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Open-label, randomized study of the effect of rivaroxaban with or without acetylsalicylic acid on thrombus formation in a perfusion chamber

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ABSTRACT

Introduction: Rivaroxaban, a direct factor Xa inhibitor, has demonstrated effectiveness for the management of both venous and arterial thrombosis. This study was designed to investigate the antithrombotic effect of rivaroxaban, with or without acetylsalicylic acid (ASA), in an *ex vivo* perfusion chamber at both low and high shear rates.

Materials and methods: Healthy subjects (N = 51) were enrolled in a randomized, crossover (rivaroxaban 5, 10 or 20 mg with or without ASA), and parallel-group (compared with ASA plus clopidogrel) study. Thrombi formed on pig aorta strips were measured after a 5-minute perfusion at low and high shear rates with blood from the subjects by measuring D-dimer concentration (for fibrin deposition) and P-selectin content (for platelet deposition).

Results: ASA alone had no impact on thrombus D-dimer levels, whereas rivaroxaban alone at peak concentrations decreased D-dimer levels by 9%, 84% and 65% at low shear rate and 37%, 73% and 74% at high shear rate after doses of 5, 10 and 20 mg, respectively. Steady-state ASA plus rivaroxaban 5 mg caused a greater reduction in D-dimer levels (63%) than monotherapy at low shear rate. Co-administration of ASA with clopidogrel was associated with a 30% decrease in D-dimer levels at low shear rate and a 14% decrease at high shear rate. No conclusive effect on P-selectin content was observed across the treatment groups.

Conclusions: Rivaroxaban dose-dependently inhibited *ex vivo* thrombus formation under low and high shear rates. Co-administration of ASA had an additional effect on the antithrombotic action of low-dose rivaroxaban.

Introduction

Thromboembolic disorders are a leading cause of morbidity and mortality. Although the pathologies of arterial and venous thrombi differ, both fibrin formation and platelet activation play a key role in thrombogenesis. These differences in pathophysiology are reflected in the different management strategies, such that antiplatelet agents are used as the predominant antithrombin therapy for secondary prevention in patients with acute coronary syndrome (ACS), whereas anticoagulant therapy plays a key role in the prevention and treatment of venous thrombosis and related disorders [1–3]. ACS is caused by thrombogenesis after the rupture of an atherosclerotic plaque; this process involves platelet activation and aggregation and activation of the coagulation cascade, which provides the rationale for anticoagulant therapy in addition to antiplatelet therapy for secondary prevention of cardiovascular events in patients with ACS [4]. Previous studies have evaluated vitamin K antagonists (VKAs; e.g. warfarin) in addition to acetylsalicylic acid (ASA) or ASA plus clopidogrel for the prevention of cardiovascular events in patients with ACS [5–7]. The results of these studies showed that, although the combination of warfarin and antiplatelet therapy was associated with an improvement in cardiovascular outcomes compared with antiplatelet therapy alone, there was also an increase in major bleeding events. In addition, VKAs have other limitations with their use, including multiple drug–drug and food–drug interactions and unpredictable responses that necessitate routine coagulation monitoring and dose adjustments to ensure that patients maintain an appropriate level of anticoagulation [8].

New oral anticoagulants that target a single coagulation factor have been developed in an attempt to overcome some of the limitations associated with traditional anticoagulants. Rivaroxaban is an oral, direct factor Xa inhibitor that inhibits clot-bound factor Xa and factor Xa

Abbreviations: ACS, acute coronary syndrome; ANCOVA, analysis of covariance; aPTT, activated partial thromboplastin time; ASA, acetylsalicylic acid; CI, confidence interval; C_{max}, maximum plasma concentration; C_{trough}, minimum plasma concentration; CV, coefficient of variation; ETP, endogenous thrombin potential; LD, loading dose; LS, least squares; od, once daily; PT, prothrombin time; SD, standard deviation; VKA, vitamin K antagonist.

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within the prothrombinase complex, as well as free factor Xa [9,10]. Preclinical studies have shown that rivaroxaban monotherapy dose-dependently inhibited tissue factor-mediated platelet aggregation in human plasma [11] and was effective in animal models of both arterial and venous thrombosis [10,12]. Rivaroxaban has demonstrated consistent efficacy and reassuring safety in large-scale phase III clinical trials across several indications [13–20]. This includes the secondary prevention of acute coronary events, where rivaroxaban reduced the risk of death from cardiovascular causes, myocardial infarction or stroke, compared with placebo in the ATLAS ACS 2 TIMI 51 trial, without any increase in fatal bleeding events [20].

Investigation of the pharmacological profiles of new antithrombotic agents such as rivaroxaban, as well as effects on biomarkers with potential clinical relevance, can be undertaken using experimental models such as the perfusion chamber method. Originally developed by Baumgartner [21], the annular perfusion chamber was shown to be a valuable model for understanding mechanisms relating to thrombosis and haemostasis in the vasculature, allowing endothelium to be exposed to flowing blood at predetermined flow rates. In the current study and in an earlier study [22], we have employed a modified method based on that described by Badimon [23]. This cylindrical system was designed to mimic conditions in the blood vessels and provides a rapid, objective method for quantifying platelet deposition and thrombus formation [23].

