Antithrombotic Effects of DX-9065a, a Direct Factor Xa Inhibitor
A Comparative Study in Humans versus Low Molecular Weight Heparin

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Summary

Background: Recent evidence suggests that TF may play a causal role in acute coronary syndromes, and may be an important therapeutic target. Several inhibitors of TF, coagulation factors VIIa and Xa are under investigation as novel antithrombotic approaches. We compared the antithrombotic effects of DX-9065a, a new FXa inhibitor, vs. enoxaparin.

Methods and Results: The protocol was an open-label crossover study. Subjects (n = 6) participated in 3 consecutive study-arms: a) enoxaparin + ASA (1 mg/Kg s. c + 162 mg/day x 3 days), b) three escalating doses of DX-9065a (1 mg bolus + 0.25 mg/h x 2 h, followed by an additional 1 mg bolus + 0.625 mg/h x 2 h and, a final 1 mg bolus + 1.25 mg/h x 2 h), and c) the same doses of DX-9065a in Arm 2 plus ASA pre-treatment. The antithrombotic effects were assessed using the Badimon perfusion chamber at each dose level.

The administration of DX-9065a whether alone or combined with ASA significantly inhibited thrombus formation at high and low shear rate conditions while enoxaparin did not have a significant effect. Furthermore, these antithrombotic effects were obtained without significant prolongations of the standard coagulation parameters as those induced by enoxaparin.

Conclusions: The direct inhibition of FXa by DX-9065a appears to be a safe and effective new approach for preventing the thrombotic complications of atherosclerotic disease. The clinical effectiveness of the direct FXa inhibitors should be further investigated.

Introduction

Clinical and pathologic evidence indicate that acute thrombus formation over a disrupted or eroded atherosclerotic plaque plays a critical role in the onset of the acute coronary syndromes. The magnitude and stability of the formed thrombus seem to modulate the severity of the acute coronary syndromes (1). The importance of thrombosis in the pathogenesis of these syndromes is demonstrated by the significant clinical benefits associated with the use of antithrombotic agents (2).

Evidence suggests that plaque vulnerability is determined by its composition rather than stenosis severity. A lipid-rich plaque, usually eccentric and mildly stenotic (<50% stenosis), has been identified to be the “culprit lesion” in approximately 70% of patients who died from a cardiovascular cause (3). Disruption of an atherosclerotic plaque facilitates the interaction between flowing blood and the subendothelial components of the lesion; i.e. the highly thrombogenic lipid core particularly rich in tissue factor (TF).

Previous research by our group suggested that “vulnerable” plaques are not only prone to disruption, but also have the highest thrombogenic potential after disruption. The TF of lipid-rich plaques seems to modulate the high thrombogenicity of these lesions (4). Furthermore, specific inhibition of the TF pathway results in a significant reduction in thrombogenicity (5). Recent studies suggest an important role for TF in triggering and sustaining the thrombogenic stimulus (6-10).

These observations identify TF and TF pathway as an important therapeutic target in thrombosis.

Several inhibitors of TF, factors VIIa and Xa have been developed and are under investigation as novel antithrombotic approaches. Among these agents, FFR-rFVIIa (NovoSeven) and the indirect Factor Xa inhibitor Fondaparinux are undergoing clinical evaluation. FFR-rFVIIa, a modified recombinant factor VIIa with the active site irreversibly blocked by a synthetic tripeptide – chloromethyl ketone, (11) is undergoing clinical investigation within the ASIS trial (12). Fondaparinux, has been shown to improve the risk-benefit ratio for the prevention of venous thromboembolism as compared with LMWH in patients undergoing total hip replacement (13, 14).

DX-9065a, a synthetic, low-molecular-weight factor Xa (FXa) inhibitor. It neutralizes factor Xa (K i = 0.041 µmol/L) with little effect on other proteases, particularly thrombin (K i >2000 µmol/L) (15).

DX-9065a directly and reversibly inhibits FXa in a manner independent of antithrombin III and inactivates both free and thrombus-associated FXa. DX-9065a inhibits both free and clot-bound prothrombinase, and its antithrombotic efficacy has been demonstrated in different animal models of thrombosis (disseminated intravascular coagulation, venous thrombosis, arteriovenous shunt thrombosis, and vein-graft thrombosis) (16-18). These properties make DX-9065a an attractive alternative to the low-molecular-weight heparins (LMWH), however, the in vivo antithrombotic properties of DX-9065a in humans are presently unknown.

