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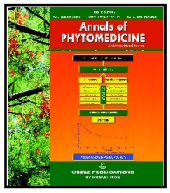

Original article

Comparative study of prothrombin complex concentrate and the combination of *Spinacia oleracea* L. extract with prothrombin complex concentrate on the reversal of apixaban anticoagulation in a rabbit model

Feras M. Almarshad
Department of Internal Medicine, College of Medicine, Shaqra University, Shaqra, Kingdom of Saudi Arabia

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Abstract

Limitation of the anticoagulants apixaban is the availability of specific antidotes with many adverse effects. Reversal allows acute bleeding events to be managed or urgent interventional procedures performed. *Spinacia oleracea* L. is a source of vitamin K, oral anticoagulants exert their effect by blocking the utilization of vitamin K, yet little is known about competitive aspects of their interaction with dietary vitamin K. This study is designed to determine in an animal model whether the leaves of *S. oleracea* (source of vitamin K) ethanol extract can potentiate the effect of prothrombin complex concentrate (PCC) in the reversal of the anticoagulation effect of apixaban. Anesthetized rabbits (n=5) were divided in to following groups, Group I: Normal saline (negative control); other groups treated with 0.04 mg/kg apixaban, followed by Group II: Normal saline; Group III: 50 IU kg^{-1} PCC; Group IV: *S. oleracea* leaves ethanol extract test doses of 150 mg/kg + 50 IU kg^{-1} PCC. After a standardized kidney incision, the volume of blood loss and time to hemostasis were determined. From the above study, we may conclude that the herbal leaves of *S. oleracea*, which may reduce the prothrombin time and blood loss and potentiate the effect of prothrombin complex concentrate in reversing apixaban. Further, investigation is warranted.

Keywords: Apixaban, oral anticoagulant, reversal, vitamin K, prothrombin complex concentrate

1. Introduction

With increasing awareness, the direct use of oral anticoagulants (DOACs) has been steadily increasing in recent years. Unfortunately, the reversal of anticoagulation attained with DOACs is not well studied yet. With the known mechanisms of a few newer agents, no universal antidote is available for reversal. Laboratory assays that can measure the anticoagulation status of patients on DOAC therapy are not readily available. Furthermore, the safety and efficacy of reversal agents have not been extensively investigated in the clinical setting (Feras *et al*., 2018). However, the reversal of DOAC therapy/ anticoagulation in an emergent setting requires a multifaceted approach. Approximately, 20% of patients on chronic anticoagulation require invasive or surgical procedures annually. Significant among these factors are the inherent bleeding risk associated with the procedure, and patient factors that can increase the risk of bleeding complications (Gage *et al*., 2006; Patel *et al*., 2012; Grove *et al*., 2013; Singer *et al*., 2013; Kovacs *et al*., 2015; Burnett *et al*., 2016; Douketis *et al*., 2018).

2. Materials and Methods

2.1 Animal groups

Anesthetized rabbits (n=5) were divided in to four groups, *viz*., Group I: Normal saline (negative control); other groups treated with 0.04 mg/kg apixaban, followed by Group II: Normal saline; Group III: 50 IU kg^{-1} PCC; Group IV: *S. oleracea* leaves ethanol extract test doses of 150 mg/kg + 50 IU kg^{-1} PCC. After a standardized kidney incision, the volume of blood loss and time to hemostasis were determined.

2.2 Animals

Female CHB rabbits, 3-4 months old, weighing 2.6-3.2 kg were 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 *ad libitum*. The rabbits received care in compliance with the European Convention on Animal Care, and the study was approved by the institutional ethical committee (Approval Number : SUCOM/LIRB/2019-02).

2.3 Endpoints

Prothrombin time (PT) as surrogate markers of bleeding diathesis served as secondary endpoints. In this open-label, placebo-controlled rabbit study, and the primary endpoints were the volume of blood loss and time to hemostasis up to 30 min, following a standardized kidney incision injury.

