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Full Length Article

Heparin supplement counteracts the prohemostatic effect of prothrombin complex concentrate and factor IX concentrate: An in vitro evaluation

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ABSTRACT

Introduction: Coagulation factor concentrates like factor IX (FIX) and prothrombin complex concentrate (PCC) can contain anticoagulant substances that may hamper their procoagulant effectiveness in the treatment of hemophilia B or reversal of oral anticoagulation, as well as the laboratory assessment thereof. The aim of the present study was to evaluate the influence of anticoagulant heparin supplement on the prohemostatic potential of different PCCs and FIX concentrates.
Materials and methods: Prohemostatic potential was evaluated in vitro employing PT/aPTT, thrombography (TGA) and thromboelastography (TEG) with FIX deficient plasma, vitamin K antagonist (VKA)-anticoagulated plasma and plasma anticoagulated with rivaroxaban.
Results: Most PCCs contained heparin, while heparin was detected in 1 out of 4 examined FIX concentrates. All heparin-containing clotting factor concentrates showed severely hampered prohemostatic effects when therapeutic doses were added to anticoagulated plasmas. Upon heparin removal, comparable prohemostatic effects were observed. Of importance is the notion that the anticoagulant effect of heparin was enhanced by rivaroxaban, resulting in a 7 fold increased PT sensitivity towards heparin in the presence of 500 μg/L rivaroxaban.
Conclusions: Compositional differences between clotting factor concentrates should be taken into account. Therapeutic levels of heparin may be co-infused when treating emergency bleeds with high prohemostatic drug doses, particularly those recommended in the reversal of non-VKA anticoagulants such as rivaroxaban by PCC. Given the relative short half-life of heparin compared to vitamin K-dependent clotting factors, an anticoagulant heparin effect shortly after concentrate infusion should be considered clinically and while interpreting laboratory coagulation parameters.

1. Introduction

Clotting factor concentrates are clinically widely used drugs for the treatment and prevention of clotting factor deficiency-related hemorrhage. Among them are factor IX (FIX) concentrate and prothrombin complex concentrate (PCC). FIX concentrate is used for the treatment of Hemophilia B [1, 2]. PCC is used for the rapid reversal of VKA-anticoagulation [3, 4] and might be useful in situations requiring rapid reversal of anticoagulation by direct thrombin and FXa inhibitors [5] as well as in the treatment of trauma-induced coagulopathy [6] or hemorrhagic complications in patients with liver disease [7]. FIX concentrates can either be of human plasma origin or purified from the culture supernatant of mammalian cell lines transfected with human FIX cDNA, so called recombinant FIX. PCC is a mixture of partially purified human plasma vitamin K-dependent coagulation factors (FII, FVII, FIX, FX, protein C, protein S) and currently no recombinant products are marketed. PCCs are usually supplemented with antithrombin and most but not all PCCs also contain heparin supplement. These anticoagulant supplements are added during manufacturing to eliminate the enzymatic activity of any activated clotting factor in the product. FIX concentrates are not supplemented with antithrombin, but a few brands do contain heparin.

PCCs were introduced in clinical practice in the 1970's and were initially intended for the treatment of hemophilia B. FIX concentrates were introduced in the 1980's. A major point of concern in the early days of PCC utilization were thrombotic complications associated with the use of these concentrates [8]. Thrombogenicity has been attributed to the presence of procoagulant phospholipid contaminant and activated coagulation factors in the product [9–13]. Thromboembolic complications associated with PCC treatment may also be related to zymogen overload in the circulation, particularly that of prothrombin, and to relatively low levels of the anticoagulant proteins C and S [12, 14]. To minimize

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thrombogenicity, heparin and antithrombin were advised as supplements in PCC together with a balanced clotting factor composition mimicking relative in vivo molar ratios [15]. With the inclusion of coagulation inhibitors and other manufacturing improvements and the implementation of thrombogenicity release tests, today's PCCs may be considered safer than earlier products [16]. For lower risk hemophilia B treatment, high purity plasma-derived and recombinant FIX concentrates were developed [17–23].

While during the introduction and further development of PCCs and FIX concentrates attention has been paid to the thrombogenicity of these concentrates, the potentially adverse effect of heparin supplement on the in vivo procoagulant efficacy of the drug was largely ignored. An in vitro effect, on the other hand, has been recognized and has led to prescriptions in the European Pharmacopoeia to neutralize heparin when performing release testing on heparin containing clotting factor concentrates (i.e. potency determinations, presence of activated coagulation factors) [24–26]. Influence of heparin has also been observed while performing thromboelastography (TEG), rotational thromboelastometry (ROTEM) and thrombin generation tests [6, 27–29]. One of the first reports pointing to this issue was from Takeyama et al. in 2007, showing reduced procoagulant activity of the PCC Proplex® in ROTEM when >1.25 IU/mL was added to FIX deficient reconstituted blood. Influence of heparin was suggested by the notion of restored procoagulant activity in the presence of the heparin antagonist protamine sulfate [27]. Another survey using ROTEM showed inhibition of fibrin clot formation by a heparin-containing PCC (Prothromplex®) but not by heparin free PCC (Cofact®) when 0.3–1.25 IU/mL were added to normal plasma. Also in that study, the hemostatic effect of heparin containing PCC was restored in the presence of protamine sulfate [28]. Using the thrombin generation assay and employing diluted normal plasma as a model for coagulopathy, Grottke and co-workers observed that no or low heparin containing PCCs (Cofact®, Beriplex®) restored coagulation while PCC's with relatively high heparin levels (Octaplex®, Prothromplex®) exhibited an anticoagulant effect [6]. Similar observations were recently reported for PCC spiked plasma and whole blood from outpatients with ventricular assist devices receiving phenprocoumon when analyzed by TEG and thrombin generation assays [29]. Studies on the usefulness of PCCs as reversal agent for the non-vitamin K antagonist direct oral anticoagulants (DOAC) has generated controversy that may relate to the employed methods to determine reversal as well as to compositional differences between PCC preparations studied [30]. For dabigatran reversal assessment by thrombin generation, a disturbing influence of heparin supplement was recently postulated [31].

