A current view of vascular thrombosis emphasizes the importance of cellular surface biochemistry and the integrated contribution of platelets, monocytes and endothelial cells. Initiation of thrombosis occurs on a tissue factor-bearing cell (monocytes, macrophages, dysfunctional or injured endothelial cells), while amplification (or priming) requires activation of platelets and coagulation proteases. The final phase, propagation is dependent on thrombin generation occurring on platelet surfaces (1) (Fig. 1).

The cell-based model of vascular thrombosis also highlights sequenced or biochemical stages rather than a more traditionally held view of separate coagulation pathways or cascades (intrinsic, extrinsic). Accordingly, tissue factor is considered the key protein for initiation of thrombosis, wherein its ability to complex with factor VIIa (fVIIa) and activate fX ultimately results in thrombin generation. Even small amounts of thrombin substrate are important biochemically because of its ability to activate platelets, fIV, fVIII, fIX and fXI through a series of bioamplification steps. With increasing concentrations of locally generated thrombin available (burst thrombin generation phenomenon), soluble fibrinogen is converted to insoluble fibrin followed rapidly by polymerization and FXIIIa-mediated covalent bond stabilization.

Thrombin, the primary procoagulant effective enzyme in hemostasis and pathologic vascular thrombosis is a potent platelet activator and possesses a diversity of effects on inflammatory cells, smooth muscle cells and vascular endothelial cells. Human thrombi contain an abundance of active thrombin (2) and thrombin activity has been identified in the subendothelial extracellular matrix following coronary intervention (3, 4).

**Factor X**

Factor X (fX) (Mr 58800) is a vitamin K-dependent glycoprotein synthesized in the liver and subsequently secreted into the plasma as a precursor to an active serine protease fXa. The human protein is composed of a light chain (Mr

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**Summary**

The development of anticoagulants for treating patients with atherothrombotic disorders of the arterial circulatory system has focused, either directly or indirectly, on thrombin – a pleuripotential effector enzyme with prothrombotic and proinflammatory properties. The pivotal role of factor (f) Xa in thrombin generation, coupled with its direct cellular effects and widely recognized limitations of currently available anticoagulants, has led to the development of pharmacologic inhibitors of this important protease. The following review focuses on DX-9065a – first in a class of direct, selective and reversible fXa antagonists – and its potential applications in the management of patients with cardiovascular disease.

**Keywords**

DX-9065a, Factor Xa, antagonist
The gene for human factor X is located on chromosome 13 at q 32-qter in approximately 27 kb of DNA. The mRNA for factor X is approximately 1500 nucleotides long and includes 1475 nucleotides that code for a prepro leader sequence of 40 amino acids, a light chain of 139 amino acids, a connecting peptide and 303 amino acids that constitute the heavy chain (5, 6).

Factor X is activated by excision of a small peptide from its heavy chain. The cleavage of an alanine–isoleucine peptide bond by either TF-fVIIa or fVIIIa-fIXa complex liberates the 52 amino acid peptide, providing a potentially measurable marker of fX activation (7). Under optimal conditions (high concentrations of TF), the TF-fVIIa complex can activate fX and, in essence, bypass the contribution of fVIIIa-fIXa.

The prothrombinase complex (fV a, fXa, phospholipid, calcium) which converts prothrombin to thrombin, is a membrane-dependent process that requires several binding steps. fXa has a γ-carboxyglutamic acid domain that is responsible for calcium and phospholipid binding and two epidermal growth factor (EGF)-like domains that participate in fV a binding. Prothrombin activation occurs in two stages. The first involves a rapid cleavage at Arg274-Thr275. Meizothrombin accumulates as the principle intermediate of the first phase and remains proteolytically active as a membrane-bound substrate until its release following a second cleavage at Arg543-Ile544. The membrane dependency of prothrombinase (and serine proteases including fXa) provides protection from circulating inhibitors (8). In addition, membrane binding limits the degrees or freedom for interacting molecules and restricts them to reactions in two dimensions (rather than three in fluid phase).

**Factor Xa: cellular binding and biological effects**

Factor Xa binds to human umbilical vein endothelial cells via a single class of binding sites with a dissociation constant value of 6.6 +/− 0.8 nM and density of 57460 +/− 5200 sites per cell. The binding kinetics are considered “pseudo” first order with association and dissociation constants of 0.15 × 10⁶ m⁻¹s⁻¹ and 4.0 × 10⁻⁴ s⁻¹, respectively. FXa binding to vascular endothelial cells is not influenced by thrombin, fVa, antithrombin or tissue factor pathway inhibitor but is blocked by antibodies specific for effector cell protease receptor (EPR-1), supporting its role in fXa-endothelial cell interactions (9, 10). The binding of fXa is associated with the following events: 1) increased intracellular calcium; 2) increased phosphoinositide turnover; 3) tissue factor expression; 4) tissue plasminogen activator release; 5) plasminogen activator inhibitor release; 6) interleukin –6 and 8 release; 7) cellular proliferation (11), 8) expression of E-selectin, ICAM-1 and VCAM-1; and, 9) nitric oxide release (12). The ability of indirect and direct antagonists to inhibit fXa-mediated cellular effects (without impacting its surface binding capacity) suggests strongly that catalytic activity is the determining feature (13, 14). Macrophages, localized within atheromatous plaques can synthesize fXa (15). An ability of fXa to promote smooth muscle cell proliferation suggests that local prothrombotic responses may also influence arterial remodeling following injury (16). The mitogenic response to fXa probably involves protease activated receptor-2 (PAR-2). Functional PAR-2, an autoactivating tethered ligand, is widely distributed in human vascular endothelial cells and smooth muscle cells (17): FXa also exerts mitogenic effects through platelet-derived growth factor (18).

