Study of Octaplex dosing accuracy: An in vitro analysis

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Summary

Prothrombin complex concentrates (PCC) are recommended for urgent warfarin reversal. However, disagreement exists regarding the proper dosing strategy (i.e. fixed vs. weight-based). We measured the in vitro effect of PCC dosing on international normalised ratio (INR) and factor activity. Plasma from warfarin-anticoagulated patients with stable INRs was collected. PCC doses of 1,000, 2,000 and 3,000 IU were added to the samples, and INR and factor activity were analysed before and after PCC. Twenty-three of thirty subjects enrolled had complete data for analysis. INRs were below 1.5 in all samples post-1,000 IU, and decreased further with subsequent doses (p<0.001). Factors II, VII, and X increased with consecutive doses (p<0.01). Linear correlation was seen between INR and factors II, VII and X. Factor IX did not increase consistently nor show correlation with INR reversal. Weight-based dosing was then estimated; INRs were all <1.2 (0.9–1.2) and activity >0.50 IU for factors II, VII and X (0.96–1.52, 0.51–1.45 and 0.81–1.38, respectively). Factor IX did not uniformly correct above 0.50 IU (0.31–1.31). We confirm in vitro that 1,000 IU of Octaplex® is able to correct INR to <1.5 but factors were not uniformly >0.50 IU until 2,000 IU, and not >1.00 IU until 3,000 IU. This suggests that INR correction alone may not accurately reflect factor activity, and lends support for weight-based dosing.

Keywords

Thrombosis, vitamin K-dependent factors, factor concentrates

Introduction

Warfarin, a vitamin K antagonist, is the most commonly used oral anticoagulant today, with an estimated usage in 1–2% of adults (1). Its diverse therapeutic indications include stroke prevention in atrial fibrillation, venous thromboembolism treatment and prophylaxis (i.e. deep-vein thrombosis and pulmonary embolism), mechanical heart valves, and cerebrovascular disease (2). Safe and effective use of warfarin must be regularly monitored using the international normalised ratio (INR). Despite this, it is estimated that patients’ INRs are within therapeutic range approximately only 60% of the time (3), and this may even represent an artificially elevated value due to increased surveillance in the context of clinical trials (4). Subtherapeutic anticoagulation puts patients at risk of thromboembolic events, while supratherapeutic INRs increase the risk of haemorrhagic complications such as life-threatening intracranial, gastrointestinal and genitourinary bleeding (1, 5, 6). Periprocedural management of patients on warfarin must focus on both safe reversal of anticoagulation and minimisation of associated thrombotic risk (7). Indeed, patients treated with vitamin K antagonists have an approximate 3–7% annual risk of requiring rapid anticoagulation reversal to treat major bleeding events or in preparation for urgent surgery (8).

Interventions for warfarin reversal vary depending on the degree of INR elevation, as well as on the presence and severity of bleeding. The INR remains the metric for assessment of correction of warfarin-associated coagulopathy despite its lack of validation for use in reflecting restored haemostasis. Simply withholding warfarin and/or reducing the weekly dose may be sufficient to correct a supratherapeutic INR in the absence of haemorrhage (9). Vitamin K may be administered at various doses, depending on the INR and presence of bleeding, but its action is not immediate (9, 10). When more urgent INR reversal is needed, active factor replacement is required, usually through transfusion of fresh-frozen plasma (FFP). However, due to its multiple potential complications, new more purified, safe and rapid options are becoming available (10–12). Octaplex® (Octapharma Canada Inc., Scraborough, ON, Canada) is a four-factor prothrombin complex concentrate (PCC) available in Canada since 2007. It is a human plasma-derived product that has undergone solvent detergent treatment and nanofiltration for viral, bacterial and parasite inactivation and removal. Approved uses include the correction of acute bleeding and perioperative prophylaxis in acquired vitamin K-dependent factor deficiency, or in cases of warfarin overdose when rapid correction is required (13). It contains lyophilised forms of factors II, VII, IX, X, as well as protein C and protein S. The
Canadian National Advisory Council (NAC) in 2008 recommended a single fixed dose of 1,000 IU (based on factor IX activity) for immediate INR reversal. Updated NAC guidelines from June 2011 recommend a single fixed dose of 1,000 IU for an INR < 3.0, 2,000 IU for an INR between 3.0 – 5.0, and 3,000 IU for an INR > 5.0 (14). PCC dosing is still a matter of debate and some centres use weight-based dosing, citing improved results over fixed-dose protocols (15). Furthermore, the relationship between the post-PCC INR and actual factor activity remains unclear (16), as does the minimum factor activity required for proper haemostasis (17).

