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Effect on perfusion chamber thrombus size in patients with atrial fibrillation during anticoagulant treatment with oral direct thrombin inhibitors, AZD0837 or ximelagatran, or with vitamin K antagonists

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ABSTRACT

Introduction: AZD0837 and ximelagatran are oral direct thrombin inhibitors that are rapidly absorbed and bioconverted to their active forms, AR-H067637 and melagatran, respectively. This study investigated the antithrombotic effect of AZD0837, compared to ximelagatran and the vitamin K antagonist (VKA) phenprocoumon (Marcoumar®), in a disease model of thrombosis in patients with non-valvular atrial fibrillation (NVAF).

Methods: Open, parallel-group studies were performed in NVAF patients treated with VKA, which was stopped aiming for an international normalized ratio (INR) of ≤2 before randomization. Study I: 38 patients randomized to AZD0837 (150, 250 or 350 mg) or ximelagatran 36 mg twice daily for 10–14 days. Study II: 27 patients randomized to AZD0837 250 mg twice daily or VKA titrated to an INR of 2–3 for 10–14 days. A control group of 20 healthy elderly subjects without NVAF or anticoagulant treatment was also studied. Size of thrombus formed on pig aorta strips was measured after a 5-minute perfusion at low shear rate with blood from the patient/control subject.

Results: Thrombus formation was inhibited by AZD0837 and ximelagatran. Relative to untreated patients, a 50% reduction of thrombus size was estimated at plasma concentrations of 0.6 and 0.2 μmol/L for AR-H067637 and melagatran, respectively. For patients receiving VKA treatment, the thrombus size was about 15% lower compared with healthy elderly controls.

Conclusions: Effects of AZD0837 and ximelagatran on thrombus formation were similar or greater than for VKA therapy and correlated with plasma concentrations of their active forms.

Oral direct thrombin inhibitors (DTIs) have been developed and recently introduced for the treatment and prevention of systemic thromboembolism [1]. As a principal advantage over therapy with vitamin K antagonists (VKAs), these drugs offer a predictable anticoagulant effect with an improved safety profile, and they do not require frequent coagulation monitoring and dose adjustments. Thrombin has a central role in the coagulation cascade and is responsible for clot formation via its potent activation of platelets and formation of the fibrin network [2,3]. The novel oral anticoagulant AZD0837 is a prodrug, which, after oral administration, is rapidly absorbed and bioconverted to its active form AR-H067637 [4], a selective and reversible DTI [5]. Animal studies have demonstrated that the active form inhibits thrombus formation in rat venous and arterial thrombosis models with no or minor increases in bleeding [6]. In clinical Phase II studies, treatment with AZD0837 for the prevention of stroke and systemic embolic events in patients with atrial fibrillation has shown promising safety and antithrombotic effect [7,8].

Investigation of pharmacological profiles of novel antithrombotic agents in experimental disease models and effects on biomarkers with potential clinical relevance may guide dose selection. The perfusion chamber method, originated by Badimon et al. [9], has been used

Abbreviations: NVAF, Non-valvular atrial fibrillation; VKA, Vitamin K antagonist; INR, International normalized ratio; DTI, Direct thrombin inhibitor; TTA, Total thrombus area; IR, Immediate-release; PK, Pharmacokinetic(s); PD, Pharmacodynamic(s); APTT, Activated partial thromboplastin time; F1+2, Prothrombin fragment 1+2; TAT, Thrombin–antithrombin complex; ACT, Activated coagulation time; TCT, Thrombin clotting time; PAP, Plasmin–antiplasmin complex; FPA, Fibrinopeptide A; β-TG, β-thromboglobulin; CRP, C-reactive protein; ICAM-, Intracellular adhesion molecule 1; VCAM-1, Vascular cell adhesion molecule 1; C_{max}, Maximum plasma concentration; t_{max}, Time to maximum plasma concentration; AUC_τ, Area under the plasma concentration–time curve during the dosing interval at steady state; $t_{½}$, Half-life; CI, Confidence interval; ULN, Upper limit of normal; CV, Coefficient of variation; SD, Standard deviation.

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to assess the antithrombotic effect of new anticoagulants in healthy subjects [10–12]. A comparison of drugs can be performed in this human disease model, using a perfusion chamber that gives a low shear rate equivalent to flow conditions in the venous system and assumed to simulate the thrombus formation in the cardiac atria of the heart in patients with atrial fibrillation.

The primary aim of the present study was to investigate the effect of oral AZD0837 treatment on thrombus formation in patients with non-valvular atrial fibrillation (NVAF). Low-shear-rate perfusion chambers with denuded pig aorta as the thrombogenic surface were used. The first study compared the effect of AZD0837 with ximelagatran, which is an oral DTI that has been studied previously. Ximelagatran is also a prodrug, which is bioconverted to the active form, melagatran. The patients received AZD0837 at different doses and ximelagatran at a therapeutic dose regimen used in clinical Phase III studies for prevention of stroke in patients with NVAF [13,14]. The second study compared AZD0837 with dose-adjusted VKA (target international normalized ratio [INR] 2–3). The size of the thrombus formed on the thrombogenic surface of the perfusion chamber was evaluated by measurement of D-dimer concentration of the plasmin-degraded thrombus. This approach is more accurate than the traditional morphometric analysis of *ex vivo* thrombus formation in studies employing collagen-coated parallel-plate perfusion chambers [15]. In study I, total thrombus area (TTA) was also evaluated by morphometric analysis of selected slices of the paraffin-embedded thrombus, which was the method used in previous perfusion chamber studies with denuded porcine aorta strips [10–12]. In addition, the effects on coagulation-time assays and biomarkers of thrombogenesis were investigated. For comparison, in a third study, *ex vivo* thrombus formation and biomarkers were assessed in healthy elderly subjects without NVAF or anticoagulant treatment.

