Diagnosis, treatment and long-term effects of venous thromboembolism
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Citation for published version (APA):
Cheung, Y. W. (2016). Diagnosis, treatment and long-term effects of venous thromboembolism

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Chapter 10

In-vivo reversal of the anticoagulant effect of rivaroxaban with four-factor prothrombin complex concentrate

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Abstract

Background: Four-factor prothrombin complex concentrate (PCC) 50 IU/kg is able to swiftly restore haemostatic parameters in healthy subjects on rivaroxaban. We hypothesised that lower dosages of PCC may already be sufficient to restore normal haemostasis.

Methods: In this double-blind, crossover, placebo-controlled study, we compared the effect of PCC 37.5 IU/kg, PCC 25 IU/kg, and placebo on thrombin generation (endogenous thrombin potential, ETP) and prothrombin time in 6 healthy subjects receiving rivaroxaban 15 mg twice daily for 2.5 days.

Results: Fifteen minutes after infusion of PCC 37.5 IU/kg, ETP increased from 47±16% to 64±22% (p=0.03; pre-rivaroxaban ETP: 92±14%). ETP over 24 hours was higher than after placebo (p=0.001). PCC 25 IU/kg did not modify ETP within 15 minutes (53±11% to 59±12%; p=0.14) and was not different from placebo over 24 hours (p=0.31). ETP reached pre-rivaroxaban levels within 6 hours after PCC 37.5 IU/kg infusion and within 12-24 hours after PCC 25 IU/kg infusion. Both dosages restored rivaroxaban-induced prothrombin time prolongation after 15 minutes (p<0.001). Placebo did not have an effect on coagulation parameters.

Conclusion: 37.5 IU/kg of PCC leads to partial restoration of thrombin generation, whereas 25 IU/kg does not. PCC 37.5 IU/kg may be insufficient for immediate full reversal of peak therapeutic rivaroxaban levels.
Introduction

Rivaroxaban is one of the direct oral factor Xa inhibitors approved for the prevention and treatment of thromboembolism.\(^1,2\) More than 8 million patients have received rivaroxaban worldwide since its registration\(^3\) on the basis of phase III trials comparing it with conventional anticoagulant therapy (heparin followed by vitamin K antagonist).

A concern of treatment with rivaroxaban is the lack of a specific reversal agent that can restore haemostasis in case of major bleeding. Although specific antidotes are under development, regulatory approval is dependent upon ongoing phase III clinical trials\(^4\) and those are not yet available in clinical practice. Meanwhile, patients with major bleeding on rivaroxaban are being managed with supportive measures and non-specific prohaemostatic agents, such as non-activated prothrombin complex concentrate (PCC).\(^5,6\) The latest European Heart Rhythm Association guidance document\(^6\) suggests that patients with life-threatening bleeding on rivaroxaban should receive PCC 25 IU/kg repeated once or twice, although that dosage has never been tested in humans\(^7,8\) and was partially or not effective in animal models.\(^9,10,11\)

Two recent in vivo studies showed that the anticoagulant effect of rivaroxaban measured by thrombin generation (Endogenous Thrombin Potential, ETP) was normalised within 15 minutes by 4-factor PCC 50 IU/kg Cofact (Sanquin Blood Supply, Amsterdam, the Netherlands)\(^7\) and within 6-8 hours by 4-factor PCC 50 IU/kg Beriplex P/N (CSL Behring, Marburg, Germany).\(^8\) However, after ETP normalisation an increase beyond baseline (e.g. pre-rivaroxaban) values in thrombin generation was observed, suggesting an “overshoot” that may lead to increased thrombosis risk.\(^7\) PCC administration for anticoagulation reversal in patients receiving vitamin K antagonists is associated with a low, but quantifiable risk of thromboembolic complications of 0.7-1.8% per administration.\(^12\) Therefore, if a dose-dependent effect of PCC occurs as observed in animal models\(^9,11\), using a lower dosage could reduce the risk of thromboembolic adverse events with a similar efficacy profile and lower costs.

The objective of our study was to assess the effect of a single administration of non-activated 4-factor PCC at the dosages of 37.5 and 25 IU/kg on the anticoagulant effect of steady-state therapeutic-dose rivaroxaban in healthy subjects.

