Thromboelastographic Evaluation of the Effects of Recombinant Factor VIIa on Dilutional Coagulopathy

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ABSTRACT

Introduction: Rotational thromboelastography (ROTEG) has been proposed as a monitoring tool that can be used to monitor treatment of hemophilia with recombinant factor VIIa (rFVIIa). In these studies special non-standard reagents were used as activators of the coagulation. The aim of this study was to evaluate if standard ROTEG analysis could be used for monitoring of effects of recombinant factor VIIa (rFVIIa) on dilutional coagulopathy.

Methods: The study was performed in vitro on healthy volunteers. Prothrombin time (PT) and ROTEG analysis were performed after dilution with 33% hydroxy ethyl starch and also after addition of rFVIIa to the diluted blood. Results: PT was impaired with INR changing from 0.9 before dilution to 1.2 after dilution while addition of rFVIIa to diluted blood lead to an overcorrection of the PT to an INR value of 0.6 (p=0.01). ROTEG activated with the contact activator ellagic acid was impaired by hemodilution (p=0.01) while addition of rFVIIa had no further effects. ROTEG activated with tissue factor (TF) was also impaired by hemodilution (p=0.01) while addition of rFVIIa lead to further impairment of the coagulation (p=0.01). Conclusions: The parameters affected in the ROTEG analysis were Clot Formation Time and Amplitude after 15 minutes while the Clotting Time was unaffected. We believe these effects to be due to methodological problems when using standard activators of the coagulation in the ROTEG analysis in combination with rFVIIa and not to be due to a true impairment of coagulation.

KEY WORDS
Thromboelastography, rFVIIa, dilution, HES, ROTEG,
INTRODUCTION

Patients undergoing massive hemorrhage experience dilutional coagulopathy with crystalloid and/or colloid resuscitation. If hemorrhage progresses, packed red cells (RBC) are transfused together with crystalloids and/or colloids. Regarding the coagulation this is not optimal, but the patients often develop a dilutional coagulopathy, sometimes worsened by hypothermia. The common approach to this is transfusion of fresh frozen plasma (FFP) and platelets, but bleeding might continue, with an often fatal outcome. Prophylactic use of fresh frozen plasma (FFP) or platelet transfusion is not of proven benefit to prevent hemorrhage in massively transfused patients[1]. Hemorrhage, complicated by the development of coagulopathy, is therefore still the major cause of death in trauma patients arriving alive in the hospital.[2, 3].

A novel approach to treat these patients is the use of recombinant factor VIIa (rFVIIa) to improve the coagulation[4]. Recently there have been several case publications of successful treatment of coagulopathic trauma patients and surgical patients[5-7].

Dilutional coagulopathy can be detected by the use of thromboelastographic measurements[8-11]. Rotational thromboelastography (ROTEG) is a recent development of thromboelastography. ROTEG gives a viscoelastic measurement of clot strength in whole blood. It is presented as a graph representing clot strength during the build-up and lysis of a clot (figure 1). From the graph several variables describing different parts of the coagulation process are derived and measured numerically (ref). It thus gives a more comprehensive picture of the coagulation than standard tests, but is on the other hand less validated and standardised than the more common coagulation tests.
In this study we have investigated the hypothesis that rFVIIa improves coagulation in blood hemodiluted in vitro with HES and that these effects could be monitored with ROTEG.

MATERIAL AND METHODS

The local ethics committee of the University of Lund approved this study on healthy volunteers. Volunteers were not allowed to take any medication in the 14 days prior to the study day. Informed consent was obtained from the participants and a total of eight were recruited. All participants had an indwelling intravenous catheter placed into the brachiocephalic vein, from which the blood samples were drawn with sterile disposable 5-ml syringes (Luer; Codan Medical Aps, Rødby, Denmark). A first 5-ml blood sample was discarded before every blood sample for the experiment described below. No tourniquet was used on the arm when samples were drawn.