Leukocyte proliferation has been observed following fXa activation (19). In turn, pro-inflammatory cytokines are released which activate fX (fXa) (20). FXa also promotes recruitment of mast cells and their secretion of vasoactive mediators including histamine and serotonin (21).

**Regulation of Factor Xa and tissue factor activity on vascular surfaces**

Vascular thromboresistance is a vital component of circulatory homeostasis. Because tissue factor is recognized as the predominant initiator of arterial thrombosis in atherosclerotic vascular disease, tissue factor pathway inhibitor (TFPI) has emerged as the preeminent regulatory protein in physiologic thromboresistance.

Human TFPI is a modular protein composed of three tandem kunitz-type domains flanked by peptide fragments (22). The K₁ domain inhibits fVIIa complexed to tissue factor, while...
the K₂ domain inhibits fXa; no direct protease activity has been identified for the K₃ domain. The primary site for TFPI synthesis is within vascular endothelial cells, with the coronary arteries possessing the greatest quantity of TFPI mRNA (23) and potential to release protein stored within intracellular pools in response to shear stress (24), thrombin (25), activated monocytes (26) and exogenous heparin. Work performed in our laboratory shows that continuous exposure of vascular endothelial cells in culture to unfractionated heparin caused progressive TFPI depletion. Although the clinical relevance has not been elucidated, heightened thrombin generation both during and after unfractionated heparin administration may reflect an acquired state of impaired vascular thromboresistance to tissue factor (27) that predisposes to recurrent thrombotic events and treatment failure.

The available information supports a proinflammatory and prothrombotic environment in atherosclerotic coronary artery disease that is governed by the cellular effects of tissue factor and fXa. Unfractionated heparin, through its platelet activating potential and detrimental effects on vascular thromboresistance, in all likelihood limits the compound’s overall role in acute coronary syndromes.

**DX-9065a**

DX-9065a is the first in a class of small molecule, direct, specific and reversible fXa inhibitors. It is a synthetic, non-peptide amidinoaryl derivative (molecular weight 571.07) (Fig. 2) with rapid and reversible binding kinetics for fXa (Ki = 0.041 μM) but not for thrombin (Ki >2000μM). The binding geometry between DX-9065a and fXa is determined by two interaction sites. The naphthamidine group is fixed in the S₃ pocket (a common structural motif of esterase enzymes) of fXa by a typical salt bridge to Asp₁₈₉, while the pyrrolidine ring binds to a unique aryl binding site (S₄) of fXa (28, 29). Unlike a majority of inhibitor complexes with serine proteases, Gly²¹⁶ (S₄) does not contribute to hydrogen bond formation. In contrast to typical thrombin binding motifs, the S₉ site of fXa is not accessible to DX-9065a (30, 31). In addition, thrombin Glu⁹² evokes an electrostatic repulsion for the carboxyl group on DX-9065a, providing a biochemically-based explanation for the high Ki.

This particular feature was considered highly favorable by the scientific development team and clinician-scientists involved in early phase study design for several reasons. First, it provided, for the first time, a selective fXa antagonist that could be utilized to test the overall relevance of fXa (and its direct inhibition) in cardiovascular thrombotic disorders. Second, an ability to preserve enough thrombin for hemostatic purposes was anticipated – potentially “separating” antithrombotic efficacy from bleeding risk (previously thought to be impossible based on the experience with heparin compounds, indirect fXa antagonists and most direct thrombin inhibitors).