We conducted the present study with two objectives: 1) to measure the in vitro effect of incremental doses of Octaplex on INR normalisation and clotting factor repletion in the plasma of subjects anticoagulated with warfarin; and, 2) to characterise the relationship between the INR and actual factor activity correction.

Materials and methods

Study participants

Adult patients (age ≥18 years) attending the Thrombosis Clinic at the London Health Sciences Centre, a tertiary care centre in London, Ontario, Canada, were invited to participate in the study. Patients were eligible for inclusion if they were taking warfarin and had stable therapeutic INRs (range 2.0 – 3.5) for at least four weeks prior to inclusion in the study. Exclusion criteria included concurrent use of other anticoagulants, hereditary coagulopathy, hepatic impairment, or if they did not provide a signed informed consent. The study was approved by the Research Ethics Board at the University of Western Ontario (#16801E).

Laboratory methods

Plasma samples were collected in 3.2% (0.109 m) sodium citrate polypropylene tubes (BD Vacutainer; Becton Dickinson, Franklin Lakes, NJ, USA), centrifuged at 1,500 x g for 20 minutes at room temperature, and aliquots were frozen at −70°C until tested. Plasma volumes (PV) for each patient were calculated using the formula recommended by the International Council for Standardization in Hematology (18). Body surface area (BSA) was calculated using the Dubois formula.

Prothrombin times and INRs were measured using a recombinant human thromboplastin with an international sensitivity index of approximately 1.0 (HemosIL RecombiPlasTin; Instrumentation Laboratory, Bedford, MA, USA) on an ACL TOP instrument (Instrumentation Laboratory). One-stage clotting assays were used to calculate factor activity. Factor II, VII and X activities were analysed using prothrombin time (PT)-based assays, with the reagents Thromborel S (Siemens Healthcare Diagnostics; Marburg, Germany) and plasma deficient in factors II, VII and X, respectively (Precision Biological; Dartmouth, NS, Canada). Factor IX activity was analysed using an activated partial thromboplastin time (aPTT)-based assay, using a synthetic phospholipid reagent with micronised silica as the activator (APTT-SR, Instrumentation Laboratory). Factor assays were done on an ACL 9000 coagulation analyzer (Instrumentation Laboratory). Internal and external quality controls are routinely used in our laboratory as per current provincial standards. Plasma samples were analysed for INR and factor II, VII, IX and X activities, both at baseline and following ex vivo incubation with a volume of Octaplex simulating 1,000, 2,000 and 3,000 IU. To calculate the simulated Octaplex test dose volume we used the formula

\[ TD (\mu l) = \frac{OD (ml) \times Va (\mu l)}{Vp (ml)} \]

where TD = test dose volume (μl), OD = simulated Octaplex dose volume (ml) for 1,000, 2,000 and 3,000 IU (40, 80, and 120 ml, respectively), Va = sample aliquot volume (μl), and Vp = the calculated plasma volume (ml). The Octaplex aliquot calculation protocol was first validated using the plasma from a severe haemophilia B patient and ensuring the expected amount of factor IX was retrieved.

Data analysis

Data are presented as medians and interquartile ranges (IQR). Given the relatively small sample size, results were tested for normality using Skewness and Kurtosis analysis. This analysis showed that data was not normally distributed, and therefore post-treatment INR and factor activity levels were compared to the untreated baseline values using the Friedman’s test for related samples. Individual group comparisons were done using the Wilcoxon’s signed ranks test. Correlations were evaluated using the Spearman’s correlation coefficient. Two-sided p values of ≤0.05 were considered as statistically significant. Data were analysed using Microsoft Excel 2007 (Microsoft Corp. Redmond, WA, USA) and PASW 18 (SPSS Inc., Chicago, IL, USA).

Results

Thirty participants were recruited between October 1, 2010 and January 31, 2011. Complete data and sufficient plasma samples were available for 23 patients which were included in the final analysis. There were 12 women and 11 men with a median age of 59 years (IQR 45.5–70.5). The median weight was 84 kg (IQR 79–94) and BSA was 1.96 m2 (IQR 1.875–2.105).