Based on these observations, we designed a human open-label, crossover study to compare the antithrombotic effects of the factor Xa inhibitor, DX-9065a alone and with aspirin (ASA) to LMWH plus ASA.
Methods

Subjects

Six healthy, non-smoking, normolipemic, male volunteers, 22 to 45 years of age (mean 31 ± 10 yrs), who had not taken aspirin or any other antiplatelet agent in the previous 10 days were identified and enrolled into the study. Upon screening, hematology, haemostatic, chemistry, and hepatic profile examinations were within the normal range. The Institutional Review Board for Human Subjects Research approved this study, and all patients gave written, informed consent prior starting the study.

Study Protocol

The protocol was an open-label crossover study. All the subjects participated in the 3 study arms:
Arm 1: One dose of enoxaparin in combination with ASA,
Arm 2: Three escalating doses of DX-9065a without ASA pre-treatment, and
Arm 3: The same doses of DX-9065a in Arm 2 after ASA pre-treatment.

There was a washout period of at least three months between each study arm.

Enoxaparin was dosed subcutaneously at 1 mg/kg body weight (as in ESSENCE trial) (19). In Arms 1 and 3, ASA was orally given at a dose of 162 mg/day for 3 days prior to the study visit. Three consecutive, escalating doses were administered in the DX-9065a arms: A) 1 mg intravenous (IV) bolus + 0.25 mg/h IV infusion for 2 h, B) an additional 1 mg bolus + 0.625 mg/h × 2 h and, C) a final 1 mg bolus + 1.25 mg/h × 2 h. Perfusion experiments were performed each day prior to the administration of the drug regimen corresponding to each arm and at 4 h post-enoxaparin dosing in Arm 1, and 1 h and 45 min after initiation of each of the 3 DX-9065a doses in Arms 2 and 3.

DX-9065a and enoxaparin were obtained from Daiichi Pharmaceutical Co., Ltd. (Tokyo, Japan) and Aventis Pharmaceutical Products Inc. (Collegeville, Pa) respectively.

Antithrombotic Assessment

The antithrombotic effects of the different regimens under study was assessed by determining the changes in thrombus formation in comparison to baseline values using the previously validated Badimon perfusion chamber (11, 20, 21). The perfusion chamber system is a cylindrical flow channel that allows flowing blood, pumped directly from the subject’s vein, to flow over an exposed thrombogenic substrate. Porcine tunica media served as thrombogenic substrate. For each perfusion study, blood was circulated through the chambers connected in series at a constant flow maintained by a peristaltic pump to 20, 40 and 80 ng/ml after the administration of 3 consecutive dose increments (bolus and 2 h infusion). As indicated in the figure, the objective was achieved, and as expected the combination with ASA did not have any effect on plasma levels of DX-9065a.

Blood Sampling for Laboratory Assessments

For each arm, subjects were asked to fast overnight and were admitted the next morning to the Mount Sinai General Clinical Research Center for blood sampling. An 18-gauge IV catheter was placed into the brachial vein of the non-dominant arm for blood collection. To ensure IV patency, normal saline was administered at a rate of 10 cc/h between blood draws. For the DX-9065a arms a 21-gauge IV catheter was placed in the dominant arm for drug infusion.

Blood samples for evaluation of coagulation and haemostatic parameters were collected at pre-selected time points. Samples for Prothrombin time (PT), Activated Partial Thromboplastin Time (aPTT), Thrombin Time (TT), Ecarin Clotting Time (ECT), and prothrombin fragment F1+2 were drawn in 3.2% sodium citrate tubes. Samples for anti-Xa activity were collected in SCAT tubes (containing PPACK, EDTA, and aprotonin; Hematological Technologies Inc., Essex Junction, Vermont). Finally, DX-9065a plasma levels were drawn in 4.5-nmol/L EDTA tubes (Becton Dickinson, Franklin Lakes, NJ). After sample collection, platelet-poor plasma was obtained by centrifugation (30,000 g × 30 min at 4°C). After separation into aliquots, the plasma samples were kept frozen at -70°C until analysis.

Coagulation and Haemostatic Parameters

aPTT, PT, and TT measurements were performed using an automated coagulometer (STA-R, Diagnostica Stago, Parsippany, NJ). ECT was determined with a ball-type coagulation analyzer (ST4, Diagnostica Stago, Parsippany, NJ). Using a standardized lyophilized ecarin reagent (Pentapharm, Basel, Switzerland).