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**Pre-clinical evaluation**

**Plasma-based and buffer systems**

The importance of prothrombinase complex assembly and activity in vascular thrombosis provides a basis for their evaluation in experimental systems as an indirect measure of relative antithrombotic potential for pharmacologic agents which target individual coagulation proteases and/or thrombin. DX-9065a has been evaluated in several in vitro and ex vivo systems, and has been shown to inhibit both prothrombinase assembly and activity in a concentration-dependent manner (32). Utilizing a human plasma-based model of clot-bound prothrombinase (prothrombin, fXa, phospholipid, antithrombin and calcium in plasma), DX-9065a inhibited thrombin generation (and activity) more effectively than pentasaccharide (IC₅₀ 20.5 μM) – an indirect fXa antagonist (Fig. 3). Similar observations were made in a buffer-based system containing fXa, phospholipid, fVa and calcium, suggesting that DX-9065a equally inhibits clot-bound and free (on microvesicles) prothrombinase activity (and subsequent thrombin generation) (33). Although the overall clinical relevance of this property, which stems from molecular size and accessibility to fXa in the presence of activated prothrombin, is unknown, attenuated thrombin generation and activity at sites of thrombus propagation may be more readily achieved in pathologic conditions characterized by platelet, monocyte, endothelial cell and coagulation protease activation. In contrast to antithrombin-dependent anticoagulants, which are unable to inhibit clot-bound fXa, the accessibility of fXa contained within an assembled prothrombinase complex to DX-9065a is supported by the direct correlation between Ki for fXa (~10-20 nM) and IC₅₀ for thrombin generation in plasma-based systems (34). The ex vivo incubation of atheroma extracts and platelets elicits a rapid (and profound) increase in prothrombinase activity and thrombin generation. DX-9065a, at a concentration of 100 ng/mL effectively, inhibited thrombin generation in this model (35) providing an explanation for a somewhat higher dose (and concentration) requirement in patients with advanced coronary artery disease (described in subsequent sections).
Cellular effects

Thrombin and fXa are known to exert leukocyte activating properties including cytokine-induced neutrophil chemoattractant (cINC) protein release from macrophages. DX-9065a reduced cINC production in a rat model of hepatic ischemia and perfusion (36). In a similar model, DX-9065a attenuated the release of monocyte tissue factor mRNA in a dose-dependent manner (37). Considered collectively, these observations suggest that microvascular thrombosis following ischemia-mediated organ injury contributes to cytokine production which, in turn, is tempered by antithrombotic therapy targeting fXa and/or thrombin.

The effects of fXa and thrombin on cellular proliferation have been discussed in a previous section. In a rat model of vascular injury, DX-9065a given subcutaneously in doses ranging from 2.5 to 10 mg/kg reduced proliferation of vascular smooth muscle cells (38). DX-9065a also reduced thrombin generation on lung adenoma cells and subsequent thrombin-mediated cellular proliferation (39).

Animal models

Thrombosis

DX-9065a has been evaluated in a wide variety of animal thrombosis models to determine its antithrombotic effects (Table 1). In mice, DX-9065a reduced tissue-factor induced disseminated intravascular coagulation (DIC) and resulting mortality with an ED$_{50}$ of 56 ± 7 mg/kg (oral dose). Utilizing venous stasis and arteriovenous shunt models in rats, Herbert and colleagues (40) identified ED$_{50}$ s for DX-9065a of 1.2 ± 0.7 mg/kg (IV) and 8.1 ± 3.5 mg/kg (IV), respectively. Similar observations were reported in rat endotoxin and thromboplastin-induced disseminated intravascular coagulation (DIC), and copper wire (41) AV shunt models (42). A lower ED$_{50}$ was demonstrated with a rabbit stasis model (0.03 ± 0.01 mg/kg IV). A rat model of mesenteric arteriolar (platelet-rich) and venule (fibrin-rich) thrombosis induced by helium-neon laser vascular injury identified effective inhibition by DX-9065a administered either intravenously (minimum effective dose 3.89 mg/kg) or orally (minimum effective dose 25.9 mg/kg) (43). DX-9065a also inhibited thrombosis in rabbit jugular vein balloon injury and partial stasis models as effectively as pentasaccharide and prevented reocclusion following fibrinolysis achieved with tissue plasminogen activator (38). It was also more effective than unfractionated heparin in a rabbit inferior vena cava-teflon graft model (44). A femoral AV shunt model in baboons that includes platelet-rich (arterial) and fibrin-rich (venous) components was employed to investigate the full antithrombotic potential of DX-9065a (45). The low molecular weight synthetic compound inhibited fXa bound to vascular grafts, platelet deposition and fibrin accumulation (45).

Anticoagulation profile

The preclinical experience with DX-9065a has included plasma measurements of coagulation, thrombin activity and thrombin generation in a variety of animal species. It is important to acknowledge species difference in DX-9065a-mediated anti-Xa activity that also translates to differences in coagulation
measurement profiles; for instance the concentration of DX-9065a required to double the prothrombin time (PT) is 22.2 uM in wister rats and 0.46 uM in squirrel monkeys (46). This suggests the possibility of FXa structural differences at its active center (in contrast to thrombin which exerts structure-function similarities across species). Never-the-less, the preclinical work provides a reference point for coagulation measurements that may be affected by DX-9065a following administration in humans.

