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Rivaroxaban and warfarin achieve effective anticoagulation, as assessed by inhibition of TG and *in-vivo* markers of coagulation activation, in patients with venous thromboembolism

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ABSTRACT

Introduction: Rivaroxaban is non-inferior to warfarin for the treatment of venous thromboembolism, with regard to clinical efficacy and safety. The *ex-vivo* effects of warfarin versus therapeutic dose rivaroxaban on *in-vivo* markers of coagulation activation and thrombin generation remain undefined. The aim of this study was to compare the effects of warfarin and therapeutic dose rivaroxaban on *ex-vivo* thrombin generation (TG), and the *in-vivo* markers of coagulation activation, prothrombin fragment 1.2 (F1.2), thrombin-antithrombin complex (TAT), and D-dimer.

Methods: Eighty-five patients with venous thromboembolism were studied, 45 on warfarin, target INR 2.5 and 40 on rivaroxaban 20 mg once daily.

Results: Anticoagulation was in therapeutic range in 71% (32/45) warfarin and 65% (26/40) rivaroxaban treated patients. 8 patients on warfarin and 9 patients on rivaroxaban had subtherapeutic INR and rivaroxaban levels respectively. Both rivaroxaban and warfarin reduced endogenous thrombin potential (ETP) and peak thrombin, and prolonged lag time and time to peak, compared to normal controls ($p < 0.0001$). The lag time and time to peak TG were longer, and peak thrombin was lower in patients receiving rivaroxaban ($p < 0.0001$) compared with warfarin, although warfarin-treated patients had lower ETP ($p = 0.0008$). *In-vivo* coagulation activation markers were within the normal ranges in all rivaroxaban-treated patients (including those with levels considered to be subtherapeutic) and in 37/45 warfarin-treated patients who had an INR ≥ 2.0. The warfarin-treated patients with subtherapeutic INRs exhibited slightly raised F1.2 and/or TAT.

Conclusion: In conclusion, both rivaroxaban and warfarin provided effective anticoagulation, as assessed by inhibition of TG and makers of *in-vivo* coagulation activation.

Introduction

Several oral direct acting inhibitors (ODI) of coagulation, including thrombin inhibitors (dabigatran etexilate [Pradaxa®]) or factor Xa inhibitors (rivaroxaban [Xarelto®], apixaban [Eliquis] and edoxaban [Lixiana®]) have been licensed for specific clinical indications. Currently, rivaroxaban is approved worldwide for the prevention of venous thromboembolism (VTE) following elective knee or hip replacement; treatment of deep venous thrombosis (DVT) and/or pulmonary embolism (PE), prevention of recurrent DVT and PE; and prevention of stroke and systemic embolism in patients with non-valvular atrial fibrillation (AF)[1].

Thrombin is a pivotal component of the haemostatic mechanism, with increased *ex-vivo* thrombin generation (TG) a key marker of thrombogenic potential and also with predictive value for the development of recurrent VTE [2,3]. In patients with acute thrombosis, *in-vivo* markers of coagulation activation such as prothrombin fragment 1.2 (F1.2), thrombin-antithrombin complex (TAT), and D-dimer are frequently elevated, and anticoagulation reduces the levels of these markers [4]. F1.2, as the activation peptide originating from the factor Xa-mediated activation of prothrombin, has been reported to be a sensitive marker of anticoagulation [5,6], and evidence supports the use of F1.2 and D-dimer in identifying patients at increased risk of VTE recurrence who need continued anticoagulation, or a different intensity anticoagulant regime [7–9]. *In-vitro* experiments, where rivaroxaban has been spiked into normal plasma, as well as *ex-vivo* data from patients receiving prophylactic rivaroxaban, have shown that rivaroxaban affects various parameters of the TG assay using the calibrated automated thrombogram (CAT) system. A greater inhibitory effect was observed on the initiation phase (lag time) and the peak TG compared to that on the

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area under the curve i.e. endogenous thrombin potential (ETP) [10,11] compared to fondaparinux *in-vitro;* and patients on prophylactic dose rivaroxaban 10 mg daily undergoing elective total hip/knee replacement showed a greater decrease of TG, F1.2 and TAT (but not D-dimer) than those on dalteparin [12].

Clinical outcome measures in patients receiving warfarin or rivaroxaban have been assessed in a number of different multicentre prospective clinical trials involving many thousands of patients, including those with VTE [13–15]. Rivaroxaban has been shown to be non-inferior to warfarin with regard to relevant clinical efficacy and safety outcome measures. However, the *ex-vivo* effects of warfarin versus therapeutic dose rivaroxaban on TG and *in vivo* markers of coagulation activation remain undefined.

We aimed to compare *ex-vivo* TG (using the CAT system) and *in vivo* markers of coagulation activation (F1.2, TAT complex and D-dimer) in patients on long term anticoagulation with warfarin or rivaroxaban following VTE.

Methods

Patients

The study included 45 patients on warfarin with a target International normalized ratio (INR) of 2.5 (range 2.0-3.0) and 40 patients on therapeutic dose rivaroxaban 20 mg daily for the prevention of recurrent VTE following a first episode of DVT or PE. Patients on rivaroxaban had previously switched from therapeutic dose warfarin to rivaroxaban because of: allergy to warfarin, lack of availability of phenindione, lifestyle and difficulty in monitoring warfarin due to needle-phobia. Rivaroxaban had been administered for at least 3 weeks (3-12 weeks) and taken with food, which increases the absorption of the drug (2). None of these patients were taking other anticoagulants, antiplatelet agents, or any other drugs known to interact with rivaroxaban. None of the patients had an episode of acute VTE within the last 3 months or acute or chronic infections/inflammation. Duration of anticoagulation in patients groups varied from 4 to 6 months. Patient demographic details are shown in Table 1. All patients in both anticoagulant treatment groups had a creatinine clearance over 50 mL/min [16] and normal liver function tests.