Thrombin generation was assessed by measuring prothrombin fragment F1+2 by using a commercially available kit (Enzygnost F 1+2 macro, Dade Behring, Marburg, Germany). Dr. E.G Bovill (Colchester Research Facility, University of Vermont), measured anti-Xa activity using a Rotachrom chromogenic anti-Xa assay (Diagnostica, Stago, France). Results of this assay are expressed in heparin anti-factor Xa units as measured against the manufacturer’s heparin standard curve.

Direct measurement of DX-9065a was performed by liquid chromatography/mass spectroscopy (Bianalytical Systems, Inc., West Lafayette, Indiana).

Template bleeding time was measured using a disposable standard device (Surgicut Junior, International Technidyne Corporation, Edison, NJ) after a sphegmomonometer cuff on the upper arm was inflated to 40 mm Hg. Bleeding time measurements were performed at baseline (after three doses of ASA in Arms 1 and 3), 3 h and 45 min after enoxaparin administration in Arm 1, and 1 h 45 min after initiation of the third and highest dose of DX-9065a in Arms 2 and 3.

Statistical Analysis

Results are expressed as mean ± standard error of the mean unless indicated otherwise. Repeated measures analyses of variance (ANOVAS) were Arm by time within-measures designs. Statistical significance was considered as a 2-tailed probability <0.05.

Results

Plasma DX-9065a Levels and Anti-Xa Activity

Plasma levels of the factor Xa inhibitor DX-9065a achieved through the duration of the perfusion experiments corresponding to Arms 2 and 3 are presented in Fig. 1. The doses used in the experimental design were selected on basis of achieving theoretical plasma levels corresponding to 20, 40 and 80 ng/ml after the administration of 3 consecutive dose increments (bolus and 2 h infusion). As indicated in the figure, the objective was achieved, and as expected the combination with ASA did not have any effect on plasma levels of DX-9065a.
More importantly, as shown in Fig. 1, anti-Xa activity after the administration of DX-6905a followed an identical pattern to plasma drug levels. Similarly, the addition of ASA did not exert any additional effect on anti-Xa activity. The strong correlation existent between plasma DX-9065a levels and anti-Xa activity may play a potential role in determining anticoagulant response.

**Effect on Blood Thrombogenicity**

The antithrombotic effects of the different drugs regimens are presented in Figure 2. The antithrombotic properties are expressed as changes in thrombus formation as a percentage of the corresponding baseline.

**High shear rate conditions (Fig. 2, right panel):** Under rheologic conditions typical of high shear rates, the administration of enoxaparin in combination with ASA did not have any effect on platelet-thrombus formation (13,835 ±1,227 μm² for baseline versus 13,018 ±1,041 μm² for post-treatment, p = NS).

Arm 2 of the study involved the administration of DX-9065a alone. Mean baseline thrombus area was similar to the baseline obtained prior to enoxaparin administration. The factor Xa inhibitor already induced a significant inhibition in thrombus formation after the first dose.

![Fig. 1](image1.png)

*Fig. 1* Plasma levels of DX-9065a (right) and Factor Xa inhibition (left) through the study. Plasma levels of DX-9065 are expressed as ng/ml. Anti Factor Xa activity is expressed as heparin anti-factor Xa units (see text). * Indicates each of the perfusion studies after administration of the different regimens

![Fig. 2](image2.png)

*Fig. 2* Antithrombotic effects of the different drug regimens at low (right) and high (left) shear rate conditions. Data are expressed as percentage of change vs. the corresponding baseline values; (X ± SEM; * p< 0.05)
Arm 3 involved the administration of the same doses of DX-9065a as in the second arm but the subjects were pre-treated with ASA. The results on platelet thrombus formation showed a similar pattern to the second arm. The first dose of the factor Xa inhibitor already achieved a statistically significant reduction in thrombus formation (8,533 ± 681 μm² for the first dose versus 12,177 ± 571 μm² for baseline, p = 0.003). Likewise, increasing doses of DX-9605a resulted in larger reductions in thrombus formation (6,937 ± 296 for the second DX-9065a dose and 7,591 ± 564 μm² for the third DX-9065a dose, p = 0.001 and p = 0.003 respectively). Although an additive effect of the combination of Dx-9065a with ASA seems to be present in our study, the number of subjects involved does not allow reaching a definitive conclusion.