Early work by Hara and colleagues (47), using a rat model showed that DX-9065a did not inhibit platelet aggregation in response to collagen, adenosine diphosphate, epinephrine or thrombin. In contrast, DX-9065a given either as an IV bolus (0.78-2.34 mg/kg) or continuous infusion (2.34-7.78 mg/kg/h) prolonged the prothrombin time (PT) (1.4-1.8-fold increase above control value), activated partial thromboplastin time (aPTT) (1.4-2.2-fold increase above control value) and exhibited measurable anti-Xa activity (chromogenic method). Unlike direct thrombin inhibitors, DX-9065a did not prolong the thrombin time (44).

DX-9065a infused at either low (15 ug/min) or high (120 ug/min) rates into baboons yielded dose-dependent prolongation of the aPTT (range 37-60 seconds) without changes in the bleeding time. It also reduced thrombus-dependent elevations in plasma thrombin-antithrombin complex (thrombin generation) and fibrinopeptide A (thrombin activity, fibrin formation) levels (48). The ID₅₀ for inhibiting thrombin generation (thrombin-antithrombin complexes) in a rat copper wire AV shunt model was 0.27mg/kg/h in DX-9065a-treated animals and 0.13 mg/kg/h for argatroban-treated animals, supporting a favorable antithrombotic profile for direct compared with indirect thrombin inhibition (47); however, an ability to prevent thrombosis without a marked increase in bleeding time was achieved most effectively with DX-9065a (compared to heparin compounds, heparinoids, and direct thrombin inhibitors) (Fig. 4).

DX-9065a reduces thrombin generation in vitro, but in contrast to the direct thrombin inhibitor argatroban, exhibits minimal effects on the initial thrombin forming time (49). This may provide an explanation for the observed preservation or hemostatic capacity and lack of a prolonged bleeding time in animal models. The incidence of major bleeding has been low in phase I and II clinical trials.

The responsible mechanisms have not been elucidated; however, only a small amount of thrombin is required for

![Figure 4: The dose-dependent effects of a selective FXa inhibitor (DX-9065a), a selective thrombin inhibitor (argatroban) and AT III-dependent anticoagulants on thrombosis in an AV shunt model and corresponding bleeding times. [From reference #48; with permission.]](image-url)
platelet activation (primary haemostasis), while larger concentrations are needed for the conversion of fibrinogen to fibrin (secondary haemostasis).

**Clinical evaluation**

**Healthy volunteers**

The pharmacokinetic and pharmacodynamic profiles of DX-9065a in humans have been determined through five phase I clinical trials involving a total of 113 individuals. Dosing regimens tested included: 0.625 to 2.5 mg (IV bolus over 1 minute), 5-30 mg (IV over 1 hour), 10-20 mg (IV over 4 hours) and 96 mg (IV over 48 hours). Plasma concentrations decreased in a bi-tri exponential manner (50) (Fig. 5A and 5B) with plasma half-lives ranging from 40 minutes following a single bolus to 5 hours after a continuous 48 hour infusion (concentration-dependent kinetics). The pharmacokinetic characteristics of DX-9065a reflect three compartment dynamics and retention in the liver with slow release. The unique pharmacokinetic profile of DX-9065a is evidenced by changes in plasma concentration in response to varying dosing strategies. Following an IV bolus (10 sec or 1 minute) peak concentrations (Cmax) were achieved rapidly (high bioavailability) and decreased rapidly. By 5 minutes, the plasma concentration was 45% of Cmax; at 15 minutes, 18%; at 30 minutes, 10%; and at 1 hour, 6%. Following a continuous infusion for 1 hour, plasma concentration decreased by 50% of Cmax in approximately 25 minutes, 25% in 1 hour and 20% at 2 hours. Following a 4 hour infusion, the plasma concentration decreased to 50% of Cmax in 1 hour and 35% in 2 hours (50).

The major route of elimination for DX-9065a is via renal mechanisms (75-80%) with a bi-phasic urinary profile which supports tubular secretion as a contributing mechanism. Low affinity protein (albumin) binding occurs in plasma (60-80%) and there is no significant biotransformation of the parent compound (51, 52).

The pharmacokinetic profile of DX-9065a has potential advantages and disadvantages in the clinical arena. Intravenous bolus administration, followed by a short infusion, provides rapid fXa inhibition that dissipates rapidly – a strategy that is particularly attractive in the setting of percutaneous coronary interventions. In contrast, prolonged infusions as might be used for acute coronary syndromes, particularly if given to patients with impaired hepatic and/or renal performance (excluded from phase I and II trials) could cause high-intensity anticoagulation (and increased bleeding risk) for prolonged periods of time. Under these circumstances, dose modifications and coagulation monitoring would be required.

**Coagulation profile**

Coagulation responses to DX-9065a administered either as a single IV bolus or continuous infusion in healthy volunteers were determined in a series of experiments. Prothrombin time (PT) increased by 40-80% within 1 minute following bolus doses ranging from 0.62 mg to 2.5 mg (Daiichi Pharm Co. Ltd. Data on file). The prolongation was brief, with the PT returning to baseline in 30-40 minutes. A similar but more modest response was observed for the aPTT. In contrast, the Xa clotting time prolonged by 2-2.5-fold and evidence of Xa inhibition persisted for several hours. The overall impact of DX-9065a on plasma coagulation measurements was dose-dependent.