Methods

Study participants

Patients with atrial fibrillation and anticoagulant treatment

Caucasian patients with persistent or permanent NVAF, and with at least one additional risk factor for stroke, were enrolled into two studies – 46 patients to study I (study code D1250C00011) and 32 patients to study II (study code D1250C00025). Medical history was obtained and a complete health examination that included a physical examination, monitoring of vital signs, electrocardiogram and laboratory screen was done at the pre-entry and follow-up visits.

Study I: Thirty-eight patients (33 men and 5 women) with a mean age of 64 years were randomized to receive drug treatment; 35 completed the study.

Study II: Twenty-seven patients (22 men and 5 women) with a mean age of 65 years were randomized to receive drug treatment; 25 completed the study (16 of these patients had previously been included in, and completed, study I).

Elderly subjects without atrial fibrillation or anticoagulant treatment

Study III: Twenty Caucasian subjects (12 men and 8 women) with a mean age of 75 years were included (study code D1250M00002). A medical history and information about use of medication were registered and a physical examination including a laboratory screen was performed. The study was performed to obtain control data from elderly subjects without NVAF or anticoagulant and antiplatelet treatment within 10 days before the experimental day.

All three studies were approved by the Ethics Committee of the Medical University of Vienna and the Allgemeines Krankenhaus, Vienna, Austria. Studies were conducted in compliance with the Declaration of Helsinki, including current revisions, and Good Clinical Practice guidelines. Written informed consent was obtained from all participants. For study I, enrolment began in January 2005 and the last patient completed the study in March 2006. For study II, enrolment began in June 2006 and the last patient completed the study in December 2006. For the elderly control subjects (study III), the first subject was enrolled in July 2006 and the last subject completed the study in August 2006.

Study Design

Patients with atrial fibrillation and anticoagulant treatment

Studies I and II followed an open, randomized, parallel-group design and enrolled patients with NVAF who were treated with VKAs. Treatment with antiplatelet agents (including acetyl salicylic acid) was not allowed within 10 days before randomization, and treatment with fibrinolytic agents was not allowed within 30 days before randomization. After enrolment, VKA treatment was discontinued and when the international normalized ratio (INR) was ≤ 2 the patients were randomised to study drug (in study II it was also required that after the partial wash-out INR should increase at least 0.3 for patients receiving VKA during the study period). The minimum change in INR after partial VKA washout was selected to allow the presence of an additional therapeutic drug effect following randomization and study drug treatment. A complete VKA washout was not possible for ethical reasons due to the risk of thromboembolic events.

Study I: Patients were randomized to either AZD0837 at a dose of 150 mg (n = 12), 250 mg (n = 2) or 350 mg (n = 11), or ximelagatran 36 mg (n = 13), twice daily for 10 to 14 days. All doses were given as immediate-release (IR) tablets. After an amendment, the 250 mg treatment group was added. However, due to a premature discontinuation of the study when ximelagatran was withdrawn from the market because of safety concerns, only two patients were included in this group.

The measurements of thrombus formation in the perfusion chamber were conducted at steady state (after 10 to 14 days of treatment) on three occasions: i) at trough (before the dose given in the morning on the last treatment day), ii) at peak plasma concentrations (3 hours after dosing on the last treatment day) and iii) in the ximelagatran group 12 hours after the last dose and for the AZD0837 treatment groups 24 hours after the last dose.

Study II: Patients received either AZD0837 (IR tablets) 250 mg twice daily (n = 14) or VKA (phenprocoumon, Marcoumar®, Roche, Austria; n = 13) titrated to an INR of 2–3 for 10–14 days.

The measurements of thrombus formation in the perfusion chamber were conducted on four occasions: i) before cessation of previous VKA treatment at an INR of 2–3, ii) after cessation of VKA and partial washout, achieving an INR of ≤ 2 and following randomization on the last treatment day, iii) before dose and iv) at 3 hours after dosing.

For both studies, a parallel-group design was chosen as the variability in the primary variable was expected to be related to the perfusion chamber method itself, rather than to interpatient variability in the response to the drug, and to minimize the length of the study period for each patient. Due to practical reasons, and as the assessment of the primary variable was obtained by objective laboratory methods, these were open-label studies. A treatment period of a minimum of 10 days ensured that steady-state pharmacokinetics (PK) of the drugs under study were achieved without a residual effect of preceding VKA therapy on the thrombin inhibitors.

Elderly subjects without anticoagulant treatment

Study III: In the control group with elderly subjects without anticoagulant treatment, venous blood sampling and a perfusion chamber experiment were conducted on a study day up to 21 days after the health screening.