Potent arterial antithrombotic effect of direct factor-Xa inhibition with ZK-807834 administered to coronary artery disease patients

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Summary
It was the objective of this study to evaluate the anti-thrombotic potency of direct factor-Xa inhibition with ZK-807834 in stable coronary patients, using an ex-vivo model of arterial thrombus formation. Tissue factor pathway is important in atherothrombosis. Direct factor-Xa blockade may more potently reduce thrombosis and prevent coronary events. Badimon Perfusion Chamber 5-minute quantitative studies have shown 40–55% arterial thrombus reduction with abciximab, 23% with clopidogrel, but none with heparin. Coronary patients (n=18, 59 ± 9 years, 55% males) were blindly randomized to four groups receiving 24-hour infusion of a low, medium or high dose of direct factor-Xa inhibitor ZK-807834, or placebo. Arterial thrombus formation was measured in Badimon Chamber at baseline, end-of-infusion [EoI], and four hours and eight hours after EoI, and factor-X activity, prothrombin time [PT] ratio and plasma drug levels were measured simultaneously. For the low-, medium- and high-dose ZK-807834 groups, mean percent-reduction in thrombus size from baseline to EoI were 29%, 34% and 68%, respectively (p<0.001), and at 8-h post EoI were 11%, 19% and 27%, respectively (p<0.01). Mean PT-ratio prolongation showed a strong linear relationship (Pearson’s r=0.93) with ZK-807834 plasma concentration. Mean percent-reduction in factor-X activity from baseline was 13%, 42% and 58%, respectively. Placebo had no effect on thrombus size or factor-X activity. In conclusion, direct factor-Xa inhibition with ZK-807834 markedly reduces ex-vivo arterial thrombus formation and factor-X activity in a dose-dependent manner. Plasma levels of ZK-807834 show a strong linear correlation with PT ratio. This direct factor-Xa inhibitor may reduce the need for additional potent glycoprotein IIb/IIIa inhibition.

Keywords
Coagulation, coronary disease, myocardial infarction, thrombosis, platelets

Introduction
Thromboembolic diseases, especially acute coronary syndromes (ACS), are the major cause of morbidity and mortality worldwide. Clinical manifestations of coronary atherosclerotic disease are caused by plaque disruption or erosion with platelet-thrombus formation onto deep plaque components which stimulate thrombin generation (1–4). Mural thrombi further increase thrombotic risk by physical obstruction (increases in shear force), release of vasoconstrictors (thromboxane, serotonin, and thrombin), incorporation of tissue factor with activator complexes, and accelerated production of thrombin which propagates thrombus formation by interlinking platelets and forming fibrin (4). Reduction of thrombus growth and distal embolization is an important objective for the treatment of ACS.

Thrombin is the primary procoagulant enzyme for pathologic thrombosis and physiologic hemostasis (5). Benefits derived from blocking its generation, along with adjunctive platelet inhibition with aspirin, thienopyridines, and glycoprotein IIb/IIIa inhibitors have been established. New therapeutic approaches to reduce thrombosis by directly targeting coagulation factors in activator complexes reduce thrombin production and can inhibit both platelet- and fibrin-thrombus formation in arteries (6–10). Therapy with heparin plus aspirin, followed by warfarin plus aspirin is more effective than aspirin alone for preventing thrombotic events during hospitalization and for the next four years (11–13). But heparins are less potent, can not inhibit coagulation factors in activator complexes, and have a narrow therapeutic window and a highly variable and weak dose-response relationship (4, 14, 15).
Factor Xa, positioned at the confluence of intrinsic and extrinsic coagulation pathways, may be an optimal therapeutic target. Direct inhibitors of factor Xa, such as tick anticoagulant peptide (6, 16), antistasin (17) and DX-9065a (18, 19), have been evaluated in animal and human studies. Direct factor-Xa inhibitors have established antithrombotic efficacy in model systems and have promising hemostatic safety profiles (20–22). Factor-Xa inhibition is potent, highly selective, safe and more efficacious than standard heparin and low-molecular-weight heparin in animal models (6, 23–25).

Based on these observations, this human study was designed to assess the relationship between direct factor-Xa inhibition with ZK-807834 (a.k.a., CI-1031; Xa Ki = 0.11 nM) at three doses and the antithrombotic effects, in patients with stable coronary artery disease (CAD) on aspirin. The study was performed using the Badimon ex-vivo perfusion chamber, mimicking the in-vivo rheologic conditions of a modestly stenosed coronary artery with deep injury into the tunica media. The hypothesis was that direct factor-Xa inhibition with ZK-807834 will reduce ex-vivo thrombus formation in a dose-dependent manner and will be safe and easy to monitor.