A one-hour infusion of DX-9065a in doses ranging from 5mg to 30mg caused 2.5, 2.1 and 3.9-fold prolongations (compared to baseline) of the PT, aPTT and Xa clotting time. A rapid decline in coagulation response occurred within 30 minutes of

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**Figure 5:** A) Plasma concentration-time profiles of DX-9065 after a single 1-minute bolus injection of DX-9065a. Values are mean ± of six subjects. B) Plasma concentration-time profiles of DX-9065a after a single 1-hour infusion of DX-9065a. Values are mean ± SD of six subjects. [From reference #50; with permission.]
infusion discontinuation; however, evidence of impaired coagulation was evident for approximately 8 hours, correlating directly with plasma DX-9065a concentrations. There was no evidence of a cumulative anticoagulant effect with repeated 1 hour infusions given every 12 hours.

A 1 mg bolus followed by a 1.5 mg continuous infusion over 4 hours yielded a modest rise in coagulation measurements (20-40% increase) shortly after the bolus with an initial decline over the subsequent 30 minute and low level anticoagulation during the infusion. A return to baseline was evident within several hours.

**In vitro studies**

Studies performed at the Laboratory for Vascular Biology Research, University of Massachusetts Medical School were designed to characterize the anticoagulant effects of DX-9065a in combination with heparin compounds and the drugs potential reversibility (Becker, unpublished data). This information was particularly useful approaching phase II studies.

Plasma-based INR increased with DX-9065a (concentrations up to 0.4 µg/mL). The anticoagulant effect was attenuated by the addition of fresh frozen plasma, particularly at low to moderate concentrations of DX-9065a (up to 0.2 µg/mL). Thus provision of FX substrate (8.0 ug/ml plasma) represents a readily available means to reduce DX-9065a-mediated anticoagulation when hemostatic capacity must be restored. Additional *in vitro* studies revealed that unfractionated heparin and the low molecular weight preparation enoxaparin combined with DX-9065a provoked an additive anticoagulant effect in low drug concentrations (0.5 U/mL). A greater intensity of anticoagulation was observed at higher concentrations (≥ 1.0 U/mL).

The anticoagulant response was particularly robust when assessed using whole blood PT, INR and ACT measurements. The anti-Xa effects followed a similar pattern.

**Ex vivo studies**

Patients (n=22) undergoing percutaneous coronary intervention (PCI) had blood samples obtained after either unfractionated heparin alone or combined with a platelet GPIIb/IIIa receptor inhibitor were administered. In a separate study, 20 patients had samples obtained after receiving clopidogrel (a platelet ADP receptor antagonist). A small volume of blood was then added to tubes containing DX-9065a in concentrations ranging form 0-0.8 µg/ml. As with the previously described *in vitro* experiments, combined unfractionated heparin and DX-9065a provoked a robust whole blood PT, INR and ACT response, reaching the upper limit of measurable results at 0.4 µg/ml and 0.2 µg/ml, respectively. The combination of DX-9065a, unfractionated heparin, and a platelet GPIIb/IIia inhibitor led to a modest prolongation (approximately 20 seconds by ACT measurement) of coagulation compared to DX-9065a and unfractionated heparin. Clopidogrel did not exert a significant effect on whole blood anticoagulation measurements (Becker, unpublished data).

The antithrombotic effect of DX-9065a under conditions of low and high shear stress were assessed in experiments using the Badimon Flow Chamber (thrombus formation on a mounted vascular substrate). A total of six healthy male volunteers participated in 3 consecutive study arms: a) enoxaparin (1 mg/kg sc) plus aspirin (162 mg) for 3 days; b) three escalating doses of DX-9065a (1 mg bolus + 0.25 mg/h x 2h, followed by an additional 1 mg bolus +0.625 mg/h x 2h and a final 1 mg bolus + 1.25 mg/h x 2h; and c) the same dose as in study (b) plus aspirin pretreatment. The administration of DX-9065a inhibited thrombus formation, particularly under high shear stress conditions in a dose-dependent manner, while enoxaparin did not have a significant effect when tested in this particular *ex vivo* model (53).

Subcutaneous administration of DX-9065a has been evaluated in a total of 36 healthy male subjects. Single doses of 2.5, 5.0 and 10 mg were administered and peak plasma DX-9065a concentrations determined 1 hour later using a chromogenic Xa assay (referenced to a calibration curve constructed with 0 to 0.5 µg/ml DX-9065a) were 39 ± 12, 79 ± 20.1 and 187.5 ± 28.9 ng/ml, respectively. DX-9065a concentrations fell below measurable levels 4 to 8 hours after injection (54).