All patients were tested for antiphospholipid antibodies (aPL) and were negative. As patients were already on warfarin, lupus anticoagulant (LA) tests were performed using the Taipan/Ecarin time ratio (which shows little impact of warfarin) and/or dilute Russell Viper Venom Time (DRVVT) using equal volume mixtures with normal plasma, to correct for warfarin-induced coagulation factor deficiency, in accordance with recommendations by the International Society on Thrombosis and Haemostasis (ISTH) and British Committee for Standards in Haematology (BCSH) guidelines on LA detection [17,18]. All patients were tested for aPL following at least 6 weeks of initiation of anticoagulation.

TG was also assessed in 51 normal controls (median age 40 [range 23-70]). The study was approved by the Research Ethics Committee at University College London Hospitals NHS Trust (Reference number: 13/EM/0150).

Table 1
Patients' demographics
Baseline characteristics of the two study groups.

	Warfarin (n = 45)	Rivaroxaban (n = 40)
Age in years: median (range)	47 (28-70)	46 (34-68)
Male/female	28/17	26/14
Intensity of anticoagulation	Target INR 2.5 (range 2.0-3.0)	Rivaroxaban 20 mg daily
Type of thrombosis DVT	25/45 (55.5%)	23/40 (57.5%)
PE	12/45 (26.7%)	11/40 (27.5%)
DVT + PE	8/45 (17.8%)	6/40 (15%)
Creatinine clearance (mL/min): Median (range)	75 (63-92)	76 (62-95)

Blood Sampling

Venous blood samples were collected following informed consent at a single time-point using a 21 gauge butterfly needle, with minimal venous stasis, within 2-4 hours of the last dose of rivaroxaban. Blood was drawn into 5 mL citrate Vacutainer® (BD, Oxford, UK) containing 0.5 mL of 0.105 M buffered sodium citrate which gave a ratio of 1 part anticoagulant to 9 parts blood. Platelet poor plasma (PPP) was prepared within 1 hour of collection by double centrifugation (2000 g for 15 minutes) at ambient temperature and 0.75 mL aliquots were transferred to 2.0 mL cryo-tubes (Sarstedt Ltd, Beaumont Leys, Leicester, UK) and stored at -80 °C for up to 12 weeks. Immediately prior to analysis the samples were thawed at 37 °C.

Coagulation Assays

Prothrombin time (PT) was performed only on samples from patients taking warfarin, using a single lot number of Innovin (Siemens Healthcare Diagnostics, Marburg, Germany), on a CS-5100 analyser (Sysmex UK Ltd, Milton Keynes, UK) and employing an instrument-specific ISI value (0.99).

Rivaroxaban Levels

Rivaroxaban levels were measured using an amidolytic anti-Xa assay (Biophen DiXaI; Hyphen BioMed, Neuville-Sur-Oise, France), in combination with Biophen rivaroxaban calibrators, covering a range of 0 to 500 ng/mL. The assay was performed on a Sysmex CS-2000*i* analyser (Sysmex UK Ltd) and samples were tested in duplicate. Two quality control preparations (Hyphen BioMed, C1: rivaroxaban level 40-140 ng/mL and C2: rivaroxaban level 220-380 ng/mL) were tested at the beginning and end of the analytical run with all patients samples processed on a single occasion.

Ex-vivo thrombin generation was measured using the CAT system (Thrombinoscope BV, Maastricht, The Netherlands) as described by Hemker et al [19] in conjunction with PPP reagent (Stago, Asnieres, France) which gave reaction concentrations of 5 pmol/L tissue factor (TF) and 4 μmol/L phospholipid. These experimental conditions were selected based on our own previous experience [20] and that of others [21]. Since the samples tested were from patients on anticoagulation, the use of a low concentration tissue factor reagent (i.e. 1 pmol/L or less) was not appropriate as there would have been minimal or no thrombin generation. In addition, as we used 5pM TF, blood collection into corn trypsin inhibitor was not necessary [22]

All TG reactions for both samples and calibrators were tested in triplicate. The following parameters of the TG curve were recorded: lag-time; time to peak TG, peak thrombin and ETP. Normal ranges for TG were established using 51 normal controls (NC) none of whom were on any oestrogen preparations. The intra- and inter-assay imprecision, using normal plasma were 2.5% and 4% respectively.

In-vivo coagulation activation markers: D-dimer was measured by an immunoturbidometric technique (Innovance D Dimer; Siemens Healthcare Diagnostics) on a CS-5100 analyser (Sysmex UK Ltd). F1.2 and TAT complex were measured by enzyme-linked immunosorbent assay (Enzygnost® F1.2 and Enzygnost® TAT respectively; Siemens Healthcare Diagnostics). Normal cut-off values for D dimer, F1.2 and TAT were: <550 μg/L FEU, <229pmol/L and <4.2 μg/L, respectively.

Statistical Analysis

Data analysis was performed using Analyse-it software version 2.26 (Analyse-It Software Ltd, Leeds, UK). Correlations between each haemostatic marker and TG parameters in relation to plasma