Diagnosis, treatment and long-term effects of venous thromboembolism
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Chapter 9

In-vivo increase in thrombin generation by four-factor prothrombin complex concentrate in apixaban-treated healthy volunteers

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Chapter 9

Abstract

Background: Four-factor prothrombin complex concentrate (PCC, Cofact, Sanquin Blood Supply) 50 IU/kg increased thrombin generation beyond baseline values in healthy, rivaroxaban-treated subjects.

Objective: Assess whether infusion with doses of 37.5 and 25 IU/kg of PCC reverses the anticoagulant effect of high dose apixaban, another oral direct factor Xa inhibitor.

Methods: In a randomised, double-blind, placebo-controlled, crossover study, 6 healthy subjects received twice-daily apixaban 10 mg for 3.5 days followed by a single bolus of PCC 37.5 IU/kg, PCC 25 IU/kg, or placebo. The primary outcome was the effect of PCC 15 minutes after infusion on thrombin generation (endogenous thrombin potential [ETP]); secondary outcomes were the immediate effect of PCC on prothrombin time (PT) and the effect of PCC compared with placebo over 24 hours on ETP and PT.

Results: Fifteen minutes after infusion of 37.5 IU/kg and 25 IU/kg PCC, ETP increased from 41±11% to 56±23% (p=0.06) and from 44±12% to 51±15% (p=0.03), respectively. ETP significantly differed over time between PCC 37.5 IU/kg and placebo during 24 hours after infusion (p<0.01). Both PCC dosages restored apixaban-induced PT prolongation after 15 minutes (p<0.01) which was sustained over 24 hours.

Conclusion: Both PCC 37.5 IU/kg and 25 IU/kg improved coagulation parameters in healthy subjects, suggestive of partial reversal of the anticoagulant effect of apixaban. This implies that PCC might be considered in patients with apixaban-associated bleeding. However, ETP was not immediately restored to pre-apixaban levels suggesting that these dosages are too low to instantly and fully restore haemostasis at peak apixaban levels.
Introduction

Apixaban is one of the direct oral anticoagulants (DOAC) that directly inhibits factor Xa and is registered for treatment and prevention of venous thromboembolism (VTE) and for stroke prevention in patients with atrial fibrillation (AF). Apixaban was evaluated in a fixed dose regimen without the need for frequent laboratory testing and dose adjustments and thereby simplifies oral anticoagulant therapy compared with vitamin K antagonists (VKA). Although anticoagulation is highly effective in the prevention and treatment of thromboembolism, its use is associated with an increased risk of bleeding. VKA therapy tops the list of drugs of which adverse drug reactions (i.e. bleeding) leads to hospital admission, indicating that anticoagulation associated bleeding is a frequently observed problem. In case of life-threatening bleeding complications, the effect of VKA on coagulation parameters (i.e. INR) can be immediately reversed with prothrombin complex concentrate (PCC).

Specific reversal agents for factor Xa inhibitors including apixaban are under development but will probably not be available in routine clinical practice in the next few years. In the absence of specific reversal agents, non-specific prohaemostatic agents could be useful. Previously, four-factor PCC 50 IU/kg (Cofact, Sanquin Blood Supply, Amsterdam, the Netherlands), containing zymogen factor X, completely reversed coagulation parameter changes in healthy subjects treated with a supra-therapeutic dose of rivaroxaban, another direct factor Xa inhibitor. However, in that study thrombin generation (expressed as endogenous thrombin potential [ETP]) increased beyond pre-rivaroxaban values. Observations from animal models showed that lower PCC doses seem equally effective in rivaroxaban associated bleeding.

The ability of PCC to reverse the anticoagulant effect of apixaban has not been assessed in humans. However, as both rivaroxaban and apixaban are factor Xa inhibitors, it is plausible that PCC also reverses the anticoagulant effect of apixaban. If so, the lowest effective dose may be preferred to minimise the risk of prothrombotic complications and to reduce costs.