**Patients with coronary artery disease**

The Xa Neutralization for Atherosclerotic Disease Understanding (XaNADU-1B) trial (55) was designed to study DX-9065a in patients representative of those encountered in clinical practice. Accordingly, men and women (n=73) between the ages of 55 and 75 years with evidence of coronary artery disease (prior myocardial infarction, coronary revascularization, or angiographic evidence) were randomized to a 1 mg IV bolus and 72 hour continuous IV infusion of either placebo or DX-9065a in doses designed to achieve plasma concentrations of 15 ng/ml, 50 ng/ml, 100 ng/ml or 200 ng/ml. All patients also received aspirin.

The pharmacokinetic and pharmacodynamic parameters for DX-9065a are summarized in Figure 6. Five minutes after a 1 mg bolus, the median plasma DX-9065a concentration was 67.9 ng/ml. By 16 hours (after initiation of continuous infusion) all groups had reached their pre-specified target concentration. Over the subsequent 56 hours, concentrations increased in all study groups and exceeded target levels by an average of 30% at completion of the 72 hour infusion. DX-9065a concentrations decreased by 50%, (relative to Cmax) at 4 hours and had declined to 25% by 24 hours. Low levels of DX-9065a
were detectable in plasma 7 days after completion of the infusion.

The pharmacokinetic modeling estimates from XaNADU-1B are summarized in Table 2. Based on a three compartment model $t_1/2(\alpha)$ was 0.14-0.3 hours, $t_1/2(\beta)$ was 1.9-3.2 hours, and $t_1/2(\gamma)$ was 76.6-98.9 hours. Independent predictors of pharmacokinetic response were dose, age, body weight, female sex and baseline creatinine.

All plasma-based pharmacodynamic measurements paralleled DX-9065a drug concentrations during both infusion and elimination phases of XaNADU 1B. At the highest DX-9065a concentration (200 ng/ml), the maximum PT/INR, aPTT and aXa levels were prolonged by 40%, 25% and 80%, respectively. Anti-Xa activity correlated highly with plasma drug concentration ($r=0.95$). PT/INR and aPTT also correlated closely with plasma levels ($r=0.77$ and 0.56, respectively) (56). Maximal suppression of thrombin generation, as assessed by change in F1.2, was seen with DX-9065a concentrations above 200 ng/ml (57).

The potential of anti-Xa measurements over other plasma-based tests relates to its close correlation with plasma DX-9065a concentration. In XaNADU 1B, anti-Xa levels (Chromogenic assay) correlated strongly with plasma DX-9065a levels measured by high performance liquid chromatography/mass spectrometry ($r=0.97$) throughout drug infusion (58). Although there are several methods for determining fXa inhibition, the available data support competition-based assays (Rotachrome™, Coatest™) over inhibition-based (Stachrom™) or chronometric (Hep Test™) assays (59).

Whole blood coagulation tests were evaluated in XaNADU 1B and yielded greater sensitivity (to DX-9065a) compared with plasma-based measurements. The PT/INR increased during drug infusion in relation to plasma DX-9065a concentrations with a lower threshold (to observe prolongation of PT/INR) of 14.6 ng/ml (60).

### Safety measures

There were no TIMI major or minor hemorrhagic events in XaNADU 1B. Minor bleeding occurred in approximately 40% of patients according to the GUSTO criteria. While there were no statistically significant differences compared with placebo, a trend toward greater minor hemorrhagic events was observed in the highest DX-9065a group. Thrombocytopenia, worsening

![Figure 6: The relationship between plasma concentrations of DX-9065a and anti-Xa activity during the initial hours of drug administration in XaNADU 1B. [From reference #55; with permission.]](image)

Table 2: Pharmacokinetic profile of DX-9065a in patients with stable coronary artery disease. [From reference #58; with permission.]

<table>
<thead>
<tr>
<th>Group</th>
<th>DX-9065a Target (ng/mL)</th>
<th>72-hour Weight-Adjusted Dose (mg)</th>
<th>$\alpha$t (h)</th>
<th>$\beta$t (h)</th>
<th>$\gamma$t (h)</th>
<th>$C_{max}$ (72-h) (ng/mL)</th>
<th>AUC (ng*h/mL)</th>
<th>MRT (h)</th>
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<tr>
<td>1</td>
<td>15</td>
<td>12.1 ± 2.5</td>
<td>0.14</td>
<td>1.93</td>
<td>76.57</td>
<td>20.4 ± 3.1</td>
<td>2219 ± 403</td>
<td>64.2</td>
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<td></td>
<td></td>
<td></td>
<td>(0.02)</td>
<td>(0.18)</td>
<td>(19.57)</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>50</td>
<td>37.8 ± 9.2</td>
<td>0.24</td>
<td>2.54</td>
<td>81.46</td>
<td>64.8 ± 11.0</td>
<td>6154 ± 1094</td>
<td>63.1</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(0.15)</td>
<td>(1.17)</td>
<td>(16.4)</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>3</td>
<td>100</td>
<td>78.9 ± 15.2</td>
<td>0.25</td>
<td>2.67</td>
<td>96.68</td>
<td>138.6 ± 24.6</td>
<td>14187 ± 4568</td>
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<td></td>
<td></td>
<td></td>
<td>(0.05)</td>
<td>(0.44)</td>
<td>(19.42)</td>
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<tr>
<td>4</td>
<td>200</td>
<td>150.6 ± 29.5</td>
<td>0.30</td>
<td>3.20</td>
<td>82.95</td>
<td>266.9 ± 77.1</td>
<td>24737 ± 8940</td>
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<td></td>
<td>(0.20)</td>
<td>(0.57)</td>
<td>(7.88)</td>
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</tbody>
</table>