2.4 Drugs

Apixaban (Eliquis, Pfizer), prothrombin complex concentrate (Kcentra, CSL Behring), Ketamine (Delphis Pharma), Xylazine (Xylamed, Bimeda), Ethanol (Merk Ethanol).

Author for correspondence: Dr. Feras M. Almarshad
Assistant Professor, Department of Internal Medicine, College of Medicine, Shaqra University, Shaqra, Kingdom of Saudi Arabia
E-mail: feras-mmm@hotmail.com

2.5 Plant material

The fresh leaves of *S. oleracea* L. were collected from the farm area and authenticated by the Botanist of the Institution and sample specimen were stored for further reference (Specimen Number : FMA 1545).

2.6 Anesthesia

5 mg kg^{-1} i.v. ketamine and 0.5 mg kg^{-1} i.v. xylazine 2% were used for induction of anesthesia. Inhaled anesthesia was maintained with isoflurane. After a 20-min stabilization period, a carotid artery catheter was used to make measurements of hemodynamic, coagulation and hematological parameters.

2.7 Dose-finding

Apixaban dose selection was based upon *in vitro* spiking experiments. Rabbits received 0.01, 0.02, 0.03, 0.04 or 0.05 mg kg^{-1} i.v. Apixaban doses *in vivo*, and plasma samples from the treated animals were collected and spiked *in vitro* with PCC at concentrations of 0.16, 0.31, 0.62, 0.94 and 1.25 IU ml^{-1}, equivalent to those resulting *in vivo* from 12.5, 25, 50, 75 and 100 IU kg^{-1} PCC doses, respectively. Peak thrombin generation was measured in the plasma samples (Xueping *et al*., 2016).

2.8 Kidney incision

When 5 min had elapsed after PCC infusion, a standardized kidney injury was created in the form of a 15 mm long and 5-mm deep scalpel incision at the lateral kidney pole. The 30 min observation period for blood loss and time to hemostasis began immediately after the incision. Blood samples were collected at baseline, prior to PCC infusion, just before kidney incision and at the end of the 30 min observation period.

2.9 Measurements

Assays of PT were performed with a Schnitger and Gross coagulometer (Heinrich Amelung GmbH, Lemgo, Germany) using the Thromborel (Dade Behring, Marburg, Germany) reagents. Blood loss was measured as the volume of blood collected from the kidney incision site with a syringe. Time to hemostasis was recorded as the interval from the kidney incision until the cessation of observable bleeding or oozing.

3. Results

3.1 Apixaban dose selection

Apixaban doses *in vivo* progressively extinguished thrombin generation. Nearly maximal inhibition was observed at the 0.04 mg kg^{-1} apixaban dose. That finding is consistent with the results from a rabbit model of venous thrombosis, in which dabigatran was shown to exhibit approximately maximal inhibition of clot formation at a 0.04 mg kg^{-1} dose.

3.2 Laboratory measurements of coagulation

PT at 10 min ranged from 8.0 to 13.7 s in the negative control group. PT was prolonged in all animals receiving 0.04 mg kg^{-1} apixaban plus saline. There was a decrease in PT in Group IV containing *S. oleracea* ethanol extract test doses of 150 mg/kg + 50 IU kg^{-1} PCC as compared to group III containing 50 IU kg^{-1} PCC.

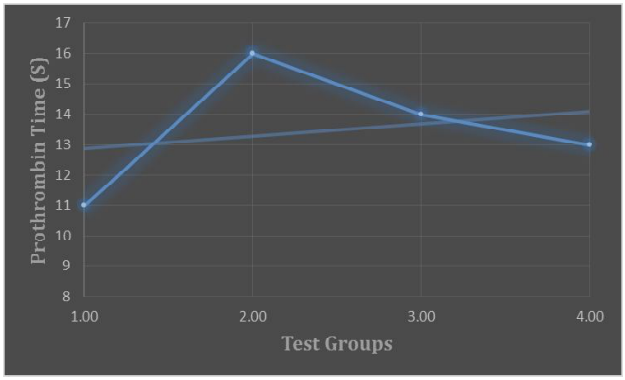

Figure 1: Effect of test groups on prothrombin time.

