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Correlation of Coagulation Markers and 4F-PCC-Mediated Reversal of Rivaroxaban in a Rabbit Model of Acute Bleeding

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ABSTRACT

Introduction: Rivaroxaban is an oral, selective direct factor Xa inhibitor approved for several indications in patients at risk of thrombotic events. One limitation of its clinical use is the lack of data pertaining to its reversal in situations where urgent response is critical (e.g. acute bleeding events or emergency surgery).
Materials and methods: This study assessed the effectiveness of a four-factor prothrombin complex concentrate (4F-PCC; Beriplex®/Kcentra®) for the reversal of rivaroxaban-associated bleeding in an *in vivo* rabbit model, and evaluated the correlations between *in vitro* coagulation parameters and haemostasis *in vivo*.
Results: Administration of single intravenous doses of rivaroxaban (150–450 μg/kg) resulted in increased and prolonged bleeding following standardised kidney incision. Pre-incision treatment with 4F-PCC (25–100 IU/kg) resulted in a dose-dependent reversal of rivaroxaban (150 and 300 μg/kg)-associated increases in time to haemostasis and blood loss; no reversal was seen at the highest rivaroxaban dose (450 μg/kg). Of the *in vitro* biomarkers tested, thrombin generation and whole-blood clotting time correlated well with *in vivo* measures of 4F-PCC-mediated effects. Thrombin generation was highly reagent-dependent, with the assay initiated using the phospholipid-only reagent being the most predictive of effective haemostasis *in vivo*.
Conclusions: In summary, in a rabbit model of acute bleeding, treatment with 4F-PCC reduced bleeding to control levels following rivaroxaban 150 μg/kg and 300 μg/kg administration.

Introduction

Acting at the point of convergence of the intrinsic and extrinsic coagulation pathways, factor Xa (FXa) is a serine protease that plays a key role in the coagulation cascade by converting prothrombin to thrombin [1,2]. These features of FXa make it a target for the development of new anticoagulants, and in recent years several new direct oral FXa inhibitors have been licensed for a number of indications related to the prevention of thrombotic events. Rivaroxaban (Bayer HealthCare AG, Leverkusen, Germany), one of these FXa inhibitors, is currently approved for the prevention of stroke and systemic embolism in patients with non-valvular atrial fibrillation (AF), the treatment of deep vein thrombosis (DVT) and pulmonary embolism (PE), the prevention of recurrent DVT and PE, DVT prophylaxis in patients undergoing hip or knee surgery, and the prevention of atherothrombotic events after an acute coronary syndrome [3].

While newer oral anticoagulants (NOACs), such as rivaroxaban, do not require routine monitoring owing to their predictable pharmacokinetic and pharmacodynamic profiles, this may still be helpful in emergency situations (e.g. prior to an emergency surgery or in the case of a life-threatening bleeding event), when assessment of bleeding risk and subsequent rapid anticoagulation reversal is critical. Clinical studies of rivaroxaban demonstrated a good correlation between rivaroxaban plasma levels and inhibition of FXa activity or prolongation of the prothrombin time (PT) [4–6]. However, other standard coagulation assays (e.g. activated partial thromboplastin time [aPTT], thrombin time [TT], ecarin clotting time [ECT]) may be of less relevance for monitoring the anticoagulant effects of rivaroxaban [6,7].

Despite the advantages offered by rivaroxaban over traditional anticoagulants, bleeding complications (including spontaneous and perioperative bleeding) remain a significant concern for clinicians. This concern is further compounded by the lack of validated reversal strategies for direct FXa inhibitors at present [8,9]. Based on data from clinical trials that compared NOACs and warfarin in patients with AF, the rates of major bleeding events appear to be in a similar range in NOAC- or warfarin-treated patients (2.1–3.6% *versus* 3.1–3.4%, respectively)

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[10–13]. Limited data are available regarding the incidence of NOAC-associated bleeding events in real-world clinical practice. Taking into account the growing number of patients receiving NOACs, there is an urgent need for strategies to manage these challenging clinical situations. Owing to the relatively short half-lives of NOACs, interruption of treatment may be sufficient in most non-emergency situations (e.g. prior to elective surgery or in patients with minor bleeding) [14, 15]. However, in cases of life-threatening bleeding, or when urgent anticoagulation reversal is required (e.g. prior to emergency surgery), current guidelines suggest administering haemostatic agents, such as recombinant activated factor VII (rFVIIa) or activated/non-activated prothrombin complex concentrates (PCCs) [14–21]. Owing to their potentially lower prothrombotic potential, non-activated PCCs may be preferable to rFVIIa and activated PCCs [21,22].

The aim of this study was to evaluate the potential of a non-activated four-factor PCC (4 F-PCC; Beriplex® [known as Kcentra® in the USA]; CSL Behring GmbH, Marburg, Germany) to reverse the effects of rivaroxaban in an *in vivo* rabbit model. As well as investigating treatment effects on blood loss and time to haemostasis following acute injury, this study aimed to evaluate the correlations between *in vitro* coagulation assays and *in vivo* measures of haemostasis, and determine which coagulation assay would best predict the effectiveness of 4 F-PCC treatment for reversal of rivaroxaban-associated bleeding.