Mean values ± SD or (SE). Area under the curve (AUC) calculated by trapezoidal rule. MRT indicated mean residence time.
renal insufficiency, and/or hepatic insufficiency (transaminase elevation) were not reported.

Patients undergoing percutaneous coronary intervention

XaNADU-PCI Pilot Study

The XaNADU-PCI Pilot Study was designed to investigate the effects of DX-9065a on the prevention of thrombosis during elective PCI in patients treated concomitantly with aspirin, clopidogrel and, in most instances, a GPIIb/IIIa receptor antagonist. A total of 175 North American patients between the ages of 18 and 75 years were randomized in sequential stages to receive either DX-9065a, in escalating doses, or UFH (4:1 ratio). In those given DX-9065a, treatment was initiated as a double (1.0mg or 2.5 mg) bolus and continuous intravenous infusion designed by pharmacokinetic simulation to achieve plasma concentration of greater than (lower 95\textsuperscript{th} percentile) 75 ng/ml, 100 ng/ml, or 150 ng/ml. The last stage of the study included patients who had recently received either UFH or enoxaparin (LMWH) within 72 hours. For patients assigned to UFH, target ACTs of 200 to 300 seconds and 250-350 seconds were recommended for those receiving and not receiving a concomitant GP IIb/IIIa receptor antagonists, respectively (61). The majority of patients (85.3\%) did receive concomitant GP IIb/IIIa antagonists.

Thrombotic events and safety profiles among patients participating in the XaNADU PCI Pilot study are summarized in Tables 3 and 4, respectively. Stage 2 (DX-9065a concentrations greater than 75 ng/ml was discontinued following a single life-threatening coronary arterial thrombotic event. The subsequent stages completed without observing an excess of either ischemic/thrombotic or hemorrhagic events in patients receiving DX-9065a. The rate of ischemic complications in the 4\textsuperscript{th} stage was modestly increased, likely reflecting the higher risk patients population enrolled. Interpretation of differences in ischemic events and bleeding is complicated by the small numbers and non-randomized nature of comparisons. Thrombocytopenia (<100,000/mm\textsuperscript{3}) was uncommon (3.4\% overall) and occurred predominantly in patients receiving GP IIb/IIIa receptor antagonists.

Coagulation measurements

Activated clotting time (ACT) increased to a modest degree among patients receiving DX-9065a. Peak levels (median values; 25\textsuperscript{th}, 75\textsuperscript{th} percentiles) for the 75 ng/ml, 100 ng/ml, 150 ng/ml, and heparin-DX 150 ng/ml groups were 173 (155, 198), 174 (155, 216), 191 (161, 252) and 172 (150, 206) seconds, respectively.

In a select group of patients (approximately 7 in each DX-9065a study arm) additional coagulation testing was performed.

| Table 3: Thrombotic events in the XaNADU-PCI Pilot Study. [From reference #61; with permission.] |
|--------------------------------------------------|------------------|------------------|------------------|------------------|------------------|
| Phase 1 | Phase 2 | Phase 3 | Phase 4 | All Phases |
| DX 100 | DX 175 | DX 150 | Heparin-DX 150 | Heparin | Total |
| n=45 | n=8 | n=45 | n=45 | n=34 | n=175 |
| Death | 0 | 0 | 0 | 0 | 0 | 0 |
| MI | 5/45 (11.1\%) | 1/6 (16.7\%) | 6/45 (13.3\%) | 2/34 (5.9\%) | 14/175 (8.0\%) |
| Urgent Revascularization | 0 | 0 | 1/45 (2.2\%) | 0 | 1/175 (0.6\%) |
| Abrupt Closure | 0 | 0 | 0 | 0 | 0 | 0 |
| No Reflow | 0 | 0 | 1/45 (2.2\%) | 0 | 1/175 (0.6\%) |
| Distal Embolization | 0 | 0 | 1/45 (2.2\%) | 0 | 1/175 (0.6\%) |
| Thrombus | 0 | 1/6 (16.7\%) | 1/45 (2.2\%) | 2/45 (4.4\%) | 4/175 (2.3\%) |
| Thrombotic Event (at least one of the above events) | 5/45 (11.1\%) | 1/6 (16.7\%) | 1/45 (2.2\%) | 6/45 (13.3\%) | 15/175 (8.6\%) |

P-value is between the DX for each phase and overall heparin group.
Whole blood INR (median, 25th/75th percentiles) increased within 1 minute of the first bolus and ranged from 2.20 (1.60, 2.60) in the 75 ng/ml group to 4.10 (3.40, 5.00) in the heparin-DX 150 ng/ml group. Peak values were observed 5 minutes after the 2nd DX-9065a bolus and prolongation of the INR persisted for 8 hours from the time of study drug discontinuation. In each group the INR decreased to below 2.0 by 60 minutes from study drug termination.