The objective of our study was to assess the effect of a single administration of non-activated four-factor PCC at the dosages of 37.5 IU/kg and 25 IU/kg on the anticoagulant effect of a high therapeutic dose of apixaban in healthy subjects.
Materials and Methods

Study design

The study was performed as a single-centre, randomised, double-blind, placebo-controlled, crossover study at the Academic Medical Center (AMC) in Amsterdam. After providing written informed consent, subjects received twice-daily (bid) apixaban 10 mg from Day -3 to Day 0 to achieve steady-state drug levels (Figure 9.1). The final apixaban dose was taken on day 0 in the morning without food consumption. Three hours after the last dose, subjects received either a single bolus of PCC 37.5 IU/kg, PCC 25 IU/kg, or a similar volume of saline (placebo). Subjects were randomised for the order of reversal method. After a wash-out period of 15-30 days, all subjects returned to the same prescription of apixaban and crossed-over for the method of reversal. All subjects subsequently received all the reversal methods (Figure 9.1). After 20 days from the last visit, patients had the final study visit.

Oral apixaban (Eliquis, Bristol-Myers Squibb/Pfizer, Middlesex, United Kingdom) was given in a dose of 10 mg bid, the initial dose for the treatment of acute VTE in the phase III AMPLIFY trial. Adherence to study medication was evaluated at each infusion by checking the blister packaging. Apixaban was stored in the hospital pharmacy and provided at baseline.

Four-factor PCC (Cofact, Sanquin Blood Supply, Amsterdam, the Netherlands) was supplied by the manufacturer. A vial of 500 IU PCC contains 500 IU of Factor IX, 280 to 700 IU of Factor II, 140 to 400 IU of Factor VII, 280 to 700 IU of Factor X, 222 to 780 IU of protein C, 20 to 160 IU of protein S, and antithrombin, without any heparin added. PCC was reconstituted by an independent researcher in the hospital pharmacy according to manufacturer’s instructions and subsequently administered by blinded investigators.

The study was conducted in accordance with the Declaration of Helsinki (60th version Fortaleza, October 2013) and according to guidelines for Good Clinical Practice. The study protocol was reviewed by the institutional review ethics board and registered at the Dutch Trial Registry (www.trialregister.nl; identifier, NTR3559). Subjects were recruited by public advertisement located at the AMC (Amsterdam) and received a financial reimbursement coherent with the time spent on the study.

Study population

Subjects were included if they met the following inclusion criteria: male sex, age between 18 and 50 years, normal laboratory screening tests, no abnormalities at physical examination, normal vital signs, and ability to provide written informed consent. Laboratory screening included renal and hepatic function, hepatitis B, C, and HIV serology, complete blood cell count, prothrombin time (PT), and activated partial thromboplastin time (aPTT). Exclusion criteria were a history of allergic reaction to blood products, a personal or family history of coagulation disorders, participation in any other investigational intervention
study within the past 30 days, medication use within 14 days before the start of the study, unhealthy use of alcohol, and any concomitant condition that, as judged by the investigator, would have placed the subject at increased risk of harm if he participated in the study. During the study, the consumption of nicotine, alcohol, and recreational drugs was recorded.

**Study procedures, sample collection and analysis**

Blood samples were collected at screening visit, at baseline visit on Day -3, at Day 0 immediately before the PCC/saline infusion and 15, 30, 60, 120, 240, and 360 minutes after the end of infusion, and at Day 1 (T=24 hours, Figure 9.1). A peripheral venous catheter was placed to administer PCC or saline, and a second catheter was inserted for blood withdrawal on the infusion days. The lines for blood samples were flushed with saline and the first 5 mL blood discarded at each blood withdrawal. Tubes of 5 mL with 3.2% sodium citrate were used and samples were double spun within 2 hours from withdrawal to produce platelet-poor citrated plasma. Samples were then stored at -80°C until analysis.