Materials and Methods

The study was conducted using a rabbit model previously utilised to assess dabigatran and edoxaban reversal [23,24]. Study animals received care in compliance with the European Convention on Animal Care and procedures were approved by the local animal welfare authority.

Study Agents

4 F-PCC (Beriplex® [known as Kcentra® in the USA]; CSL Behring GmbH, Marburg, Germany), containing factors II, VII, IX and X and coagulation proteins C and S [25], was reconstituted in water for injection at room temperature and used immediately. The diluent was injected into the 4 F-PCC vial at an appropriate volume to obtain a final concentration of 28 IU/mL, as instructed by the label. Rivaroxaban (Bayer HealthCare AG, Leverkusen, Germany) was dissolved in a solution of polyethyleneglycol (PEG) 400/H_2O/ethanol (50%/40%/10%) (CSL Behring GmbH, Marburg, Germany). To achieve a clear solution at the 1.0 mg/mL dose, the solution was warmed to 37 °C and placed in an ultrasound bath for 60 min.

Study Endpoints

The main objective of this unblinded, randomised rabbit study was to evaluate *in vivo* the ability of a 4 F-PCC to reverse the effects of rivaroxaban in an acute bleeding model. In addition to the primary endpoints of time to haemostasis and volume of blood loss, this *in vivo* study evaluated treatment effects on the following coagulation parameters: PT, aPTT, whole-blood clotting time (WBCT) and thrombin generation using different reagents. Rivaroxaban plasma levels were assessed based on FXa inhibition.

Animals

Female Chinchilla Bastard rabbits, 3–4 months of age, weighing 2.5–3.2 kg, were obtained from Bauer (Neuental, Germany) and housed individually in wire-steel cages at 21 − 23 °C and 50% relative humidity under a 12 h/12 h light/darkness cycle. The animals had free access to tap water and were fed rabbit pellets (Deukanin, Deutsche Tiernahrung Cremer GmbH & Co. KG, Düsseldorf, Germany) *ad libitum*. The animal groups were distributed within and between racks in a manner that allowed equalisation of environmental influences across the study.

Treatment

The full details of treatment group assignment are described in Table 1. Animals were assigned to 10 groups (n = 5 animals per group). Sample size for the treatment groups were chosen to allow statistical analysis of the results obtained based on previous experience ofNOAC reversal in this rabbit model. Animals received a single intravenous (iv) administration of increasing doses of reconstituted rivaroxaban (150, 300 and 450 μg/kg) with the aim of inducing a clear bleeding signal after kidney incision; the vehicle control group received vehicle solution instead of rivaroxaban. At 3 min after rivaroxaban administration, the animals received an iv administration of 4 F-PCC at dose levels of 25, 50 and 100 IU/kg or an iv bolus of an equal volume of 0.9% (w/v) isotonic saline instead of 4F-PCC. Doses of 4 F-PCC were selected based on the recommended clinical dose for VKA reversal (maximum dose 50 IU/kg) [24] and multiples thereof.

Anaesthesia

Anaesthesia was induced using 4.5–8.5 mg/kg iv ketamine and 0.5–1.1 mg/kg iv xylazine 2%. Animals were intubated and ventilated over the study period; inhaled anaesthesia was maintained using isoflurane (Isofluran CP®, CP-Pharma Handelsgesellschaft mbH, Burgdorf, Germany). Following a 20-min stabilisation period, a carotid artery catheter (22G needle; B Braun, Melsungen, Germany), fixed by a ligature around the artery, was used for blood sampling purposes. The catheter was flushed with saline after each blood sampling.

Kidney Incision

A standardised kidney incision was performed as described previously [23]. Briefly, at 5 min after 4 F-PCC infusion (8 min post rivaroxaban administration), a standardised kidney incision (measuring 15 mm long and 5 mm deep) was created, using a scalpel, from the upper to the lower kidney poles. The 30-min observation period for the assessment of blood loss and time to haemostasis began immediately after the kidney incision.

Assessments

Blood samples for determination of rivaroxaban plasma levels and coagulation parameters were collected at baseline, after rivaroxaban administration but prior to 4 F-PCC administration (t = 3 min), after 4 F-PCC administration and immediately before kidney incision (t = 8 min) and at the end of the observation period (t = 40 min). Approximately 1.65 mL of blood was drawn at each time point and collected in a 5 mL single-use polystyrol tube. A whole blood sample of 0.50 mL was used to determine the WBCT. The remainder was

Table 1
In vivo study: treatment group assignment.

Rivaroxaban dose (μg/kg)	4F-PCC dose (IU/kg)	Animals (n)
0[a]	0[b]	5
150	0[b]	5
150	25	5
300	0[b]	5
300	25	5
300	50	5
300	100	5
450	0[b]	5
450	50	5
450	100	5

[a]vehicle (polyethylene glycol/water/ethanol 50%/40%/10% solution) administered; [b]isotonic saline 0.9% (w/v) administered; rivaroxaban was administered at t = 0 min, 4F-PCC was administered at t = 3 min. 4F-PCC, four-factor prothrombin complex concentrate; IU, international unit.