Anti-Xa levels ranged from 0.47 (0.42, 0.52) to 0.60 (0.56, 0.93) U/ml one minute after the first DX-9065a bolus, remaining above 0.30 U/ml up to the time of study drug completion (Fig. 7). Anti-Xa levels declined rapidly thereafter with a 50% reduction by 60 minutes from drug termination.

Whole blood INR and anti-Xa measurements (peak) were, on average, 30-40% higher in stage 4 (heparin-DX 150 ng/ml) than stage 3 (DX-9065a 150 ng/ml), supporting an additive anticoagulant effect when DX-9065a is administered to patients who have received heparin within the past several hours (confirming prior in vitro observations).

The XaNADU PCI Pilot demonstrated that percutaneous coronary intervention is feasible using DX-9065a as an anticoagulant. Clinically acceptable thrombotic complications and hemorrhage rates were observed when DX-9065a was administered on a background of potent antiplatelet therapy including aspirin, clopidogrel, and, in most cases GP IIb/IIIa antagonists (61). Whether DX-9065a’s specificity for fXa, allowing for some degree of thrombin generation provides a mechanism for preserved hemostasis (and a lower risk for bleeding) can only be determined through further investigation (phase III clinical trials).

Patients with non-ST-segment acute coronary syndromes

A larger phase II clinical trial, XaNADU ACS was conducted (62). In XaNADU ACS, 402 patients from the United States, Canada, and Japan with non-ST-segment elevation acute coronary syndromes were randomized to either unfractionated heparin or one of two doses of DX-9065a. The DX-9065a dosing regimens were designed to achieve plasma concentrations of 100ng/ml and 200ng/ml respectively. The trial protocol recommended an early invasive management approach. The primary efficacy endpoint of death, myocardial infarction, urgent revascularization, or recurrent ischemia on continuous ST-segment monitoring occurred. The primary safety endpoint was major bleeding. Enrollment was completed in October 2003 and results were recently presented at the 2004 American College of Cardiology Meetings.

Weight adjusted DX-9065a dosing regimens achieved the desired target concentrations. The achieved anti-Xa levels, using a rotachrome assay and heparin standard, were 0.23 U/ml with low-dose DX-9065a and 0.41 U/ml with high-dose DX-9065a. The primary efficacy endpoint in XaNADU ACS occurred in 34%, 34%, and 31% of patients assigned to unfractionated heparin, low dose DX-9065a, and high-dose DX-9065a respectively. Although there was no effect on the primary efficacy endpoint, there were consistent, although not statistically significant, trends toward lower rates of death, myocardial infarction, urgent revascularization, and symptomatic ischemia among patients assigned to high-dose DX-9065a compared to
Becker, et al.: DX-9065a development in cardiovascular disease

heparin. Along with this trend toward a lower rate of ischemic complications, patients receiving DX-9065a in XaNADU-ACS also tended to have lower rates of major bleeding than those receiving heparin. Major bleeding occurred in 3.3% of patients receiving heparin compared to 0.8% of patients receiving low-dose DX-9065a and 0.9% of patients receiving high-dose DX-9065a. Although the results with DX-9065a in XaNADU-ACS are promising, they require confirmation in larger, adequately powered, phase II and phase III trials before meaningful conclusions regarding the potential role of DX-9065a as an anticoagulant in patients with acute coronary syndromes can be drawn.

Concluding comment

A cell-based model of coagulation, when applied to coronary arterial thrombosis places fXa at the center of several fundamental stages including initiation, priming and propagation. In addition, the pleuripotential cellular properties of fXa which contribute to cellular proliferation and inflammation provide additional support for current enthusiasm to develop targeted pharmacologic antagonists for clinical use. DX-9065a, the first in a class of direct, selective and reversible fXa inhibitors, has shown promise in animal models of thrombosis, as well as preclinical and clinical studies involving patients with stable coronary artery disease, acute coronary syndromes, and those undergoing PCI. Information gathered through the developmental stages of DX-9065a will provide valuable insights regarding the role of fXa in atherothrombosis and the potential benefits derived from its direct inhibition.

References


Figure 7: Anti-Xa activity in patients participating in the XaNADU PCI Pilot Study – measurements were made 15 minutes after the 2nd bolus of DX-9065a. In a portion of phase IV, patients receiving unfractionated heparin were eligible for enrollment. A cumulative anti-Xa effect was evident.