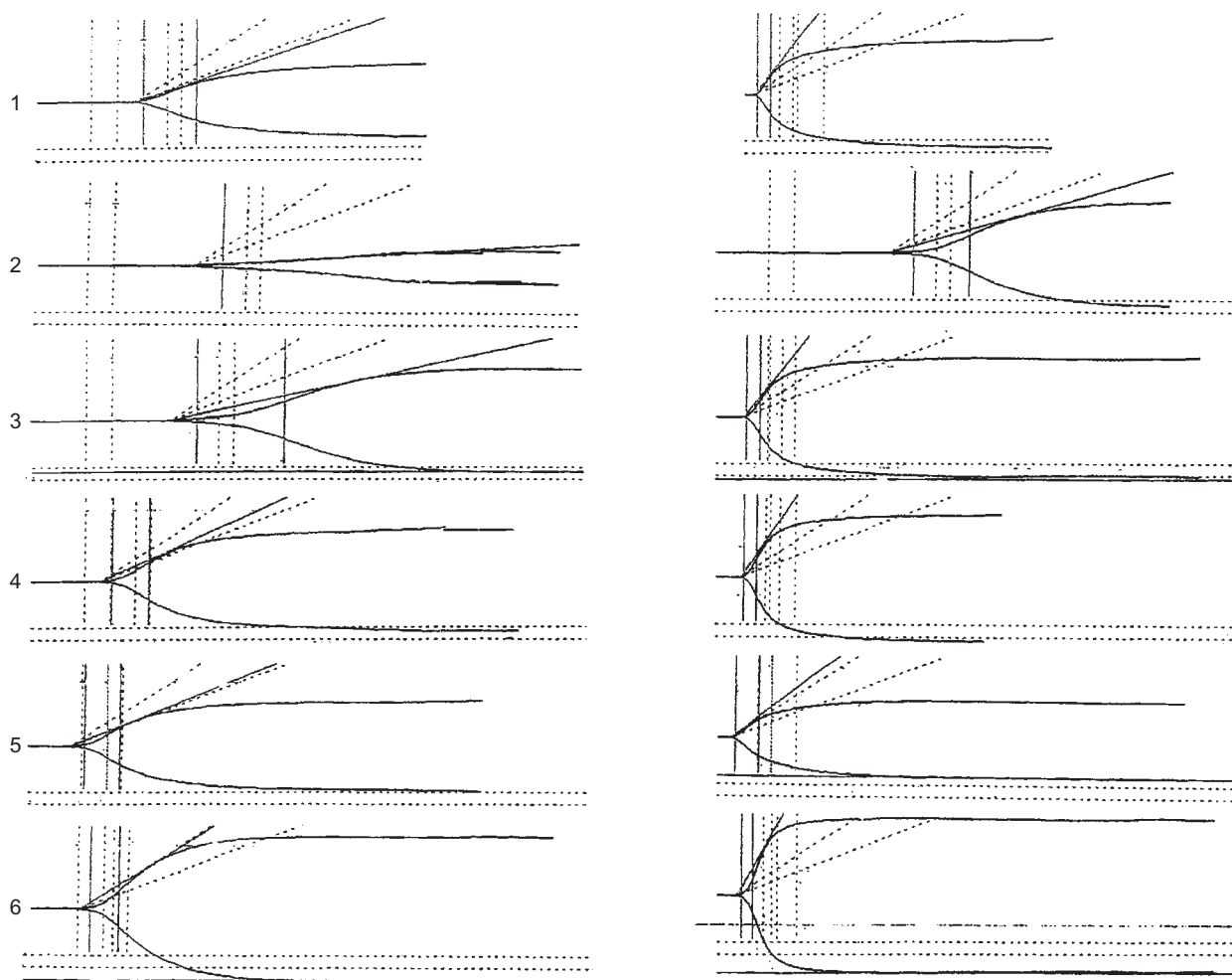
## Results

All six study patients received a single bolus injection of 80  $\mu\text{g}/\text{kg}$  rFVIIa before surgery; none of them required additional doses during the operation. Study patients and control patients did not differ significantly in baseline TEG variables (Table 1), but study patients had more severe liver disease (Child-Pugh B and C in three patients each) than controls (Child-Pugh A and B in three patients each). The results of coagulation tests and TEG recordings are presented in Table 1. Differences in baseline coagulation variables between the two groups were statistically significant for PT values ( $P = 0.037$ ) and platelet counts ( $P = 0.025$ ). In contrast with the controls, a significant shortening of PT ( $P = 0.028$ ), aPTT ( $P = 0.028$ ),  $r$  ( $P = 0.046$ ) and  $k$  ( $P = 0.043$ ) values, and a significant incline of the  $\alpha$  angle ( $P = 0.028$ ) were noticed after the bolus injection of rFVIIa. Although an increase of MA values was observed after the administration of rFVIIa in five out of six patients (Fig. 1), the difference with baseline values did not reach the level of statistical significance ( $P = 0.075$ ). TEG revealed no signs of fibrinolysis:  $A_{60}$  was  $< 5\%$  in all patients. Platelet counts and plasma fibrinogen levels did not change significantly in both groups.

**Table 1.** Coagulation variables in six study patients 30–60 min before versus 30–60 min after administration of 80  $\mu$ g recombinant activated factor VII (rFVIIa), as compared with six controls

Variable (normal value)	Study group			Control group		
