Management of antithrombotic therapy in venous and arterial thromboembolism

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RECOMBINANT FACTOR VIIa REVERSES THE ANTICOAGULANT EFFECT OF THE LONG-ACTING PENTASACCHARIDE IDRAPARINUX IN HEALTHY VOLUNTEERS

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ABSTRACT

We investigated whether the anticoagulant effect of idraparinux, a selective long-acting factor Xa inhibitor, could be neutralized by recombinant factor VIIa (rFVIIa) in healthy male volunteers. We performed a randomized, placebo-controlled trial, comparing idraparinux (7.5 mg s.c.) followed at 3 h by rFVIIa (90 μg/kg i.v.)(n=6), or idraparinux (7.5 mg s.c) followed at 171 h by rFVIIa (90 μg/kg i.v.)(n=6). rFVIIa, given 3 h after idraparinux, significantly reversed the increased thrombin generation time (TGT), the increased aPTT and PT, and the reduced F1+2 levels, although no clear effect of rFVIIa on the endogenous thrombin potential (ETP) was observed. One week after idraparinux, injection of rFVIIa resulted in a similar relative reduction of the remaining increased aPTT, PT, and TGT, with correction to pre-idraparinux values. A clear increase of F1+2 was observed and a small increase in ETP. We conclude that rFVIIa has significant effects on the idraparinux inhibited thrombin generation and clotting parameters. These results suggest that rFVIIa may be useful in case of serious bleeding complications in idraparinux treated patients.
INTRODUCTION

Recently, a novel class of anticoagulants has been introduced in the prophylaxis and treatment of venous thromboembolism. This class of pentasaccharides causes a selective factor Xa inhibitory effect by binding to antithrombin, resulting in a 300-fold increase of antithrombin activity against factor Xa, with no direct inhibition of thrombin. The first developed synthetic pentasaccharide, fondaparinux, has a half-life of approximately 17 h after subcutaneous (s.c.) injection and was shown to have a superior efficacy to the current standard in the prevention of venous thromboembolism after orthopaedic surgery\(^1\)-\(^3\). Through chemical modification, a new synthetic pentasaccharide has been developed; idraparinux (SanOrg34006). This compound has a similar factor Xa inhibitory effect as fondaparinux, but exhibits a prolonged half-life, resulting stable therapeutic anticoagulant levels without the need for monitoring when administered once weekly. Currently, idraparinux is being compared against (low-molecular weight) heparin and vitamin K antagonists (VKA) in various thromboembolic diseases. A recently performed clinical trial, comparing several doses of idraparinux against standard VKA treatment in patients with symptomatic deep vein thrombosis, has been satisfactorily completed\(^4\). Phase III trials with idraparinux in the treatment of venous and arterial thromboembolic disorders and in patients with atrial fibrillation are planned.

Although stable and long-acting anticoagulation by idraparinux is an advantage in many clinical circumstances, there may be situations in which the anticoagulant effect needs to be counteracted, for instance when an acute indication for surgery occurs or in case of serious bleeding complications. In a previous study, we investigated whether recombinant factor VIIa (rFVIIa) was able to overcome the anticoagulant effect of the pentasaccharide fondaparinux in healthy volunteers\(^5\). In that study, we observed that a single bolus injection of rFVIIa was able to reverse the inhibited thrombin generation in healthy volunteers treated with 10 mg fondaparinux.

In the present study, we investigated whether rFVIIa was able to reverse the inhibitory effects of peak levels of idraparinux on thrombin generation and coagulation tests in healthy volunteers. The effect of rFVIIa was tested at peak plasma levels of idraparinux (3 h after administration), as well as at trough therapeutic plasma levels (7 days after administration). A dose of 7.5 mg idraparinux was administered, which was 3-fold higher than the dose currently used in clinical trials.
MATERIALS AND METHODS

Subject selection
Healthy male subjects (age 18 to 45 years), with a body mass index between 18 and 30 kg/m\(^2\) and a maximum weight of 100 kg, were eligible. Subjects with a personal or family history of thrombosis or bleeding disorders were excluded. All subjects gave written informed consent. The study was approved by the Medical Ethics Committee of the Academic Medical Center, Amsterdam, the Netherlands. The study protocol was conducted in accordance with the International Conference on Harmonization of Good Clinical Practice Guidelines\(^6\). All screened subjects were included in the study.