Overall, there were differences between baseline and post-Octaplex INR values and factor activities when assessed with the Friedman’s test (p< 0.001 for all comparisons). Factor activities (IU) and INR values at baseline and post-Octaplex addition are shown in Table 1. INR values corrected to 1.5 or less in all samples after ex vivo incubation with 1,000 IU of Octaplex. Comparisons of
250 Patriquin et al. Study of Octaplex dosing accuracy

baseline INRs to post-Octaplex INRs were statistically significant at each incremental dose increase (p < 0.001 for all comparisons). Similar significant changes were noted when comparing baseline factor activity for factors II, VII, IX and X to activity following incubation with the Octaplex doses (p < 0.001). However, factor IX activity post-3,000 IU was not different from factor IX activity post-1,000 IU (p = 0.637) and was significantly lower than factor VII activity post-3,000 IU compared with the Octaplex doses (p < 0.01). However, factor IX activity for factors II, VII, IX and X to activity following incremental increases available. As such, the simulated weight-based dosing was as follows: ≤ 40 kg = 1,000 IU; 41–80 kg = 2,000 IU; and, ≥ 81 kg = 3,000 IU (recommended maximum dose) (13). Based on our simulated weight-based dosing guidelines, six of our 23 subjects had weights between 41 and 80 kg and therefore would have received 2,000 IU of Octaplex. The remainder of the enrolled subjects had weights ≥ 81 kg, correlating with a simulated dose of 3,000 IU. After ex vivo incubation with an ideal weight-based Octaplex dose, we observed increases in all factor activities and normalisation of the INR, and all changes were statistically significant (Table 1). The INR corrected to < 1.2 in all samples (range: 0.90–1.20), and factors II, VII and X levels increased to > 0.50 IU in all samples as well (ranges: 0.96–1.52, 0.51–1.45, and 0.81–1.38, respectively). However, though factor IX levels increased significantly post-ideal weight-based Octaplex, not all samples corrected to at least 0.50 IU (range: 0.31–1.31). However, using our weight-based regimen, the 20th percentile of factor IX activity based on weight was 0.51 IU and the 15th percentile 0.47 IU. This means that for 15% of the samples the FIX activity was of 0.47 IU or less. Again, the correlation between INR and factor levels was linear for all but factor IX (Table 2).

Table 1: Factor activities and INR values in samples of anticoagulated patients before and after in vitro addition of Octaplex.

<table>
<thead>
<tr>
<th>Factor</th>
<th>Baseline</th>
<th>Post - 1,000 IU</th>
<th>Post - 2,000 IU</th>
<th>Post - 3,000 IU</th>
<th>Post - weight-based dose</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Median</td>
<td>Interquartile range</td>
<td>Median</td>
<td>Interquartile range</td>
<td>Median</td>
</tr>
<tr>
<td>Factor II (IU)</td>
<td>0.30</td>
<td>0.25</td>
<td>−0.34</td>
<td>0.65&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.58</td>
</tr>
<tr>
<td>Factor VII (IU)</td>
<td>0.30</td>
<td>0.25</td>
<td>−0.43</td>
<td>0.55&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.48</td>
</tr>
<tr>
<td>Factor IX (IU)</td>
<td>0.42</td>
<td>0.35</td>
<td>−0.51</td>
<td>0.59&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.49</td>
</tr>
<tr>
<td>Factor X (IU)</td>
<td>0.15</td>
<td>0.13</td>
<td>−0.19</td>
<td>0.55&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.49</td>
</tr>
<tr>
<td>INR</td>
<td>2.30</td>
<td>2.10</td>
<td>−2.70</td>
<td>1.30&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.20</td>
</tr>
</tbody>
</table>

IU, international units; INR, international normalised ratio. <sup>a</sup>p-value for comparison vs. baseline < 0.001. <sup>b</sup>p-value for comparison vs. 1,000 IU < 0.001. <sup>c</sup>p-value for comparison vs. 1,000 IU < 0.01. <sup>d</sup>p-value for comparison vs. 2,000 IU < 0.001.