**Low shear rate conditions (Fig. 2 left panel):** When the perfusion studies were performed under low shear rate conditions, the first observation was the clear effect of rheology on platelet-vessel wall interaction and thrombus formation. As such, baseline values at low shear rate were significantly lower than those obtained at high shear rate conditions for the same subjects. As expected, the difference was highly significant (13,835 ± 1,277 μm² for high shear rate versus 4,534 ± 487 μm² for low shear rate, p = 0.0001).

The administration of enoxaparin did not exert an antithrombotic effect at low shear rates (4,488 ± 268 μm² for post-treatment versus 4,534 ± 487 μm² for baseline, p = NS). Despite a trend towards a dose-dependent antithrombotic effect with the three doses of DX-9065a in Arm 2 (p = 0.078 at the highest dose), none of the doses achieved statistical significance. Even though the highest one was borderline when compared to baseline values (3,424 ± 372 μm², p = 0.069). The lack of significance may have been attributed to the fact that only 6 subjects were enrolled, and the significantly lower thrombus formation values obtained at low vs. high shear rate conditions.

When the FXa inhibitor was investigated in combination with ASA, the antithrombotic effects followed a similar pattern than when administered without ASA; but under these conditions, the inhibitory effect observed with the highest dose of the inhibitor reached statistically significance (5,517 ± 685 vs 7,161 ± 285 μm²; p <0.05).

**Effects on Coagulation and Hemostatic Parameters (Table 1)**

In addition to the antithrombotic effects of the different regimens being tested, we also analyzed their effect on hematologic parameters as an indication of their potential safety for clinical use. Consequently, we studied the effects of these agents on aPTT, PT, TT and ECT. In addition, we also studied the effect on ACT values since one of the first potential clinical applications of this agent would be the prevention of thrombotic complications associated to percutaneous coronary interventions (PCI); currently under investigation in the on-going Xanadu PCI Pilot trial.

In accordance with reports by other investigators, enoxaparin administration induced a prolongation of aPTT, PT, and TT (an increase of 25%, 4%, 41% respectively; p <0.05). The prolongation of these parameters peaked at 4 h post administration and corresponds to the time when enoxaparin anticoagulant activity is known to be highest. Bleeding time was also significantly prolonged vs. baseline values, but remained within the normal range. Lastly, there was no effect on ECT, ACT or prothrombin fragment F1+2 levels with enoxaparin administration.

The administration of DX-9065a, whether alone or in combination with ASA, had no effect on TT but induced a small but significant increase in both aPTT and PT. DX-9065 alone had no effect on ACT, ECT or F1.2 plasma levels. When combined with ASA, bleeding time was significantly prolonged vs. baseline values, but remained within the normal range.

The lack of effect of any of the treatments on F1.2 plasma levels, could be probably explained in basis of the normal and healthy population selected for the study. Their baseline levels of F1.2 were, as expected, within the normal range and, thus quite difficult of being significantly reduced.

| Table 1 Effect of the different drug regimens on different hematologic parameters. Data on aPTT, TT, PT, ACT and ECT are expressed as seconds. BT expressed as minutes and F1.2 as ng/ml * p <0.05 vs. its respective baseline control |
Analysis revealed that enoxaparin induced a significantly greater increase in both aPTT and TT in comparison to either DX-9065a arm. In contrast, PT prolongation was significantly greater with DX-9065a administration with and without ASA (p <0.05 after the highest dose) than with enoxaparin. However, the increases in coagulation parameters were of little clinical relevance. Lastly, DX-9065a administration with and without ASA significantly prolonged bleeding time similarly to enoxaparin, and the values remained within the normal range.

Discussion

In this open-label, crossover study, we have investigated the antithrombotic effects of a new inhibitor of coagulation factor Xa, DX9065a, alone and in combination with ASA in six healthy male volunteers. The study was performed using the Badimon perfusion chamber and compared its effects to those observed with enoxaparin. Overall, DX-9065a showed superior antithrombotic effects at doses that were associated with only modest increases in aPTT and PT vs. baseline and similar to those induced by the administration of enoxaparin.

The role of acute thrombus formation in the onset of acute coronary syndromes and progression of atherosclerosis has been clearly demonstrated. Furthermore, antithrombotic agents have significantly reduced the mortality of acute coronary events (2). Despite the improvements achieved in this area, cardiovascular event rates remain unacceptably high.