Study design
All 12 subjects received 7.5 mg idraparinux s.c. in the abdomen. Subsequently, subjects were randomized to one of two regimens: (a) rFVIIa 3 h after idraparinux and placebo 171 h after idraparinux (rFVIIa-placebo group)(n=6); or (b) placebo 3 h after idraparinux and rFVIIa 171 h after idraparinux (placebo-rFVIIa group)(n=6). The study was double-blind for rFVIIa. Idraparinux (Organon/Sanofi-Synthelabo, Oss, The Netherlands/Paris, France) was administered as a single dose of 7.5 mg s.c. in 0.5 ml. Recombinant factor VIIa (Novo Seven® , Novo Nordisk, Copenhagen, Denmark) 90 \(\mu g/kg\), or an equal volume of placebo was administered as a single intravenous (i.v.) bolus injection.

Blood sampling
Blood samples were collected just before idraparinux administration (t=0) and 2, 3 (just before rFVIIa/placebo administration), 3.5, 4, 4.5, 5, 6, 7, 9, and 23 hours thereafter. On day 8, i.e. 171 h after idraparinux administration, sampling was performed before and after rFVIIa/placebo administration according to the previous time points. At each sampling, the first 5 ml blood was discarded, after which 9 ml was collected in tubes containing 1 ml citrate (final concentration 0.32%) and 5 ml was collected in K\(_3\) EDTA Vacutainer tubes. Blood was centrifuged at 2200g for 20 minutes at 18 °C. Plasma was separated, pooled and filled out in cryocup s and frozen at \(-80\) °C until analysis was performed. These procedures were completed within 1 h after blood sampling.

Assays
The thrombin generation time (TGT) was measured spectrophotometrically using the fibrin polymerization method. Thrombin generation was initiated by adding calcium and recombinant tissue factor (2500 x diluted prothrombin time concentration) and results were expressed as t \(\frac{1}{2}\) max (time to reach the midpoint of clear to maximal turbid density). The endogenous thrombin potential (ETP) was performed as previously described\(^7\). Briefly, thrombin potential was determined amidolytically at 37 °C in
defibrinated plasma containing phospholipids, tissue factor, and calcium chloride. Results were expressed as a percentage of standard pooled plasma derived from 40 healthy male volunteers. Plasma concentration of prothrombin fragment 1+2 (F1-2) was measured by a sandwich-type ELISA assay (Dade-Behring, Marburg, Germany). The activated partial thromboplastin time (aPTT) and prothrombin time (PT) were determined according to standard methods. Plasma levels of factor VII antigen were determined using the Asserachrom VII:Ag assay (Diagnostica Stago, Asnieres-sur-Seine, France). Idraparinux plasma concentrations were measured by an amidolytic photometric assay method based on the anti-Xa activity of the antithrombin-idraparinux complex. Factor Xa and the chromogenic substrate S-2222 were added to the samples after which the amount of hydrolysed substrate was measured by a spectrophotometer.

**Statistical analysis**
Differences between treatment groups were compared with an analysis of covariance (ANCOVA) on the log-transformed area under the curve (AUC) divided by time span during the first 6 h after rFVIIa or placebo injection. Log-transformed baseline values were used as covariates. Additionally, t-tests were performed to identify differences between the groups per time point in the 6 h following rFVIIa injection. Paired t-tests were used to detect differences between time points within a group. A p-value of <0.05 was considered statistically significant. Data in the figures are presented as mean values ± standard deviation (SD) per group for each parameter.

**RESULTS**

**Thrombin generation and thrombin activity**
Administration of idraparinux increased the thrombin generation time (TGT) from 186±21 seconds to a maximum 629±113 sec at 2 h (p<0.001) (Figure I; placebo-rFVIIa group). The TGT gradually decreased to 490±98 sec at 23 h. Injection of rFVIIa 3 h after idraparinux resulted in an immediate decrease of the TGT from 665±133 sec to 341±42 sec 30 minutes after administration (p=0.001) (Figure I; rFVIIa-placebo group). The TGT remained significantly lower up to 4 h after rFVIIa injection compared with the placebo-rFVIIa group. This marked reduction of the TGT by rFVIIa was reflected in a significantly lower AUC between time points 3 and 9 compared with the placebo group (-31%; p=0.005) (Table I).
Figure I. Effect of recombinant factor VIIa (rFVIIa) on thrombin generation time (TGT) (mean values ±SD). rFVIIa was administered 3 h (rFVIIa-placebo group) or 171 h (placebo-rFVIIa group) after 7.5 mg idraparinux s.c. (administered at 0 hours). Significant differences between the two groups per time point (t-test, p<0.05) during the first 6 h after rFVIIa are marked with *.