Materials and Methods

Study design

The study was performed as a single-center, randomised, double-blind, placebo-controlled, crossover study at the Academic Medical Center of Amsterdam (the Netherlands).
All subjects provided written informed consent prior to screening and were subsequently randomised in blocks for the order of reversal method by an independent investigator. Rivaroxaban 15 mg was prescribed twice daily from day -2 to day 0: the final dose was taken on day 0 in the morning without food consumption, followed after 3 hours by either a single bolus of PCC 37.5 IU/kg, PCC 25 IU/kg, or a similar volume of saline (placebo). Subjects returned to the same prescription of rivaroxaban and crossed-over for the method of reversal after a wash-out period of at least 15 days (Figure 10.1).

Oral rivaroxaban (Xarelto, Bayer, Leverkusen, Germany) was used in the commercially available formulation\(^1,2\) and adherence to study medication was evaluated at each infusion by checking the blisters. Rivaroxaban was stored in the hospital pharmacy and provided at baseline. The dosage of 15 mg twice daily was chosen as it is the highest approved dosage for the initial treatment of acute venous thromboembolism\(^1,2,13,14\).

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, 280-700 IU of factor II, 140-400 IU of factor VII, 280-700 IU of factor X, 222-780 IU of protein C, 20-160 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 (59th version Korea, October 2008), the Medical Research Involving Human Subjects Act, 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 (NTR3559). Subjects were recruited by public advertisement located at the Academic Medical Center (Amsterdam) and received a financial reimbursement coherent with the time spent on the study.

Figure 10.1 | Study design

PCC infusion and blood withdrawal were performed at each session. Time points sampling: T=0 (pre-PCC), 15, 30 minutes, 1, 2, 4, 6, 24 hours after the end of PCC/saline infusion. Abbreviations: R, rivaroxaban; PCC, prothrombin complex concentrate (expressed as IU/kg).
Study population
Subjects were included after screening 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, hepatitis C, and human immunodeficiency virus serology, complete blood cells count, prothrombin time, and activated partial thromboplastin time. Exclusion criteria were a history of allergic reaction to blood products, a personal or family history of coagulation disorders, participating in any other investigational interventional study within the past 30 days. During the study, the consumption of nicotine, alcohol, and drugs was recorded.

Study procedures, sample collection and analysis
Blood samples were collected at screening visit, at baseline visit on day -2, 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 10.1).
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% 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 measures were used for study outcomes: endogenous thrombin potential (ETP; as a measure of thrombin generation) and prothrombin time. 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 generated in time during the test, 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. Prothrombin time was analyzed with an 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-FXa activity (Biophen DiXal anti-FXa assay, Hyphen, Neuville-sur-Oise, France) was used to verify adequate rivaroxaban 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.
Potential adverse events were recorded throughout the study: the final visit was scheduled after 20 days from the last infusion.
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 prothrombin time between T=0 (pre-PCC) and T=15 for each PCC dosage, 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.\textsuperscript{7}: improvement of ETP between T=0 and T=15. In that study\textsuperscript{7} pre-rivaroxaban 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 0.8, 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 (pre-PCC) and T=15 for each PCC dosage.

Data are presented as mean±SD or median (range), whatever was appropriate. For the immediate effect of 4-factor PCC, paired t-tests were performed. The effect over 24 hours 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 (IBM Corporation, Armonk NY, United States).

Results
Six subjects were enrolled and all completed the study. Subjects had a median age of 22 years (range 20-50 years), mean height of 180±7 cm, and mean weight of 83±14 kg. All subjects had normal baseline screening tests.

The mean PCC dosage per subject was 2.070±356 IU in the 25 IU/kg dosage session and 3,113±534 IU in the 37.5 IU/kg dosage session.

Effects of rivaroxaban on coagulation before PCC/placebo
After having received twice-daily rivaroxaban 15 mg from day -2 to day 0 (five doses), subjects showed statistically significant changes of both ETP and prothrombin time consistent across sessions (overall, p=0.001 for ETP and p=0.001 for prothrombin time), indicating an effect of rivaroxaban on those parameters.

Mean anti-FXa-derived rivaroxaban peak level at steady-state (day 0, T=0 pre-PCC) was 189±72 ng/mL (range 93-351) with no differences between groups (placebo: 190±52 ng/mL; PCC 25 IU/kg: 184±69 ng/mL; PCC 37.5 IU/kg: 193±102 ng/mL), suggesting rivaroxaban blood levels in line with data from literature.\textsuperscript{16}
Immediate effect outcomes

Fifteen minutes after infusion of PCC 37.5 IU/kg, ETP increased from 47±16% to 64±22% (p=0.03). After infusion of PCC 25 IU/kg, ETP modified from 53±11% to 59±12% (p=0.14; Figure 10.2; Table 10.1).