The following assays were used for study outcomes: ETP as a measure of thrombin generation and PT. The Calibrated Automated Thrombogram assays the generation of thrombin in clotting plasma using a microtiter plate–reading fluorometer (Fluoroskan Ascent, ThermoLab Systems, Helsinki, Finland) and Thrombinscope software (Thrombinscope BV, Maastricht, the Netherlands). The software calculated the ETP, the total amount of thrombin formed in time that was normalised to pooled plasma obtained from more than 200 individuals; reference values: 65-146%. The assay was carried out as described by Hemker et al. with 5 pM of tissue factor as initiator of coagulation. PT was analyzed with automated coagulation analyzer (Behring Coagulation System XP), Thromborel S reagent, and protocols from the manufacturer (Siemens Healthcare Diagnostics, Marburg, Germany); reference values: 10.7-12.9 seconds. A calibrated anti-Xa activity assay (Berichrom Heparin, Siemens) was
used to verify adequate apixaban uptake before PCC administration.

All laboratory analyses were performed by laboratory technicians, who were unaware of the type and the dosage of the reversal method.

**Study outcomes**

Pre-defined study outcomes were: 1) the effect of PCC administration on coagulation parameters, as reflected by difference in ETP (primary outcome) and PT between T=0 and T=15 for each reversal method, and 2) the effect of two different PCC dosages compared to placebo over 24 hours.

**Sample size calculation and statistical analysis**

Our sample size calculation was based on the primary outcome from the study by Eerenberg et al.: improvement of ETP between T=0 and T=15. In that study, baseline ETP values in the rivaroxaban group were 92±22% (mean ± standard deviation [SD]). Administration of rivaroxaban decreased ETP to 51±21% and PCC increased ETP to 114±26%.

Using a paired Student t-test, an alpha of 0.05 and a power of 0.80, a sample size of 6 subjects in the present crossover study was calculated for the primary outcome, the effect of two PCC dosages on ETP, as reflected by differences between T=0 and T=15 for each PCC dosage.

Paired T-tests were used to assess the immediate effect (i.e. 15 minutes after infusion) of PCC on ETP and PT within each treatment group. The effect over 24 hours between treatment groups was analyzed with linear mixed models. Two-sided p-values <0.05 were considered to be statistically significant. Data analysis was performed using IBM SPSS v21.0 (Armonk NY, USA).

**Results**

Six subjects were enrolled and all completed the study. Baseline characteristics were: mean age (± SD) of 26±7 years and weight (± SD) of 75±12 kg. All subjects had normal baseline screening tests. In the 37.5 IU/kg session, the PCC dosage ranged between 2250 and 3563 IU. In the 25 IU/kg session, the PCC dosage ranged between 1500 and 2375 IU.

**Effects of apixaban on coagulation before PCC/placebo**

After twice-daily apixaban 10 mg from Day -3 to Day 0 (seven doses), subjects showed statistically significant modifications of both ETP and PT consistent across sessions (overall, p <0.01 for ETP and p< 0.01 for PT). Mean anti-Xa-derived apixaban peak level at steady state was 330±113 ng/mL with no differences between groups, indicating sufficient oral uptake.
Immediate effect outcomes

Fifteen minutes after infusion of PCC 37.5 IU/kg, ETP increased from 41±11% to 56±23% (p=0.06). After infusion of PCC 25 IU/kg, ETP increased from 44±12% to 51±15% (p=0.03; Figure 9.2, Table 9.1).

Fifteen minutes after infusions of both PCC 37.5 IU/kg and PCC 25 IU/kg, the PT prolongation induced by apixaban normalised (13.6±0.8 seconds to 11.8±0.5 seconds: p<0.01 and 13.1±1.2 seconds to 12.0±0.8 seconds: p<0.01, respectively; Figure 9.2, Table 9.1).

No changes in both ETP and PT were observed over the first 15 minutes after infusion of saline in the placebo group (ETP: 45±10% to 44±11%, p=0.85; PT: 13.6±1.2 seconds to 13.4±0.8 seconds, p=0.44).