	30–60 min before incision	30–60 min after incision	<i>P</i> value	30–60 min before incision	30–60 min after incision	<i>P</i> value
PT (11–16 s)	21.8 (15.2–23.6)*	11.7 (8.6–15.9)	0.028	14.9 (13.4–18.2)	16.0 (13.5–19.8)	0.115
aPTT (26–36 s)	38.5 (35.8–51.9)	33.7 (28.3–34.0)	0.028	40.3 (35.7–42.0)	39.1 (36.0–45.5)	0.463
Fibrinogen (1.7–3.5 g/l)	1.7 (1.4–3.9)	1.8 (1.2–3.5)	0.093	2.7 (2.1–5.1)	2.6 (1.7–4.5)	0.248
Platelets ( $150\text{--}350 \times 10^9/l$ )	78 (38–134)**	112 (44–139)	0.465	154 (118–194)	142 (90–210)	0.6
<i>r</i> (19–28 mm)	28.5 (20.0–57.5)	9.5 (6.0–10.2)	0.046	32.0 (28.5–117.5)	31.5 (20.0–74.0)	0.176
<i>k</i> (8–13 mm)	12.5 (10.0–30.5)	4.5 (4.0–8.5)	0.043	16.8 (11.0–32.5)	20.3 (6.0–25.0)	0.917
MA (48–60 mm)	50.0 (40.7–73.5)	62.0 (39.5–79.0)	0.075	58.3 (38.5–72.0)	58.0 (15.5–78.5)	0.674
$\alpha$ angle (29–43°)	30.5 (17.5–42.5)	62.0 (47.5–68.5)	0.028	28.0 (14.0–39.5)	26.5 (6.0–55.5)	0.917

Median values (range) are presented. PT, Prothrombin time; aPTT, activated partial thromboplastin time; *r*, reaction time; *k*, kinetic time or clot formation time; MA, maximal amplitude;  $\alpha$ , angle. \**P* = 0.037 and \*\**P* = 0.025, compared with baseline values in controls



**Figure 1.** Thromboelastography tracings at baseline (left panel) and after the administration of recombinant activated factor VII (right panel) in six study patients. In all patients, reaction time *r*, kinetic time or clot formation time *k*, and angle  $\alpha$  significantly improved. Maximal amplitude increased in five out of six patients.