3.3 Blood loss

In the negative control group receiving no apixaban, blood loss during the 30 min period after kidney incision ranged from 1.0 to 8.1 ml. In all animals treated with apixaban and 5 min thereafter with saline, blood loss within the 30 min observation period was higher, ranging from 16 to 45 ml. There was a decrease in blood loss in Group IV containing *S. oleracea* ethanol extract test doses of 150 mg/kg + 50 IU kg^{-1} PCC as compared to Group III containing 50 IU kg^{-1} PCC (Figure 2).

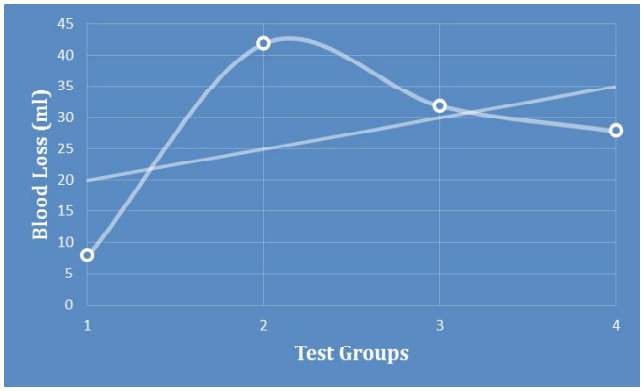

Figure 2: Effects of test groups on blood loss (ml).

4. Discussion

Despite the availability and advantages of DOAC therapy, the lack of a reversal agent is perceived as limiting their use. Study reported the potential of PCC to reverse apixaban-associated anticoagulation in a rabbit model. Blood loss and time to hemostasis following a standardized kidney incision were both markedly increased. Administration of PCC shortly after apixaban dosing significantly reduced both bleeding time and total blood loss post incision (Pragst *et al*., 2012; Herzog *et al*., 2015; Herzog *et al*., 2015). Vitamin K is the reversal of oral anticoagulants that are widely used for the treatment and prophylaxis of thromboembolic disease. Vitamin K is an essential micronutrient. The clinical effectiveness of OACs derives from their ability to block post translational γ-carboxylation of the 4 vitamin k-dependent pro-coagulants (II, VII, IX, and X). Hence, treatment with OACs results in the production of dysfunctional, under-carboxylated species of coagulation factors (Furie *et al*., 1999; Hirsh *et al*., 2003). The herbal leaves of *S. oleracea* is one of the source of vitamin K. Our aim of the study was to compare the reversal effect of PCC alone and with the

combination of ethanol extract of *S. oleracea* leaves and PCC by using standard apixaban as an anticoagulant. There was a reduction of PT and blood loss in a group where ethanol extract of *S. oleracea* leaves is combined with PCC as compared to PCC alone.

5. Conclusion

From the above study, we may conclude that the herbal leaves of *S. oleracea* (source of vitamin K), which may reduce the prothrombin time and blood loss and potentiate the effect of prothrombin complex concentrate in reversing apixaban in rabbit model. However, in light of the variable correlation of laboratory parameters with *in vivo* measures of hemostasis, there is a clear need for validated assays to guide newer oral anticoagulation reversal. The agreement of the results from this study with those seen for the rabbit model indicates that derivatives from the *S. oleracea* extracts may be an option for the urgent reversal of the anticoagulant effect of apixaban, but clinical data in human patients are required to confirm these results. Further, investigation is warranted.

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Conflict of interest

I declare that no conflict of interest exists in the course of conducting this research. I had final decision regarding the manuscript and the decision to submit the findings for publication.

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