The primary objective was to assess the antithrombotic potency and duration of three doses of ZK-807834 in patients with known coronary or peripheral arterial disease. Secondary objectives were to assess the effect of ZK-807834 treatment on factor-X activity, and the correlation of plasma drug levels with blood coagulation monitoring parameters.

Materials and methods

Study design

This was a double-blind, placebo-controlled, randomized study, to evaluate the antithrombotic responses of three doses of direct factor-Xa inhibitor ZK-807834 (Fig. 1). Eighteen patients were enrolled at the Mount Sinai Hospital in New York and randomized to placebo (2 patients), low- (5 patients) medium- (5 patients) and high- (6 patients) dose groups. All patients received background 325 mg of aspirin daily. Antithrombotic effects of ZK-807834 were assessed using the Badimon Perfusion Chamber by measuring the size of thrombus formed post-treatment and comparing that to the pre-treatment baseline. Prothrombin time [PT] ratio and plasma drug concentrations were measured concurrently. The strength of this design is the ability for each patient to serve as his/her own control. This allowed inclusion of more patients in the treated groups than in the placebo arm.

Patient population

Eligibility criterion for inclusion in the study was the presence of chronic but stable cardiovascular disease, including CAD (history of myocardial infarction, percutaneous coronary intervention, coronary artery bypass surgery, coronary angiography showing >50% luminal narrowing of at least one major coronary artery on angiography or chronic angina with positive stress test) with or without documented peripheral arterial disease (PAD) (claudication, ankle/brachial index [ABI] <0.80, prior angioplasty/stent or bypass grafting or >50% stenosis in any artery distal to the aortic bifurcation by angiogram or ultrasound, or a bruit over a peripheral artery). Exclusion criteria included clinically active atherosclerotic disease, history suggestive of recurrent blood loss or a predisposition to bleeding (including abnormal PT and activated partial thromboplastin time), prior stroke or transient ischemic attack, creatinine clearance (Cockcroft & Gault) <30 ml/min, women who were breast-feeding or pregnant, or inability to provide written informed consent. Patients on antiplatelet (except for the aspirin given as part of the study) or anticoagulant medications were also excluded, whereas any other medications were continued unchanged. All enrolled patients signed the informed consent form approved by the Institutional Review Board.

Drug dosing

The treatment was administered as an intravenous (IV) bolus plus 24-hour (h) continuous infusion, followed by an additional 24-h of in-hospital observation. A bolus of 1.7 mg, followed by 0.17 mg/h of ZK-807834 was administered to the low-dose...
group, whereas the doses were 2.9 mg + 1.15 mg/h and 2.9 mg + 2.1 mg/h for the medium- and high-dose groups, respectively. The doses of ZK-807834 were determined through an open-label, dose-escalation substudy completed earlier, in which the doses were targeted to achieve PT ratios of 1.125, 1.5 and 2.5 (low, medium and high dose, respectively).

**Antithrombotic assessment**

The ex-vivo formation of thrombus was assessed using the Badimon Perfusion Chamber. Perfusion studies were performed just prior to the initiation of treatment (baseline), at the end of the 24-h infusion, at 4 h after the end of infusion, and finally at 8 h after the end of infusion (Fig. 1). For each patient, the size of the thrombus formed at each time-point post-treatment was compared to the corresponding baseline to assess the effect of the drug. The strength of this design is that each subject serves as his/her own control.

The perfusion study setup used in this study has been described in detail in the literature (26, 27). It consisted of three small chambers connected in series, with each having a cylindrical flow channel that allowed non-anticoagulated blood directly from the patient’s vein, to flow over thrombogenic substrates. The substrates in this case were pieces of porcine aorta surgically prepared to expose the deeper, highly thrombogenic medial components of the arterial wall (26, 28, 29). The air-tight setup was submerged in a 37°C temperature-controlled water bath. Output of the chamber was connected to a distal peristaltic pump (Master-Flex 7013, Cole-Palmer Inc., Vernon Hills, IL, USA) calibrated to maintain unidirectional blood flow at the set rate. All the perfusion studies were performed for a period of 5 minutes (min) at a constant flow of 10 ml/min (calculated shear rate of 1,690/s; Reynolds number 60; average blood velocity 21.2 cm/s). The combination of all these factors mimics the rheologic conditions seen in moderately stenotic coronary arteries, and has been shown to result in consistent levels of platelet deposition (28, 30).

After completion of each perfusion study, the porcine aortic specimens were immediately fixed in 4% paraformaldehyde. Histologic sections (5 µm) were prepared from each specimen and stained with Combined Mason-Trichrome elastin (CME), which stains thrombus red. The morphology of the thrombi that form on the exposed substrates has been shown to be similar to those that formed on human arterial segments that contain lipid-rich plaques (26, 29, 31).