Fifteen minutes after infusion of PCC 37.5 IU/kg, prothrombin time decreased from 13.4±1.0 seconds to 11.5±0.8 seconds (p<0.0001). After infusion of PCC 25 IU/kg, prothrombin time decreased from 13.2±1.3 seconds to 11.6±0.9 seconds (p<0.001; Figure 10.3, Table 10.1).

No changes in both ETP and prothrombin time were observed over the first 15 minutes after infusion of saline in the placebo group (ETP: 45±17% to 42±6%, p=0.43; prothrombin time: 13.7±1.5 seconds to 13.7±1.3 seconds, p=0.88).

Sustained effect outcomes

ETP levels significantly differed over time between PCC 37.5 IU/kg and placebo during 24 hours after infusion (p=0.001), but not between PCC 25 IU/kg and placebo (p=0.31; Figure 10.2; Table 10.1). Mean ETP values reached pre-rivaroxaban levels after about 6 hours from the PCC 37.5 IU/kg dosage infusion and after 12-24 hours from the PCC 25 IU/kg dosage infusion.

Prothrombin time values were significantly different over time between each PCC dosage and placebo during 24 hours after infusion (p<0.0001; Figure 10.3, Table 10.1).

Adverse events

Three subjects experienced six adverse events, including two haematomas on the site of peripheral line placement, one recurrent epistaxis, two bleeding episodes after minor skin abrasions, and a vasovagal reaction while placing the peripheral line before infusion.
Figure 10.2 | Effect of rivaroxaban followed by two dosages of four-factor prothrombin complex concentrate or placebo on the endogenous thrombin potential (%)

Immediate effect outcome: differences between T=0 (pre-PCC) and T=15 (15 minutes after PCC infusion). ETP baseline (pre-rivaroxaban ETP): mean ETP value before rivaroxaban administration. Abbreviations: PCC, prothrombin complex concentrate; ETP, endogenous thrombin potential.

Figure 10.3 | Effect of rivaroxaban followed by two dosages of four-factor prothrombin complex concentrate or placebo on prothrombin time (seconds)

Immediate effect outcome: differences between T=0 (pre-PCC) and T=15 (15 minutes after PCC infusion). PT baseline (pre-rivaroxaban PT): mean prothrombin time value before rivaroxaban administration. Abbreviations: PCC, prothrombin complex concentrate; PT, prothrombin time.
Table 10.1 | Immediate and sustained effect outcomes

<table>
<thead>
<tr>
<th>ETP (%)</th>
<th>PCC 37.5</th>
<th>p</th>
<th>PCC 25</th>
<th>p</th>
<th>Placebo</th>
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<tbody>
<tr>
<td>Pre-rivaroxaban</td>
<td>92±14</td>
<td></td>
<td>98±13</td>
<td>97±16</td>
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<td>T=0</td>
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<td>45±17</td>
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<td>T=15 min</td>
<td>64±22</td>
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<td>59±12</td>
<td>42±6</td>
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</tr>
<tr>
<td>T=6 h</td>
<td>98±33</td>
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<td>86±10</td>
<td>60±10</td>
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<td></td>
</tr>
<tr>
<td>T=24 h</td>
<td>125±28</td>
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<td>103±18</td>
<td>83±14</td>
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<tr>
<td>T=0-24 h vs placebo</td>
<td>0.001</td>
<td>vs placebo</td>
<td>0.31</td>
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<table>
<thead>
<tr>
<th>PT (seconds)</th>
<th>PCC 37.5</th>
<th>p</th>
<th>PCC 25</th>
<th>p</th>
<th>Placebo</th>
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<tbody>
<tr>
<td>Pre-rivaroxaban</td>
<td>11.3±0.7</td>
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<td>T=0</td>
<td>13.4±1.0</td>
<td>&lt;0.0001</td>
<td>13.2±1.3</td>
<td>13.7±1.5</td>
<td>0.88</td>
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<tr>
<td>T=15 min</td>
<td>11.5±0.8</td>
<td></td>
<td>11.6±0.9</td>
<td>13.7±1.3</td>
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<tr>
<td>T=6 h</td>
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<td>11.2±0.8</td>
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<tr>
<td>T=24 h</td>
<td>11.3±0.7</td>
<td></td>
<td>11.0±0.5</td>
<td>11.8±0.7</td>
<td></td>
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<tr>
<td>T=0-24 h vs placebo</td>
<td>&lt;0.0001</td>
<td>vs placebo</td>
<td>&lt;0.0001</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

T=0 indicates the pre-PCC infusion time point.
Abbreviations: ETP, endogenous thrombin potential; PCC, prothrombin complex concentrate (expressed as IU/kg); PT, prothrombin time.