Figure II. Effect of recombinant factor VIIa (rFVIIa) endogenous thrombin potential (ETP) (mean values ±SD). rFVIIa was administered 3 h (rFVIIa-placebo group) or 171 h (placebo-rFVIIa group) after 7.5 mg idraparinux s.c. (administered at 0 hours). Significant differences between the two groups per time point (t-test, p<0.05) during the first 6 h after rFVIIa are marked with *.
### Table I.

<table>
<thead>
<tr>
<th></th>
<th>AUC(_{3-9h})</th>
<th>AUC(_{171-177h})</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>TGT</strong></td>
<td>31% (14%-45%)</td>
<td>45% (37%-52%)</td>
</tr>
<tr>
<td><strong>ETP</strong></td>
<td>0% (0%-19%)</td>
<td>11% (5%-17%)</td>
</tr>
<tr>
<td><strong>F(_{1+2})</strong></td>
<td>15% (6%-26%)</td>
<td>23% (0%-56%)</td>
</tr>
<tr>
<td><strong>aPTT</strong></td>
<td>9% (5%-14%)</td>
<td>8% (3%-14%)</td>
</tr>
<tr>
<td><strong>PT</strong></td>
<td>23% (18%-28%)</td>
<td>24% (21%-28%)</td>
</tr>
</tbody>
</table>

Legend to Table I.

Effect of rFVIIa versus placebo on coagulation parameters. Differences in the area under the curve (plus 95% confidence intervals) in the 6 h following placebo versus rFVIIa injection administered 3 h (AUC\(_{3-9h}\)) or 8 days (AUC\(_{171-177h}\)) after 7.5 mg idraparinux. TGT = thrombin generation time. ETP = endogenous thrombin potential. \(F_{1+2}\) = prothrombin fragments 1+2. aPTT = activated partial thrombin time. PT = prothrombin time.

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On day 8 (171 h after idraparinux), both groups had comparable TGT times of ±300 sec which were still higher than pre-idraparinux levels (p<0.001). Injection of rFVIIa resulted in a comparable relative decrease of the TGT as observed during peak levels of idraparinux, resulting in a TGT of 155±39 sec 30 minutes after injection (p=0.001). The TGT remained significantly lower at least up to 6 h after rFVIIa injection compared with the rFVIIa-placebo group. The AUC reduction was 45% in the placebo-rFVIIa group compared with rFVIIa-placebo group (p<0.001).

The endogenous thrombin potential (ETP) decreased from 100±21% at baseline to 48±14% at 2 h after idraparinux administration (p<0.001) (Figure II, placebo-rFVIIa group). There was a slow and moderate increase of the ETP to 60±20% during the following 23 h. With lower baseline levels in the rFVIIa-placebo group, the ETP revealed a similar proportional decrease after idraparinux injection, although the mean values at 2 and 3 h were significantly lower than the placebo-rFVIIa group. The ETP showed a small increase after rFVIIa injection of 7% at 0.5 h after administration (p=0.04), but did not exceed the ETP of the placebo-rFVIIa group. The AUC was similar in both groups (p=1.0) (Table I).

The ETP levels at day 8 after idraparinux were 63±12% for the rFVIIa-placebo group and 82±19% for the placebo-rFVIIa group. rFVIIa injection resulted in a similar moderate ETP increase of 10%, with significantly higher ETP levels in the 6 h following rFVIIa injection compared to the rFVIIa-placebo group. The AUC was 11% higher in the placebo-rFVIIa group compared to the rFVII-placebo group (p=0.002).

The plasma levels of prothrombin fragment 1+2 \((F_{1+2})\) slightly decreased after idraparinux administration from 0.65±0.14 nmol/L at baseline to a minimum of
0.52±0.11 nmol/L 7 h after administration (p=0.003) (Figure III, placebo-rFVIIa group). Injection of rFVIIa three hours after idraparinux prevented this F1+2 decrease up to 6 h after injection, resulting in a significantly 15% higher AUC in comparison with the placebo-rFVIIa-group (p=0.005) (Table I).

The injection of rFVIIa on day 8 after idraparinux resulted in a rapid and distinct F1+2 increase from 0.54±0.04 nmol/L before rFVIIa injection to a maximum of 0.98±0.4 nmol/L 4 hours later (p=0.04). Levels at 30 minutes and 1 h were significantly higher in the placebo-rFVIIa group versus the rFVIIa-placebo group, with a non-significant 23% higher AUC in the 6 h post rFVIIa in the placebo-rFVIIa group (p=0.09).

![F1+2 graph](image URL)

* = injection of rFVIIa or placebo

**Figure III.** Effect of recombinant factor VIIa (rFVIIa) on prothrombin fragment 1+2 (F1+2) (mean values ±SD). rFVIIa was administered 3 h (rFVIIa-placebo group) or 171 hours (placebo-rFVIIa group) after 7.5 mg idraparinux s.c. (administered at 0 hours). Significant differences between the two groups per time point (t-test, p<0.05) during the first 6 h after rFVIIa are marked with *.