Morphometric analysis of thrombus was conducted at tenfold magnification by an observer blinded to the treatment assignments. Images were digitized and the thrombus area on each section was measured by computerized planimetry using Image Pro Plus software. At least three sections were measured for each chamber, and the results were averaged to determine the thrombus area of that chamber. The results of the three chambers were averaged to represent the overall thrombus formation for each perfusion study. Results were expressed as percent of baseline at 0, 4 and 8 h after the end of infusion.

**Prothrombin time**

Prothrombin time was assessed before infusion and after the 24-h infusion. The PT ratio was calculated as the PT at 24 h relative to baseline. These were assessed to investigate whether the PT ratios were close to target values for each dose of the drug.

**Factor-X activity**

Blood samples were collected at baseline and at the end of 24-h infusion. Factor-X coagulant activity (FX:C) was measured at the Pfizer Global Research and Development Laboratories with a prothrombin time clot-based assay using RecombiPlasTin (Hemoliance, Lexington, MA, USA) and an automated coagulometer (MLA Electra 1400C, Medical Laboratory Automation, Inc., Pleasantville, NY, USA). Using this assay, the clotting time of test plasma (collected in 3.2% sodium citrate at a dilution of 1:9 parts blood) was compared to reference curve constructed using plasma containing known quantities of factor-X activity. Results are reported in terms of percent of baseline factor-X activity.

**Plasma drug concentrations**

Plasma ZK-807834 concentrations were measured at the Pfizer Laboratories by liquid chromatography/tandem mass spectrometry.

**Statistical analysis**

Multiple regression with an F-test was used by J. C. to test the effect of ZK-807834 dose (treated as a factor variable) on percent reduction in thrombus at the end of infusion relative to baseline (equivalent to ANOVA). A p-value of 0.05 or lower was considered statistically significant evidence of an effect. An approximate 95% confidence interval (95% CI) for the mean in each dose group was calculated as the sample mean plus/minus two standard errors estimated via the model. Pairwise comparisons were also made between each dose and placebo based on the linear regression model using the placebo group as the baseline level of the factor variable. The same approach was used for analysis of thrombus reduction at four and eight hours after infusion, as well as for other recorded variables. Pearson’s correlation coefficient was estimated to summarize the degree of association between prothrombin time (relative to baseline) and ZK-807834 plasma concentration, and a simple linear regression model with intercept at one was fitted to estimate the degree to which the PT ratio increased with increasing ZK-807834 concentration.

**Results**

Table 1 displays the baseline characteristics for the randomized patients. The characteristics of the patient population were generally comparable across the groups. One difference was the absence of current hypercholesterolemia in the placebo group which was present in all the treatment groups. However, since no new lipid-lowering medications were prescribed to any of the study patients and medications prescribed prior to enrolling in the study were not altered, any confounding effect, if present, was minimal.

None of the patients experienced any adverse event during the entire study period.

**Thrombus formation**

There was strong evidence (p<0.001) of an effect of drug dose on thrombus reduction. For the low-, medium- and high-dose
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Table 1: Baseline characteristics.

ZK-807834 groups, mean reductions in thrombus size at the end of infusion (EoI) from respective baselines were 29% (95% CI: 16% to 42%), 34% (21% to 47%) and 68% (56% to 80%), respectively. Figure 2 shows the antithrombotic effect seen in each group at each time-point while Figure 3 shows the plasma drug levels at the corresponding time-points. At 4-h post EoI the mean reductions were 20% (10% to 29%), 30% (20% to 39%) and 35% (26% to 44%) (p=0.02), and at 8-h post EoI they were 11% (2% to 21%), 19% (9% to 29%) and 27% (18% to 36%), respectively (p=0.01). The greatest inhibition in thrombus formation was observed with high-dose ZK-807834. The results demonstrate a dose-dependent inhibition of thrombus formation. No significant change was observed in the platelet counts of the patients before or after the treatments (226 ± 88 vs. 234 ± 109 x10^3/ul, p=NS).

A sub-analysis of male versus female patients did not show any significant differences in the placebo, low- or medium-dose groups. In the high-dose group, the 24-h infusion resulted in a higher mean plasma drug level in females than in males (AUC 13211 vs. 8678 respectively), which was statistically significant at 5, 8, 12 and 18 h of infusion. This was also reflected in a higher mean antithrombotic effect in females at EoI (77% vs. 59%), 4-h post EoI (40% vs. 30%) and at 8-h post EoI (31% vs. 23%). The differences in the antithrombotic effect however, were not statistically significant.