A thrombus formed on the thrombogenic surface of the perfusion chamber can be assessed by measuring the level of fibrin degradation products in the plasmin-degraded thrombus. Previous studies have shown that thrombus D-dimer concentrations directly correlate with thrombus size [22,24], making it a useful biomarker in perfusion chamber studies assessing the antithrombotic effect of drugs. Another prospective biomarker of thrombogenesis is P-selectin, which has the potential to indicate platelet disposition in plasmin-digested thrombi [25]. The objectives of this study were to evaluate the effect of rivaroxaban with and without ASA on thrombus formation (platelet and fibrin deposition) in a perfusion chamber system at low and high shear rates (representing flow conditions in the venous system and stenosed arteries, respectively). The effects of the combination of ASA and clopidogrel, and the safety, pharmacodynamics and pharmacokinetics of rivaroxaban were also investigated.

Methods

Subjects

In total, 51 healthy male or female Caucasian subjects, aged 18–55 (mean 27.9) years were enrolled in this study. All subjects had a body mass index of 18–30 kg/m^2. Subjects were excluded if they had participated in another clinical trial or donated blood during the preceding 3 months, or had a medical disorder, condition or history that could interfere with the study results or impair their ability to participate in or complete the study (e.g. coagulation disorders, disorders known to be associated with an increased risk of bleeding, history of thromboembolism, etc.). Other key exclusion criteria included regular use of therapeutic or recreational drugs; use of medication within the 2 weeks preceding the study that could interfere with any of the study drugs; relevant deviation from the normal range in clinical chemistry, haematology, blood pressure, electrocardiogram or urinalysis; a positive test for HIV or hepatitis B or C; and pregnancy or lactation in women of child-bearing age.

All subjects gave written informed consent to participate in the study before undergoing any study-specific procedures. The study was conducted in accordance with the currently accepted version of the Declaration of Helsinki, the International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use Good Clinical Practice (ICH GCP) Guideline and local regulations. The study was registered with EudraCT (2007-002345-21).

Study Design

This was a single-centre, randomized, non-blinded, parallel-group, two-way crossover study. Different dosing regimens of rivaroxaban (5, 10 or 20 mg) were investigated with and without ASA in a crossover fashion, and were compared in parallel with clopidogrel plus ASA (Fig. 1). Subjects were randomized and allocated to different treatment groups. Group A received ASA once daily from day −3 to day 0 (300 mg loading dose followed by 100 mg per day to achieve steady-state levels), together with a single dose of rivaroxaban 5, 10 or 20 mg (in separate treatment groups) on day 0. In group B, subjects received a single dose of rivaroxaban 5, 10 or 20 mg alone on day 0 (in separate treatment groups). After a washout period of ≥14 days, subjects were crossed over to the alternate treatment arm. Subjects in group C received clopidogrel plus ASA. Clopidogrel was administered once daily on four consecutive days, beginning with a 300 mg loading dose on day −3 and followed by 75 mg on days −2, −1 and 0. ASA was also given once daily on four consecutive days from day −3 to day 0 (300 mg loading dose followed by 100 mg per day). All subjects then underwent a follow-up assessment 1–2 weeks after they had received the final dose.

Pharmacodynamics

Thrombus Formation in the Perfusion Chamber at High and Low Shear Rates

The effect of rivaroxaban with and without ASA on thrombus formation was investigated using a perfusion chamber model as described previously [22,26]. Each chamber contained a piece of pig aorta stripped of the intimal layer. The low shear rate was 212 /s (which mimicked venous flow conditions) and the high shear rate was 1690 /s (which mimicked moderately stenotic arterial flow conditions). Before blood perfusion, the system was perfused with 0.9% sodium chloride to ensure that no leaks were present and to remove air bubbles. Blood from the subject's antecubital vein was drawn through an 18 G cannula with a pump (Masterflex® L/S™, Cole-Parmer Instrument Company, Vernon Hills, IL, USA). Five milliliters of blood were discarded before each perfusion, and the aorta pieces were perfused at 10 ml/min for 5 minutes, followed by a 30-second perfusion with 0.9% sodium chloride.

The size of the thrombus formed on the thrombogenic surface of the chambers was evaluated by measurement of the concentration of D-dimer (Asserachrome D-Dimer, Diagnostica Stago, Asnières-sur-Seine, France) of the plasmin-degraded thrombus. Thrombus D-dimer concentration is a measure of fibrin deposition because it is a degradation product of cross-linked fibrin that is formed when thrombin-generated clots are digested by plasmin. As such, it is directly correlated to the size of the thrombus formed in the perfusion chamber [22]. The thrombus on the pig aorta was degraded with the use of plasmin solution (0.5 ml 0.1 M phosphate-buffered saline pH 7.4 and 0.05 ml plasmin at 10 U/ml; Chromogenix, Milano, Italy). The tubes were placed in a water bath (37 °C) for 60 minutes, mixed every 15 minutes, and the reaction was stopped by addition of 50 μl aprotinin (6150 U/ml, from bovine lung; Fluka Chemie GmbH, Buchs, Switzerland). Platelet deposition was measured in terms of P-selectin content in dissolved plasmin-digested thrombi using immunoenzymoassays (Quantikine human soluble P-selectin; R&D Systems, Minneapolis, MN, USA).

D-dimer and P-selectin were measured at baseline, peak (at 3 hours after drug administrations in groups A and B, and at 4 hours after drug administration in group C), and at expected trough rivaroxaban concentrations (time 0 on day 1 for treatment groups A and B, i.e. 24 hours after rivaroxaban dosing). Pharmacodynamic biomarkers were assessed in plasma from venous blood