Finally, we looked at INR and factor correction following a hypothetical ideal weight-based dosing of Octaplex. This was estimated using our own centre’s Blood Transfusion Laboratory guidelines, in which 500 IU of Octaplex is given for approximately every 20 kg of weight, to simulate giving a patient approximately 25–50 IU/kg of Octaplex. For this study, we had only 1,000 IU increments available. As such, the simulated weight-based dosing was as follows: ≤ 40 kg = 1,000 IU; 41–80 kg = 2,000 IU; and, ≥ 81 kg = 3,000 IU (recommended maximum dose) (13). Based on our simulated weight-based dosing guidelines, six of our 23 subjects had weights between 41 and 80 kg and therefore would have received 2,000 IU of Octaplex. The remainder of the enrolled subjects had weights ≥ 81 kg, correlating with a simulated dose of 3,000 IU. After ex vivo incubation with an ideal weight-based Octaplex dose, we observed increases in all factor activities and normalisation of the INR, and all changes were statistically significant (Table 1). The INR corrected to < 1.2 in all samples (range: 0.90–1.20), and factors II, VII and X levels increased to > 0.50 IU in all samples as well (ranges: 0.96–1.52, 0.51–1.45, and 0.81–1.38, respectively). However, though factor IX levels increased significantly post-ideal weight-based Octaplex, not all samples corrected to at least 0.50 IU (range: 0.31–1.31). However, using our weight-based regimen, the 20th percentile of factor IX activity based on weight was 0.51 IU and the 15th percentile 0.47 IU. This means that for 15% of the samples the FIX activity was of 0.47 IU or less. Again, the correlation between INR and factor levels was linear for all but factor IX (Table 2).

Table 2: Correlation between factor and INR values in samples of anticoagulated patients before and after in vitro addition of Octaplex.

<table>
<thead>
<tr>
<th>Factor</th>
<th>Spearman’s rho</th>
<th>p</th>
<th>Spearman’s rho</th>
<th>p</th>
<th>Spearman’s rho</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Factor II</td>
<td>-0.784</td>
<td>&lt;0.001</td>
<td>-0.860</td>
<td>&lt;0.001</td>
<td>-0.636</td>
<td>0.001</td>
</tr>
<tr>
<td>Factor VII</td>
<td>-0.531</td>
<td>0.009</td>
<td>-0.749</td>
<td>&lt;0.001</td>
<td>-0.584</td>
<td>0.003</td>
</tr>
<tr>
<td>Factor IX</td>
<td>-0.523</td>
<td>0.010</td>
<td>-0.537</td>
<td>0.008</td>
<td>-0.091</td>
<td>0.680</td>
</tr>
<tr>
<td>Factor X</td>
<td>-0.425</td>
<td>0.043</td>
<td>-0.507</td>
<td>0.014</td>
<td>-0.124</td>
<td>0.573</td>
</tr>
</tbody>
</table>

IU, international units; INR, international normalised ratio.
Discussion

Rapid correction of warfarin-associated coagulopathy is paramount in patients with active bleeding or requiring urgent surgical intervention. However, clinical evidence is far from robust regarding safe perioperative factor levels that will ensure proper haemostasis. Expert consensus in haemophilia management recommends > 0.50 IU for dental procedures, 0.60–1.00 IU for general surgery, and at least 0.80–1.00 IU for orthopaedic surgery or clinically significant bleeds (19). In contrast, little is known regarding target factor levels in patients with multiple-factor deficiencies (e.g. warfarin therapy, liver disease), and cannot be directly extrapolated from the haemophilia literature (15). Our study confirms the efficacy of Octaplex 1,000 IU in correcting the INR to target post-treatment levels of less than 1.5 (20) in vitro in 100% of samples. For each vitamin K-dependent factor, the mean factor activity level was ≥0.50 IU following incubation with a 1,000 IU dose; however this was not the case for every individual sample. As was expected, the mean activity of factors II, VII and X increased linearly with incremental doses, and all samples had activity levels ≥0.50 IU by the 2,000 IU dose. In contrast, factor IX activity did not show a linear correlation with INR, despite the fact that PCC dosing is based specifically on factor IX activity (13, 15). This could be explained by the fact that factor IX activity was quantified using an aPTT-based assay, whereas the other factors were measured using an assay based on the PT. It is possible that the heparin present in the PCC could have interfered with the factor IX assay. Unfortunately, we did not have enough samples to corroborate this hypothesis.