**Discussion**

In our crossover study of healthy subjects receiving therapeutic-dose rivaroxaban, we observed that 4-factor PCC 37.5 IU/kg generated a significant but modest increase of thrombin generation within 15 minutes, with pre-rivaroxaban ETP levels reached 6 hours after PCC infusion. PCC 25 IU/kg had no significant immediate or sustained effect on thrombin generation. Both dosages normalised prothrombin time within 15 minutes. Combined with the results of the prior 50 IU/kg PCC study, our findings suggest a dose-dependent reversal effect of PCC on the peak anticoagulant effect of rivaroxaban.

The discrepancy between effects of PCC on ETP and prothrombin time might be explained by the intrinsic characteristics of those tests: whereas ETP is a global haemostasis parameter and is influenced by many coagulation factors, prothrombin time is very sensitive to levels of factor VII present in PCC. ETP and prothrombin time as pharmacodynamic outcomes have been studied in in vivo and in vitro studies on direct oral factor Xa inhibitor reversal with PCCs and activated PCC. Although ETP and prothrombin time might represent
acceptable estimates of rivaroxaban anticoagulant effect and rivaroxaban concentrations\textsuperscript{20,21}, respectively, both have substantial inter-individual variability\textsuperscript{22,23,24} and it is uncertain to what extent they are relevant for the ultimate desired effect: restoration of effective haemostasis and improvement of the clinical outcome of bleeding. Nevertheless, this limitation currently applies to most reversal strategies for any form of anticoagulation. Whereas several studies have demonstrated that PCC leads to faster INR normalisation over fresh frozen plasma, none have convincingly demonstrated that faster INR normalisation per se improves clinical outcome of bleeding compared with fresh frozen plasma.\textsuperscript{25} Furthermore, the utility of anti-FXa activity as a tool for monitoring low-molecular weight heparins and predicting efficacy and safety outcomes is even less proven.

PCCs have been evaluated in a few consecutive patients with rivaroxaban-associated bleeding\textsuperscript{5} and preliminary findings of their in vivo effect mostly derive from animal models.\textsuperscript{9-11} In these studies, 4-factor PCC showed to be effective for reducing the mesenteric bleeding time in rats receiving PCC 50 IU/kg (but not after PCC 25 IU/kg infusion)\textsuperscript{9}, for preventing haematoma expansion in mice in a dosage of 50-100 IU/kg (but not after PCC 25 IU/kg infusion)\textsuperscript{11}, while it was not able to normalise the blood loss and the ear immersion bleeding time in rabbits (PCC 40 IU/kg).\textsuperscript{10} Results from our in vivo study are in line with findings from those animal studies with respect to the 25 IU/kg dosage.

Our results apply to this specific 4-factor PCC (Cofact, Sanquin Blood Supply, Amsterdam, the Netherlands) and different PCCs vary in composition with respect to individual coagulation factor levels which could have the effect on coagulation assays.\textsuperscript{8} The PCC dose is quantified by the amount of factor IX but non-activated PCCs can be formulated with either four (factors II, VII, IX and X) or three coagulation factors (factors II, IX and X) and also vary with respect to levels of protein C, protein S, antithrombin, and heparin. Nevertheless, consistent data have been recently presented in two in vivo studies that studied other 4-factor PCCs at different dosages (50-25-10 IU/kg, and 50 IU/kg, respectively) in healthy subjects receiving either edoxaban\textsuperscript{18} or apixaban.\textsuperscript{19}

While most of the current guidelines recommend PCC for vitamin K antagonist-associated major bleeding reversal\textsuperscript{26,27}, guidelines for rivaroxaban reversal\textsuperscript{6,28,29,30} are based on a few experimental data. Our in vivo study reinforces the rationale for the use of PCC for the management of rivaroxaban-associated major bleeding in clinical practice until approval of a specific antidote for factor Xa inhibitors.\textsuperscript{4,31} The administration of 4-factor PCC for rivaroxaban reversal in acute bleeding should be based on its dose-dependent effect on haemostatic parameters. For immediate and full reversal of peak levels of therapeutic-dose rivaroxaban administration of PCC 37.5 IU/kg is likely to be insufficient. Such a lower dose could theoretically still be effective with a longer duration since the last rivaroxaban dose (e.g. more than 12 hours).
Reference list


2. FDA. Highlights of prescribing information. 2013. Available at www.accessdata.fda.gov