The aim of the present study was to compare the hemostatic effect of different PCC and FIX preparations in in vitro model systems, with special reference to the presence of heparin. We not only aimed at the indicated use of PCCs and FIX concentrates to correct VKA-induced anticoagulation and FIX deficiency related hypocoagulation, but we also focused on the in vitro reversal of rivaroxaban anticoagulation.

2. Materials and methods

2.1. Pooled normal and hypocoagulant plasma

One batch of normal plasma consisted of a pool of 32 single donor plasmapheresis units (Sanquin, Amsterdam, The Netherlands). Alternatively, 2 batches of pooled normal plasma were prepared from blood from 10 apparently healthy volunteers, collected into siliconized tubes containing trisodium citrate (0.129 M, 1/9 *v*/*v*). FIX deficient plasma (single batch) was from Hematologic Technologies Inc., Essex Junction, Vermont, USA. One batch of VKA-anticoagulated plasma (INR 2.7) consisted of a pool of 10 single donor units from patients on Coumadin (George King Bio-Medical Inc., Overland Park, Kansas, USA). Pooled plasma was aliquoted and stored at -80 °C. Rivaroxaban-anticoagulated plasma was prepared by spiking 1 mL normal pooled plasma with 10 μl rivaroxaban dilutions in buffered saline (50 mM Tris, 150 mM NaCl, pH 7.5, 0.5% HSA). Rivaroxaban was kindly provided by Dr. J Harenberg [32].

2.2. Clotting factor concentrates

The following plasma-derived 4-factor PCCs were examined: Cofact® (Sanquin), Beriplex® (CSL Behring, King of Prussia, Pennsylvania, USA), Octaplex® (Octapharma AG, Lachen, Switzerland), Prothromplex® (Baxter International Inc., Deerfield, Illinois, USA). Plasma-derived FIX concentrates were Nonafact® (Sanquin), Mononine® (CSL Behring) and Octanine® (Octapharma). Recombinant FIX concentrate (Benefix®) was from Pfizer Inc. (New York City, New York, USA). All concentrates are supplied as freeze-dried products. Pilot experiments revealed differences in prohemostatic potential between PCCs of different brands that may relate to differences in salt composition. To compensate for formulation differences, the freeze-dried PCC preparations were dissolved in H_2O at a 4-fold concentrated solution with respect to the manufacturer's instructions and dialyzed against citrate buffered saline (10 mM trisodium citrate, 150 mM NaCl, pH 7.4) o/n at 4 °C. All freeze-dried FIX concentrates were dissolved in H_2O as indicated on the package inserts. Dissolved concentrates were aliquoted and stored at -80 °C until further use. For each concentrate, a single vial from a single lot (batch) was used for all experiments reported in this paper. At the day of dissolving, all lots had not exceeded the stated date of expiry. They were all well in-data (5–30 months prior to expiry), except Nonafact that was dissolved 17 days prior to expiration.

2.3. FIX and heparin assays

Prohemostatic potential of the different clotting factor concentrates was examined based on their FIX activity content. FIX activity was evaluated on the Sysmex CA-7000 analyzer using a one stage clotting assay with Actin FSL (Siemens Healthcare, Erlangen, Germany). FIX activity was determined against the European Pharmacopoeia reference standard for FIX concentrates. FIX antigen was determined by an in-house ELISA against reference plasma that was calibrated using the WHO FIX standard [33]. Heparin was measured against a calibration curve prepared from the 6th international standard for unfractionated heparin (NIBSC, Hertforshire, UK) using the Biophen Heparin (AT+) anti-Xa chromogenic method from Hyphen Biomed (Neuville-Sur-Oise, France).

2.4. Removal of heparin from clotting factor concentrates

Heparin was removed from clotting factor concentrates by using the anionic exchange resin ecteola cellulose (coarse grade; Sigma-Aldrich, St Louis, MA, USA). Pilot experiments revealed that 1.25 mg ecteola cellulose was required to neutralize 1 IU unfractionated heparin (from porcine mucosa, NIBSC). Clotting factor concentrates were diluted with citrate buffered saline to 12 IU/ml FIX in order to lower the heparin concentration in the starting material. Subsequently, 0.5 ml of the diluted concentrate was incubated end over end with 10 mg ectoela cellulose for 50 min at room temperature. As a control, 0.5 ml diluted concentrate was simultaneously processed without ecteola cellulose. Following centrifugation for 10 min at 1500 rpm, the supernatant was removed and used for further analysis and experimentation. The FIX ELISA revealed no loss of FIX protein when employing this method. Upon treatment, the heparin content was ≤0.01 IU/IU FIX.

2.5. Procoagulant activities of clotting factor concentrates assessed in normal plasma

Clotting factor concentrates were pre-diluted with citrate buffered saline to 12 IU/ml FIX. Subsequently, 75 μl of these dilutions were