Prothrombin time

PT prolongation increased with increasing doses of ZK-807834. The PT ratios (EoI relative to baseline) were 1.37 (95% CI: 0.98 to 1.29), 1.44 (95% CI: 1.28 to 1.59) and 2.10 (95% CI: 1.96 to 2.24) for low, medium and high doses of ZK-807834, respectively. These can be compared to the target PT ratios of 1.125, 1.5 and 2.5, respectively (Fig. 4). There was a strong linear relationship between PT prolongation and ZK-807834 plasma concentration (Pearson’s r=0.93) for the concentrations of ZK-807834 up to 500 ng/ml seen in this study (Fig. 5). We estimated that for every 100 ng/ml of ZK-807834, the PT ratio increased by 0.24 units (95% CI: 0.21 to 0.27).

Factor-X activity

Factor-X activity decreased with increasing doses of ZK-807834. Mean percent reduction (± standard error) from baseline in factor-X activity was 0.7% (± 5%), 13% (± 3.5%), 42% (± 3.5%) and 58% (± 3.3%) for placebo, low, medium and high doses, respectively. Factor-X activity was significantly inhibited in the medium- and high-dose groups (p<0.001 for both comparisons with placebo).

Figure 2: Reduction in thrombus size. Mean and 95% confidence intervals for percent thrombus reduction at 0, 4 and 8 hours after the end of infusion (EoI) for each ZK-807834 dose. Points represent actual reductions seen at end of infusion in eighteen patients of this study.
Discussion

This is the first human study to evaluate the ex-vivo arterial antithrombotic efficacy and duration of action of the direct factor-Xa inhibitor ZK-807834. Antithrombotic efficacy was examined using the ex-vivo Badimon perfusion chamber with deeply injured artery sections at rheologic conditions mimicing moderate stenosis. Antithrombotic potency was quantitated by direct morphometric analysis of thrombus formed over 5 min as previously done with abciximab and clopidogrel in patients on aspirin (32, 33). This quantitative potency appears to reflect the relative potency of antithrombotic therapy in clinical trials (32–34).

In a porcine model of arterial thrombosis, ZK-807834, administered to yield PT ratios of 2.0–2.5 times control, inhibited thrombosis and its progression, and de-aggregated half-hour-old arterial thrombi. However, enoxaparin, an indirect FXa inhibitor which cannot bind bound-FXa, was no better than saline in the porcine arterial crush injury model in reducing growth of arterial thrombus or enhancing its de-aggregation (25). Pigs are similar to humans in that they spontaneously develop atherosclerosis and have highly thrombogenic arteries after deep arterial injury and thus appear to predict potency in humans (35).

The doses of ZK-807834 were determined through an open-label, dose-escalation substudy completed earlier. In the double-blind study, doses were targeted to achieve PT ratios of 1.125, 1.5 and 2.5 (low, medium and high dose, respectively). There appears to be a dose-dependent effect with the highest dose showing the greatest reduction of thrombus, which was evident until eight hours after the end of drug infusion. The dose-response relationship in the PT prolongation, which strongly correlates with the plasma ZK-807834 concentration, may be used to monitor drug dosing. The substantial anti-thrombotic effect lasting for eight hours after stopping infusion of the high dose should allow for the administration of a loading dose of clopidogrel and for it to start exerting its antithrombotic effect, which is evident by two hours after dose initiation (33).

The current study clearly demonstrates that ZK-807834 has strong antithrombotic potency, similar to that of platelet-fibrinogen receptor antagonist abciximab. In the Badimon Chamber
model, clinical doses of abciximab reduced thrombus formation by 40–58% compared to aspirin alone (32). ZK-807834 is also more potent than clopidogrel in human studies, which reduced thrombus formation by 23% compared to aspirin alone (33). This relative potency of clopidogrel plus aspirin versus aspirin alone was reflected in the CURE study of patients with unstable angina or non-Q-wave myocardial infarction. It showed a 20% reduction in cardiovascular death, myocardial infarction or stroke in patients treated with clopidogrel plus aspirin compared to aspirin alone (34). Based on historical comparisons, ZK-807834 is also considerably more potent than either heparin or low-molecular-weight heparin, which do not reduce arterial thrombus formation over 5 min, compared to aspirin alone under the same ex-vivo conditions, in patients with coronary disease (32).

In this small study, ZK-807834 showed good safety and was well-tolerated with no side-effects in patients with stable CAD taking aspirin. There were no bleeding complications (major or minor), nor any apparent changes in primary hemostasis. Finally, preclinical and clinical studies have demonstrated that ZK-807834 activity can be monitored routinely using PT ratios or anti-Xa assays, thus providing reliable methods to ensure safe and accurate dose titrations.

Collectively, these preclinical observations, and the present human study of ex-vivo reduction of arterial thrombosis with ZK-807834, suggest that this drug has the potential to provide significant antithrombotic benefits in treatment of patients with acute coronary syndromes and for patients undergoing percutaneous intervention.

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References