## Discussion

This is the first report describing the effects of rFVIIa on coagulation and TEG variables in end-stage liver disease patients undergoing OLT. This data demonstrates that a single bolus injection of rFVIIa improved all measured hemostatic variables except the plasma fibrinogen levels, platelet counts and MA. In advanced liver disease, the levels of all coagulation factors are lowered, excepted factor VIII [7]. Administration of rFVIIa corrected prolonged PT values ( $P < 0.028$ ), as reported from previous studies [4–6]. A prolonged aPTT, although not dependent on factor VII, also normalized in all cases ( $P = 0.028$ ), probably due to direct activation of factor X by activated factor VII [8]. As expected, plasma fibrinogen levels and platelet counts were not influenced, as reported previously [4–6]. Of TEG variables,  $r$ ,  $k$ , and  $\alpha$  angle values improved. These changes suggest faster initiating of fibrin formation ( $r$ ), increased clot stability ( $k$ ) and faster clot formation ( $\alpha$ ). MA increased as in other studies [5,6], but this change did not reach the level of statistical significance. Considering the small sample size ( $n = 6$ ), this may be due to a type I error ( $\alpha$ ), as MA improved in five out of six patients and even exceeded the upper limit of its normal range in four out of six patients. Since MA increased in almost all our patients while plasma fibrinogen levels and platelet counts remained unchanged, rFVIIa apparently not only influenced the speed of clot formation, but also the properties (i.e. the strength and stability of the formed clot).

The precise working mechanism of rFVIIa remains to be established and so the impact of rFVIIa-induced changes of TEG variables. Activated factor VII initiates thrombin formation by its interaction with tissue factor [9]. This FVIIa/tissue factor complex activates factor X directly or indirectly via factor IX. In high doses (80–100  $\mu\text{g}/\text{kg}$ ), however, activated FVII also activates factor X in the absence of tissue factor, probably by activation of factor X bound at the surface of activated platelets [10,11]. Activated factor X activates prothrombin to thrombin. Thrombin is not only responsible for fibrin formation, but stabilizes fibrin due to cross-linking by activation of factor XIII and inhibits fibrinolysis by the thrombin activatable fibrinolytic inhibitor (TAFI) [12]. Tissue factor is normally not exposed to the circulating blood and it is not present in the blood sample used for TEG recording. Coagulation in the TEG cup is initiated by contact activation of factor XI. This may be enforced by rFVIIa, activating platelet bound factor X. The changes of TEG variables after rFVIIa administration can be ex-

plained as an accelerated thrombin formation by rFVIIa. Similar changes, found after the administration of FFP and/or platelets, support this assumption. Fibrinolysis was not encountered by TEG neither in the study group, nor in controls.

This study was designed to evaluate the effects of rFVIIa on a number of coagulation parameters and TEG variables rather than clinical effects (i.e. blood loss or transfusion requirements in the setting of liver transplantation). Its design, a comparison of rFVIIa-treated patients with controls who were 1:1 matched for TEG variables, does not allow conclusions about the clinical effects of rFVIIa. These effects were evaluated in a previous, separate analysis, comparing the same study patients with another control group, 1:2 matched for clinical and laboratory variables, that were identified as determinants of transfusion requirements [13]. These results suggested a beneficial effect of rFVIIa [14]. Study patients required less transfusion of red blood cells (median, 3 U versus 9 U;  $P = 0.002$ ) and FFP (median 1 U versus 8 U  $P = 0.011$ ) than controls. Although it is attractive to correlate these findings with those here presented, suggesting a reduction of required transfusion as a result of the observed improvement of hemostatic variables, we emphasize the need for properly designed studies to establish these data. This is also true for the potential risk of thrombosis, due to rFVIIa administration. In particular, the changes of TEG variables (i.e. median values of  $r$  and  $k$  below, and of MA and  $\alpha$  above, their normal ranges) might be interpreted as reflecting a hypercoagulable state. Thus far, there is, however, no convincing evidence of an increased risk of thrombosis (or disseminated intravascular coagulation) in patients who received rFVIIa [15], but the contrary can neither be excluded. One should remain aware of this potential risk.

In conclusion, rFVIIa rapidly improved coagulation variables in liver transplant patients, including prolonged PT and aPTT values. Of TEG variables,  $r$  (initiation of fibrin formation) and  $k$  and  $\alpha$  angle (speed of clot formation) significantly improved. The observed trend of MA to increase suggests a concomitant effect on the physical properties of the clot, which can only be detected by TEG. It is probable that these changes result from augmented thrombin generation. The clinical relevance of the observed effects and relation to blood loss should be established.

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