Sustained effect outcomes

ETP levels significantly differed over time between PCC 37.5 IU/kg and placebo during 24 hours after infusion (p<0.01), but not between PCC 25 IU/kg and placebo (p=0.10; Figure 9.2, Table 9.1). Mean ETP values gradually reached pre-apixaban levels after about 6 hours from the PCC 37.5 IU/kg dosage infusion and after 12 hours from the PCC 25 IU/kg dosage infusion.

PT values were significantly different over time between each PCC dosage and placebo during 24 hours after infusion (p<0.01; Figure 9.3, Table 9.1).

Table 9.1 | Immediate and sustained effect outcomes.

<table>
<thead>
<tr>
<th>ETP (%)</th>
<th>PCC 37.5</th>
<th>PCC 25</th>
<th>Placebo</th>
</tr>
</thead>
<tbody>
<tr>
<td>T=0 minutes</td>
<td>41±11</td>
<td>44±12</td>
<td>45±10</td>
</tr>
<tr>
<td>T=15 minutes</td>
<td>56±23</td>
<td>51±15</td>
<td>44±11</td>
</tr>
<tr>
<td>p=0.06</td>
<td></td>
<td>p=0.85</td>
<td></td>
</tr>
<tr>
<td>T=0-24 hours</td>
<td>vs placebo</td>
<td>vs placebo</td>
<td>-</td>
</tr>
<tr>
<td>p&lt;0.01</td>
<td>p=0.10</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>PT (seconds)</th>
<th>PCC 37.5</th>
<th>PCC 25</th>
<th>Placebo</th>
</tr>
</thead>
<tbody>
<tr>
<td>T=0 minutes</td>
<td>13.6±0.8</td>
<td>13.1±1.2</td>
<td>13.6±1.2</td>
</tr>
<tr>
<td>T=15 minutes</td>
<td>11.8±0.5</td>
<td>12.0±0.8</td>
<td>13.4±0.8</td>
</tr>
<tr>
<td>p&lt;0.01</td>
<td>p&lt;0.01</td>
<td></td>
<td></td>
</tr>
<tr>
<td>T=0-24 hours</td>
<td>vs placebo</td>
<td>vs placebo</td>
<td>-</td>
</tr>
<tr>
<td>p&lt;0.01</td>
<td>p&lt;0.01</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Abbreviations: ETP, endogenous thrombin potential; PCC, prothrombin complex concentrate (expressed as IU/kg); PT, prothrombin time.
Figure 9.2 | Effect of apixaban followed by two dosages of four-factor PCC or placebo on the ETP (%)

Immediate effect outcome: differences between T=0 minutes and T=15 minutes.
Abbreviations: PCC, prothrombin complex concentrate (expressed as number of IU/kg); ETP, endogenous thrombin potential, ETP baseline, mean ETP value before apixaban administration.
**Figure 9.3** Effect of apixaban followed by two dosages of four-factor PCC or placebo on the PT (seconds)

Immediate effect outcome: differences between T=0 minutes and T=15 minutes

Abbreviations: PCC, prothrombin complex concentrate (expressed as number of IU/kg); PT, prothrombin time; PT baseline, mean PT value before apixaban administration.
Adverse events

One subject had numbness in his calf several days after infusion of 37.5 IU/kg PCC. An ultrasound to rule out a deep venous thrombosis was not performed as he did not mention discomfort until the next visit, at which time the complaints had completely subsided. Another subject fell on his right hip one day before he started with the third session of apixaban. He developed a haematoma of 3 by 2 centimeter and took apixaban the next day. The haematoma did not expand after apixaban intake and resolved completely. During that session the subject received 25 IU/kg PCC as reversal.

Discussion

Our study suggests that four-factor PCC in a dose of 25 IU/kg and 37.5 IU/kg immediately increases ETP (but not to pre-apixaban levels) and immediately normalises PT in healthy volunteers treated with a high therapeutic dose of apixaban at peak concentrations. The effect of PCC was sustained over a 24 hour period. These findings suggest that PCC can overcome the anticoagulant effect of apixaban and might be considered in patients with severe apixaban associated bleeding.