Dilution of the blood samples was performed with hydroxy ethyl starch (HES) 130/0.4 (Voluven®, Fresenius Co., Bad Homburg, Germany). Three different preparations of blood were examined. The first preparation contained 5 ml of undiluted blood (normal). The second preparation contained 3.3 ml of blood and 1.7 ml of HES thereby achieving a 33% dilution (dilution). The third preparation contained 3.3 ml of blood, 1.7 ml of HES and 50 µl of rFVIIa at the concentration 0.12 µg/µl (dilution + rFVIIa). The latter concentration of rFVIIa was equivalent to the concentration achieved when the dose 90 µg/kg body weight is administered in vivo. 90 µg/kg is the recommended dose in hemophilia and well within the range suggested for treatment of acute hemorrhage in non hemophilia patients[7, 12]. The reason for the chosen dilution (33%) was that it is a clinically relevant dilution that is readily achieved
during resuscitation of a patient. The dilution was performed in a polypropylene test tube and the tube was gently turned to mix the blood with the added HES and rFVIIa.

The tests performed on the different preparations were hemoglobin concentration (Hb), Prothrombin time (PT) and ROTEG analysis. For the Hb measurements a Hemocue (HemoCue Co., Ängelholm, Sweden) was used. PT measurements were performed with a Rapidpoint Coag Analyzer (Bayer AB Diagnostics, Gothenburg, Sweden) with PT-ONE test cards. The ROTEG analyses were performed on a Rotational Thromboelastograph (ROTEG, Pentapharm, Munich, Germany) and the samples were analysed 120 seconds after the blood was drawn from the intravenous catheter. Both INTEG and EXTEG analyses were performed according to standard procedure recommended by the manufacturer. In INTEG analysis the coagulation is initiated with the addition of 20 µl of the contact activator ellagic acid (Pentapharm, Munich, Germany) to 320 µl of blood pipetted from the test tube to a reaction cup used in the ROTEG. In EXTEG analysis the coagulation is activated by the addition of 20 µl of a preparation containing tissue factor (TF) (Pentapharm, Munich, Germany) to 320 µl of blood pipetted from the test tube to the reaction cup. TF activates the coagulation through binding to Factor VIIa and this is believed to be the important interaction when in vivo coagulation occurs. The parameters obtained from the ROTEG analysis were Clotting Time (CT) reflecting the initiation of the coagulation, Clot Formation Time (CFT) reflecting the rate of clot formation once the formation is initiated and A15 describing the strength of the clot 15 minutes after initiation of the coagulation (figure 1).

Statistical analysis was performed with Wilcoxon's paired test. All values are given as median (range). A p value of < 0.05 was considered statistically significant.
**RESULTS**

The insertion of venous catheters and the blood sampling were performed uneventfully. The Hb values decreased as an expected sign of dilution (table 1). PT values increased in the dilution group compared to normal and decreased to below normal in the dilution + rFVIIa group (table 1).

In neither INTEG nor EXTEG analysis we found any change in the CT between normal and dilution groups, while CFT and A15 were impaired in the dilution group (tables 2 and 3). There were no differences between the dilution and the dilution + rFVIIa groups when analysed with the INTEG analysis (table 2). However, when rFVIIa was added to the dilution a prolongation of the CFT with > 200% and an impairment of the A15 with 40% were found (table 3 and figure 1).

**DISCUSSION**

Treatment of dilutional coagulopathy is challenging and the primary monitoring tools are measurement of PT, activated partial thromboplastin time (APTT), platelet count and fibrinogen[13-15]. These tests are providing us with information regarding the activation of the coagulation process and about the absolute number of platelets. They do not provide us with information regarding the dynamic properties of blood clotting and the rate at which a clot is formed once the clotting is initiated. New monitoring methods are needed and ROTEG is a monitoring tool that could potentially be of value in these situations. To this end, it has been suggested that treatment of hemophilia patients and liver transplant patients with rFVIIa can be monitored with ROTEG where a shortening of the CT and CFT has been found in case series [16-18].
In our study we found that hemodilution in vitro with HES lead to an increase of the PT and the addition of rFVIIa leads to a prompt decrease of the PT. This is in line with previous studies, which have shown that dilution with HES lead to readily detectable changes in the coagulation system[9, 19, 20]. Previous studies have also shown a decrease or a normalisation of the PT after administration of rFVIIa[21, 22]. In this study we found an overcorrection of the PT to values below the normal range.