Recent evidence has implicated TF and TF pathway as a putative triggers for platelet activation and thrombosis (6-10). The same evidence suggests that the inhibition of TF pathway is a novel therapeutic approach in reducing the thrombotic complications of coronary disease. DX-9065a is the first of a new class of antithrombolytic agents based on the direct inhibition of FXa as their major mechanism of action.

The potential limitation of having a small number of subjects involved in our study was minimized by the experimental design. This is the first human study with a dose-finding and proof of concept as major objectives; these studies are generally carried out in a reduced number of human healthy male volunteers. To avoid the potential limitation of the reduced number of subjects involved in the study, we selected the study design in which the same subjects participate in the three arms of the study. Thus, practically abolishing the intra-group variability. In addition, the design allows for each subject to serve as their own control in each of the three arms of the study allows the expression of the data as a mean value and as a percentage of its corresponding baseline.

Our data indicated that D-9065a significantly inhibits thrombus formation at both high and low shear rate conditions. At high shear rate conditions the inhibitory effects of DX-9065a were significant even with the lowest dose tested whether alone or combined with ASA. Although not statistically significant, there was an indication of an additive effect when DX-9065a was given with ASA (Figure 2). A larger clinical trial involving more subjects would help better characterize this potentially important interaction. Interestingly, the inhibitory effects on platelet-thrombus formation by DX-9065a were achieved without having modified aPTT or PT to a clinically relevant degree. Further, under the same experimental conditions, the administration of enoxaparin at the dose used in the ESSENCE trial (19) did not have any effect on acute thrombus formation.

The superior antithrombotic efficacy of the enoxaparin versus unfractionated heparin in the treatment of ischemic stroke, deep venous thrombosis and unstable angina has been demonstrated in several clinical trials (19, 22, 23). However, in our study, the LMWH enoxaparin did not have a significant effect on acute thrombus formation. We have to take into account the significant differences in the design, follow-up and end-points in the different studies. Our study investigated the acute antithrombotic effects while the clinical trials involved a longer follow-up and clinical endpoints. Similar results were already reported by Roffer et al. (24), they experimental study showed no significant differences in the acute antithrombotic effects of Reviparin when compared with unfractionated heparin. A significant inhibition on fibrin deposition was noted in the LMWH group. The authors postulated that the reduction in the fibrin content of the thrombus may be associated to a diminished stability of the formed thrombus, and would render it more susceptible to fibrinolysis, either endogenous or promoted by adjunctive treatment.

One of the first clinical presentations in which to investigate the effectiveness of this agent would probably be the inhibition of acute thrombotic complications associated to PCI. Therefore, we also assessed the effects of DX-9065a on ACT, the parameter more widely used by invasive cardiologists for assessing the anticoagulation of the patients and making clinical decisions. In our study, none of the doses of DX-9065a induced a significant prolongation of the ACT values. These observations strongly suggest that direct inhibitors FXa show a significant partition between their “anticoagulant” surrogates, evaluated as prolongation of the usually monitored coagulation parameters, and their “antithrombotic” properties documented as an inhibition of thrombus formation. Furthermore, the preliminary data indicating that there were only modest increases in aPTT and PT suggests the possibility of administering higher doses of the factor Xa inhibitors without a concurrent increase in bleeding complications. The recently published XANADU trial describes the first human study on the safety of DX-9065a in patients undergoing elective PTCA (25).

In addition to its effects on the clotting cascade, recent reports have indicated that FXa is involved in several other cellular and molecular events including the release of cytokines, expression of adhesive molecules, and cell proliferation and migration (26). Therefore, the inhibition of FXa will not only effectively inhibit the clotting cascade but may also attenuate the mechanisms involved in the pathogenesis of various diseases such as arterial restenosis, venous corona graft disease, acute inflammatory responses, sepsis, and even cancer.

In summary, our data suggest that the inhibition of FXa should be considered a new and efficacious antithrombotic target to prevent the acute complications of thrombosis. Further, DX-9065a appears to be safe and a potentially effective treatment in reducing the thrombotic complications of atherosclerotic disease. These initial observations are encouraging and suggest that a broad range of plasma concentrations can be achieved reliably and safely. The clinical effectiveness of the direct FXa inhibitors should be further investigated.

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