The complete normalisation of PT and only partial restoration of ETP likely reflects the different intrinsic characteristics of the ETP and PT assays. The PT assay is very sensitive to the factor VII levels in PCC whereas thrombin generation is regarded as an overall markers of haemostasis. This is supported by a similar study in rivaroxaban treated healthy subjects in which four-factor PCC had a larger effect on PT than three-factor PCC (i.e. PCC without factor VII), whereas the effect on ETP was similar.9

While both PCC doses significantly increased thrombin generation, neither dose achieved an immediate ETP increase to pre-apixaban levels. This suggests that both dosages could be too low to fully restore functional haemostasis. In a recently presented conference abstract, a higher dose of 50 IU/kg PCC restored thrombin generation faster than in our study, suggesting more potency of a higher dose.15

Our study investigated one particular type of four-factor PCC, i.e. Cofact (Sanquin Blood Supply). The results of this study can therefore not be extrapolated to three-factor PCCs or activated four-factor PCCs. Moreover, there are different inactive four-factor PCCs on the market with varying compositions (e.g. slightly different coagulation factor levels, addition of heparin and/or antithrombin). The results formally only apply to the four-factor PCC tested here, a PCC with trace amounts of antithrombin but no added heparin. However, we speculate that the results may be extrapolated to other four-factor PCCs. For example, our previous study in rivaroxaban treated volunteers showed similar results of 50 IU/kg of Cofact compared with an identical study in which 50 IU/kg of another four-factor
PCC (Beriplex P/N, CSL Behring, a PCC with added heparin) was used.\textsuperscript{8,9}

This is the first study that investigated the potential of four-factor PCC to reverse the anticoagulant effect of apixaban in healthy human subjects. The enrolment of healthy, non-bleeding male subjects and the use of coagulation assays as a surrogate outcome limits the generalisability to bleeding patients. However, the strength of the present study is that it evaluated the effects on human haemostasis and that the dosages used are applicable to routine patient care. Earlier studies demonstrated that PCC can restore thrombin generation in human plasma, spiked with apixaban in different concentrations.\textsuperscript{16,17} Our study should be regarded as necessary in-vivo confirmation. In various studies in which animals were treated with apixaban and other direct factor Xa inhibitors, four-factor PCC in a dose of 25-100 IU/kg was able to reduce bleeding in most studies.\textsuperscript{10-12,18,19} The combined results of animal studies, in-vitro studies in spiked human plasma and our in-vivo study in healthy volunteers suggest that PCC may act as a reversal agent for factor Xa inhibitors.

Ultimately, the goal of apixaban reversal in bleeding patients is to improve the outcome of bleeding. Although restoration of haemostasis would intuitively improve clinical outcome, this has never been firmly established. The efficacy and safety of PCC as a reversal agent for factor Xa inhibitors should be confirmed in cohorts of patients with bleeding. Interestingly, the approval of PCC for reversal of VKA associated bleeding was mainly based on studies that used coagulation parameters as a primary outcome. The beneficial effect of PCC on the clinical outcome of VKA associated bleeding remains uncertain to date.\textsuperscript{5,20-25}

In the absence of specific antidotes for factor Xa inhibitors, the European Heart Rhythm Association guidance document suggests to use 25 IU/kg PCC or 50 IU/kg activated PCC (aPCC) as potential reversal agents in patients with DOAC-associated bleeding.\textsuperscript{26} Moreover, the risk of thrombotic complications of PCC is probably smaller than of activated factor concentrates such as aPCC or recombinant factor VIIa.\textsuperscript{27,28} While our study strengthens the suggestion to use PCC to improve haemostasis, the limited magnitude of this improvement suggests that a higher than 25 IU/kg dose should be considered in patients with very severe or life-threatening bleeding.
Reference list

1. EMA. Summary of product characteristics. 2014.