When analysing the ROTEG parameters we found that the 33% dilution with HES resulted in a prolongation of CFT values and impairment in A15 values in accordance with previous studies[9, 11, 19, 20]. After rFVIIa had been added to the diluted blood, coagulation variables remained unchanged when assessed with INTEG, but were markedly affected when assessed with EXTEG. CFT and A15 reflect the dynamic interplay between platelets and fibrin polymerisation, both being disturbed by HES hemodilution as can be seen in table 2 and 3. Addition of rFVIIa in vitro, worsening both these parameters according to EXTEG analysis, suggested that platelets or fibrinogen became dysfunctional in contrast to the clinical effect of administration of rFVIIa, where rFVIIa has been found successful in case stories of bleeding patients [4, 7, 12, 23].

The lack of effect in the INTEG analysis when adding rFVIIa to the diluted blood is most likely due to the fact that a contact activator is used to activate the coagulation in the INTEG and therefore insensitive to rFVIIa as rFVIIa initiates coagulation through interaction with TF.

The impairment of CFT and A15 in the EXTEG analysis after addition of rFVIIa to the diluted blood is harder to explain. We expected addition of rFVIIa to the diluted blood to result in a prompt improvement of the TEG parameters measured in the EXTEG analysis.
This was expected partly because TF is used as an activator of the coagulation in the EXTEG analysis and the first step in the initiation of the coagulation system is the interaction between TF and FVIIa. The previously reported improvements of coagulation in hemophilia and liver transplant patients as evaluated with ROTEG after administration of rFVIIa also lead us to believe that ROTEG parameters would be improved after addition of rFVIIa to the diluted blood[16, 17, 24]. It is however important that these studies were performed on hemophilia patients suffering from a severe deficiency of factor VIII or IX and on liver transplant patients suffering from a very complex coagulopathy[16, 17, 24]. It also seems important to dilute TF extensively to detect the effects of rFVIIa on ROTEG. Dilutions of TF up to 1:17000 have been performed by the Ingerslev group in Denmark [17, 24]. These dilutions are, however, not made with commercially available reagents that are ready to use immediately and therefore not suitable for use outside research laboratories.

Addition of supranormal amounts of rFVIIa may initiate a very rapid coagulation process with large amounts of thrombin being generated. Such a rapid thrombin generation might have lead to generation of large amounts of tissue factor pathway inhibitor (TFPI) in the test tube. When the sample from the test tube then was added to the EXTEG system containing TF, the content of TFPI in the sample might have inhibited the initiation of the coagulation expected to be initiated through interaction between TF and FVIIa. The definite answer to why the EXTEG analysis failed in the study remains, however, still unclear.

In conclusion we found that 33% dilution of blood with HES 130/0.4 lead to impairment of the coagulation when evaluated with ROTEG or PT. Addition of rFVIIa lead to overcorrection of the prolonged PT. ROTEG analysis revealed that INTEG analysis was insensitive to effects of addition of rFVIIa and that there were methodological issues and
potentially pharmacological interactions making commercially available EXTEG analysis inappropriate for monitoring of rFVIIa effects under circumstances of dilution. If ROTEG is to be used clinically for monitoring of rFVIIa treatment we believe that new and more sensitive methods than the standard ROTEG procedure need to be developed.

COMPETING INTERESTS

None of the authors have any financial or other relationships that might influence the objectivity when performing the study or preparing the manuscript.