**Clotting times**

In the placebo-rFVIIa group, idraparinux increased the aPTT from 37.4±3.8 sec to 46.5±6.4 seconds 2 h post-idraparinux (p=0.001) (Figure IVa). During the following 23 h, the aPTT slightly decreased to 42.5±4.2 seconds. Injection of rFVIIa resulted in a sharp decrease of the aPTT to 37.3±2.8 sec which remained at this level and significantly lower than the placebo-rFVIIa group up to time point 9 h. The AUC analysis in the 6 h following rFVIIa revealed a significant 9% reduction of the aPTT compared to the placebo-rFVIIa group (p=0.002) (Table I).

A persistently higher aPTT of 40.8±2.9 sec in the placebo-rFVIIa group was observed at day 8 compared to the rFVIIa-placebo group (36.0±2.8 sec). A decrease to levels slightly lower than the rFVIIa-placebo group occurred immediately after rFVIIa
injection which remained lower up to 3 h after injection (p=NS), resulting in a significant 8% lower AUC (p=0.007).

The prothrombin time increased after idraparinux administration from 13.8±0.9 at baseline to 15.4±0.6 sec at 2 h (p=0.01), and remained significantly higher compared to baseline levels up to 9 h after administration (Figure IVb; placebo-rFVIIa group). Immediately after injection of rFVIIa, the prothrombin time decreased from 15.1±0.8 to 10.5±0.7 sec (p=0.004), and remained significantly lower than the placebo-rFVIIa group at least up to 6 h following rFVIIa. This shortening of the PT resulted in a 23% AUC reduction in the 6 h after rFVIIa injection of 23% (p<0.001) (Table I).

At 1 week, the prothrombin times were similar to baseline values. A decrease to 9.8±0.4 sec was observed when rFVIIa was administered, which remained significantly lower compared to the placebo group up to 6 h post-rFVIIa injection. This was reflected in a 24% AUC reduction (p<0.001).

**Factor VII and idraparinux plasma levels**

Factor VII antigen plasma levels increased from 75% at baseline to a maximum of 195% 30 minutes post-rFVIIa injection and remained elevated up to time point 9 h (data not shown). At 23 h, the plasma levels were comparable with baseline.

When rFVIIa was administered 1 week after idraparinux, the maximum factor VII plasma level was higher (224%) than the maximum level observed 3.5 h after idraparinux (195%), and remained similarly elevated up to 6 h after injection. Both groups showed a comparable rFVIIa half-life of approximately 2.4 h. Injection of rFVIIa had no effect on the pharmacokinetic profile of idraparinux. Maximum plasma levels of idraparinux were reached at 3 h after administration (1.7 mg/L), with plasma levels at 1 week (171 h) of 0.3 mg/L (data not shown).
Figure IVa and IVb. Effect of recombinant factor VIIa (rFVIIa) on activated partial thromboplastin time (aPTT) and prothrombin time (PT) (mean values ±SD). rFVIIa was administered 3 hours (rFVIIa-placebo group) or 171 h (placebo-rFVIIa group) after 7.5 mg idraparinux s.c. (administered at 0 hours). Significant (t-test) differences (p<0.05) between the two groups during the first 6 h after rFVIIa are marked with *. 
DISCUSSION

In this study, we observed that rFVIIa significantly decreased the inhibitory effect of the long acting pentasaccharide idraparinux on thrombin generation and clotting times in healthy subjects. At both peak and trough plasma levels of idraparinux, rFVIIa produced a consistent decrease of the thrombin generation time, aPTT, and PT. Additionally, rFVIIa prevented the decrease of the in-vivo thrombin generation marker F1+2 after idraparinux administration, and markedly increased F1+2 levels during trough idraparinux levels. Only the ETP showed a moderate change by rFVIIa, at both peak and trough idraparinux levels. At peak plasma concentrations of idraparinux, the TGT, aPTT, and ETP did not return to baseline levels after rFVIIa injection, while at trough plasma idraparinux levels (at 1 week) they did. In order to correct coagulation to baseline levels during peak levels of 7.5 mg idraparinux, a higher dosage of rFVIIa may be required. However, it should be stressed that the dose used in this study was 3-fold higher than the idraparinux dose for clinical use in venous and arterial thrombotic diseases. In this study, rFVIIa resulted in a significant correction of the idraparinux inhibited coagulation assays. Therefore, a complete correction to baseline coagulation levels might be attained at steady state (trough) levels of 2.5 mg idraparinux, because these levels (±0.36 mg/L) are comparable to the concentration 1 week after a single 7.5 mg dose. Whether these effects of rVIIa on idraparinux, as indicated by these idraparinux-inhibited coagulation assays, indicate clinical efficacy, i.e. contain a clinically significant bleeding in an idraparinux treated patient is unknown. However, rFVIIa has been shown to be a potent prohemostatic agent in various situations with a seriously impaired coagulation system and during major perioperative blood loss. These results are consistent with our previous observation in which rFVIIa was able to reverse the anticoagulant effect of the pentasaccharide fondaparinux on thrombin generation and clotting assays.