In our study, median activity of approximately 0.80 IU for factors II, VII and X was not reached until the 2,000 IU dose of Octaplex, and activity ≥1.00 IU not until 3,000 IU. This is potentially concerning given that previous guidelines recommend the 1,000 IU dose for all situations, except for patients with extreme weights and/or INR in consultation with a haematologist (13). Updated guidelines from the NAC have been changed to recommend dosing based on pretreatment INR (14). Furthermore, using a weight-based dosing strategy for Octaplex, Riess et al. reported a median dose of 3,000 IU (range 900 to 8,000 IU) and found that 88% (52 of 59) achieved the primary endpoint of an INR less than or equal to pre-defined targets (21). The weight-based doses described by Riess et al. are well above those currently recommended by the NAC (13, 21). Despite the fact that 1,000 IU of Octaplex consistently reduced the INR to ≤1.5, the activity of replaced factors may not necessarily be sufficient for surgical haemostasis. As in our study, we tested plasma from stably anticoagulated patients with INRs in the range 2.0–3.5, the majority of whom would have received a fixed 1,000 IU dose irrespective of body size. In this group, 10 of 23 participants (43%) would have had at least one vitamin K-dependent factor level below 0.50 IU. Using the weight-based regimen described, all patients would have achieved factor levels (except factor IX) of 0.60 to 1.00 IU. As such, as has been argued by others, individualised PCC dosing is more appropriate for effective warfarin reversal (15). PCCs are not without potential side-effects, as was highlighted by a recent meta-analysis that reported a 1.8% incidence of thromboembolism in patients treated with four-factor PCCs (22). Increased accuracy in PCC dosing may treat/prevent bleeding complications while minimising the risk of thromboembolic events.

Several questions still remain unanswered: 1) What level of factor activity is safe to undergo surgery, especially with deficiencies in multiple factors? 2) Though changes in INR and factor activity were statistically significant, what is the incremental clinical impact? 3) Does the standard Octaplex dose actually treat the patient, or just the INR, potentially giving physicians a false sense of security? 4) What is the appropriate dose for patients with supratherapeutic INRs? Future studies should help to confirm our current findings and assess appropriate dosing in vivo. Indeed, a weakness of our study is that its in vitro design is somewhat artificial. We acknowledge that testing INR and factor activity pre- and post-PCC in patients presenting with bleeding or for urgent procedures would allow for greater clinical application but this was not practical at our institution. We also recognise that the INR has not been validated to indicate effective correction of haemostasis post-PCC, but it is still nevertheless used by many as such a marker of bleeding risk, especially in the perioperative setting (23). This paper further highlights the variable relationship between INR and factor activity.

In conclusion, our study showed that a single dose of Octaplex 1,000 IU corrects the INR to the targeted post-treatment level of ≤1.5, but also that such corrections do not directly correlate with factor activity repletion, at least with lower PCC doses. As well, we used plasma from appropriately anticoagulated patients under controlled conditions. We anticipate even greater discrepancies in vivo when PCCs are used to correct supratherapeutic INRs in patients with major bleeding or in need of urgent surgery. Future studies in these patient populations, characterising these INR-factor relationships, will further inform clinicians regarding both anticipated factor activity post-PCC, as well as the utility of the INR.

What does this paper add?

- Bleeding is a not uncommon complication of warfarin therapy.
- Management of a bleeding patient with an elevated international normalised ratio (INR) requires factor replacement therapy in addition to vitamin K and source control.
- Factor replacement with prothrombin complex concentrates (PCCs) is more effective than fresh frozen plasma at correcting INR, and is associated with significantly fewer side effects.

What is known about this topic?

- The relationship of the magnitude of INR correction and factor activity following ex vivo incubation with different doses of PCC.
- Achieving a post-PCC INR below 1.5 may not necessarily correlate with appropriate activity levels of the replaced vitamin K-dependent factors.
- Dosing of PCCs based on weight is able to more accurately and reproducibly correct the INR and elevate factor activity to appropriately safe levels.

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Thrombosis and Haemostasis 107.2/2012
in predicting haemostasis. Until then, it remains evident from our study and others that weight-based PCC dosing is more accurate and effective at correcting warfarin-associated coagulopathy.

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Conflict of interest
None declared.

References