AUTHORS CONTRIBUTIONS

ME contributed to the design of the study, performed the analyses and drafted the manuscript. PR contributed to the design of the study, to data interpretation and to preparing the manuscript. US contributed to the design of the study, performed the analyses and participated in manuscript preparation. All authors have read and approved the final manuscript.
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Table 1

Effects of dilution and addition of rFVIIa on Hb and PT values

<table>
<thead>
<tr>
<th></th>
<th>Normal</th>
<th>Dilution</th>
<th>Dilution + rFVIIa</th>
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<tbody>
<tr>
<td>Hb (g/l)</td>
<td>136 (127-147)</td>
<td>88 (81-99)*</td>
<td>88 (81-99)*</td>
</tr>
<tr>
<td>PK (INR)</td>
<td>0.9 (0.7-1.2)</td>
<td>1.2 (0.9-1.3)*</td>
<td>0.6 (0.5-0.7)* §</td>
</tr>
</tbody>
</table>

A lowering of Hb and an increase in the PT were seen as signs of dilution while the addition of rFVIIa lead to a decrease of the PT (n=8). * p=0.01 compared to normal. § p=0.01 compared to dilution. ¶ p=0.02 compared to normal.
Table 2

Coagulation variables as assessed by INTEG

<table>
<thead>
<tr>
<th></th>
<th>Normal</th>
<th>Dilution</th>
<th>Dilution + rFVIIa</th>
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</thead>
<tbody>
<tr>
<td>CT (s)</td>
<td>93.5 (82-104)</td>
<td>108.5 (87-136)</td>
<td>97.5 (68-118)</td>
</tr>
<tr>
<td>CFT (s)</td>
<td>85.5 (59-111)</td>
<td>216 (158-310)*</td>
<td>190.5 (167-368)*</td>
</tr>
<tr>
<td>A15 (mm)</td>
<td>56 (52-61)</td>
<td>43 (36-47)*</td>
<td>43.5 (33-50)*</td>
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</tbody>
</table>

Obvious signs of dilution are found in the CFT and the A15, while the addition of rFVIIa to the diluted blood does not affect the coagulation parameters (n=8). * p=0.01 compared to normal
Table 3
Coagulation variables as assessed by EXTEG

<table>
<thead>
<tr>
<th></th>
<th>Normal</th>
<th>Dilution</th>
<th>Dilution + rFVIIa</th>
</tr>
</thead>
<tbody>
<tr>
<td>CT (s)</td>
<td>51.5 (30-69)</td>
<td>63 (41-81)</td>
<td>58.5 (37-99)</td>
</tr>
<tr>
<td>CFT (s)</td>
<td>91 (67-105)</td>
<td>227 (171-332)*</td>
<td>558.5 (308-998)*†</td>
</tr>
<tr>
<td>A15 (mm)</td>
<td>57 (53-63)</td>
<td>42 (33-49)*</td>
<td>25.5 (19-37)*†</td>
</tr>
</tbody>
</table>

Obvious signs of dilution are found in the CFT and the A15. The addition of rFVIIa to the diluted blood leads to a prolongation of the CFT and the A15 (n=8). * p=0.01 compared to normal. † p=0.01 compared to dilution.
Legend figure 1

The figure shows the 3 representative tracings of the EXTEG analysis from one of the participants in the study. The horizontal scale shows time with every mark on the scale representing 10 minutes. The vertical scale shows the clot strength with every mark representing the arbitrary strength of 20. The short straight line in the beginning represents the Clotting Time (CT). The black part following the straight line represents the Clot Formation Time (CFT) and A15 is the strength after 15 minutes. Above the normal tracing with a short CT and CFT is shown. It can be seen that the clot strength is rapidly increasing after initiation of the clotting. In the middle the tracing after hemodilution with HES is found. It can be seen that the CFT is prolonged and that the strength of the clot is increasing slower. Below the tracing after hemodilution and addition of rFVIIa is found. The clotting is then severely impaired, the clot strength is increasing slower and the maximum strength is also severely impaired.
Figure 1