The mechanism of action by which rFVIIa induces thrombin generation in subjects treated with the factor Xa inhibitor idraparinux is not one of a direct neutralization of idraparinux. rFVIIa is a non-selective strong activator of the coagulation system and presumably acts by activating sufficient amounts of non-idraparinux-inhibited factor X to achieve thrombin generation. By this mechanism, rFVIIa has been shown to reverse the inhibitory effects of not only selective Xa inhibitors (idraparinux, fondaparinux), but also of other anticoagulants such as tissue-factor-factor VIIa inhibitors (rNAPc2), and vitamin K antagonists. It is therefore likely that the observed counteracting effects will be larger when more tissue factor/factor VIIa complexes can be generated such as in the case of extra-vascular damage. We observed lower peak factor VII antigen (fVIIag) levels when rFVIIa was administered shortly after idraparinux compared with the peak levels when rFVIIa was injected 1 week after idraparinux (195% versus 224%, respectively). These increased peak levels remain during 2 h post-rFVIIa injection. A
direct binding of idraparinux to rFVIIa with increased plasma clearance of this complex is not likely, as the plasma concentration of idraparinux was not affected by the injection of rFVIIa. Furthermore, we observed no effect of idraparinux on the FVIIa assay during in-vitro addition of idraparinux to control plasma (data not shown). Therefore, this in-vivo phenomenon is more likely to be caused by an up regulation of FVIIa binding proteins, which could rapidly neutralize infused rFVIIa.

As fondaparinux does not increase tissue factor pathway inhibitor (TFPI) levels\textsuperscript{15-16}, it is unlikely that idraparinux influenced TFPI. It is possible that increased antitrombin activity is responsible for this effect, as antithrombin in combination with unfractionated heparin or pentasaccharides is capable of binding to the FVIIa-tissue-factor complex\textsuperscript{17-19}. Idraparinux administration increased both the aPTT and PT (Figure IVa and IVb). These increases were small for the PT i.e. 1.7 sec, and moderate for the aPTT i.e. 9.1 sec. As with the pentasaccharide fondaparinux, aPTT and PT changes do not accurately reflect the anticoagulant effect of idraparinux, and can therefore not be used for individual patient monitoring. The relatively small changes in these assays are not consistent with the anti-Xa levels.

The effect of rVIIa on the ETP was only modest, showing marginal increases both at the peak and trough idraparinux levels. The insensitivity of the test for rVIIa-increased thrombin generation could be caused by dilution of the rVIIa effect during the long incubation time of this test for thrombin potential measurement (20 min of plasma activation).

In this small, healthy volunteer study, no adverse events were observed and both thrombin generation and clotting assays showed no overshoot of coagulation after rFVIIa injection. However, the use of rFVIIa in patients treated with idraparinux requires caution. Injection of rFVIIa results in the activation of the coagulation system through the tissue-factor dependent extrinsic pathway. In the absence of tissue factor, peak concentrations of rFVIIa are capable of directly activating factor X, and can overwhelm factor Xa inhibitors as seen in this and other studies\textsuperscript{8,13}. However, in patients treated for venous or arterial thrombosis, the increased expression of tissue factor, either in atherosclerotic arteries\textsuperscript{20-21}, intravascular thrombus, or disrupted veins, could lead to a much greater thrombin generating effect of injection of rFVIIa. The high affinity binding of tissue-factor with (r)FVIIa results in a complex with a much greater potential of factor X activation, promoting local thrombus formation at these tissue factor expressing sites. Clearly, in patients with acute coronary syndromes, the use of rFVIIa should be reserved to life-threatening cases, whereas, in case of severe bleeding, local interventions and administration of plasma are ineffective.

This study showed that rVIIa has a distinct effect on thrombin generation and clotting times in subjects treated with a single 7.5 mg subcutaneous injection of
idraparinux. These results suggest that rFVIIa may be a suitable treatment in case of serious bleeding complications in patients treated with idraparinux.

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**REFERENCE LIST**


