Theme Issue Letter to the Editor

Combined coagulation phase-directed factor Xa inhibition with heparin compounds and DX-9065a – A direct and selective antagonist

Dear Sir,

The development of novel anticoagulants for use in patients with acute coronary syndromes (ACS) has evolved steadily, emphasizing the importance of cellular surface biochemistry and the highly integrated contribution of platelets, leukocytes and endothelial cells in the thrombotic process. Accordingly, tissue factor and its primary complexing coagulation proteases, factor VIIa and Xa, represent attractive targets for pharmacologic inhibition of thrombin generation (1).

Although combined or “multi-site directed” pharmacologic therapy is an appealing construct for the attenuation of vascular thrombosis in ACS, pharmacokinetic and pharmacodynamic relationships that may potentially influence safety, efficacy and dosing must be considered carefully.

DX-9065a is the first in a class of synthetic, small-molecule, direct factor Xa inhibitors. Its antithrombotic efficacy has been demonstrated in animal models of venous thrombosis (2), arteriovenous shunt thrombosis (3, 4), vein graft thrombosis (5) and disseminated intravascular coagulation (6). In a double-blind trial of 73 patients with stable coronary artery disease (7), DX-9065a given as a 72-hour infusion was well tolerated and plasma drug concentrations correlated strongly with anti-factor Xa activity (r=0.97).

Because unfractionated heparin (UFH) and low molecular weight heparin (LMWH) represent the current standard of care for anticoagulant therapy in ACS and both inhibit factor Xa (as well as other coagulation proteases) indirectly through an antithrombin III-dependent mechanism, we conducted a series of in vitro and ex vivo experiments to determine their pharmacodynamic interactions with DX-9065a.

Blood samples were obtained by standard venipuncture from healthy donors (n=6). The initial 2 ml of blood was discarded and the remainder placed in tubes containing 3.2% sodium citrate. Following gentle mixing, samples were transferred to tubes containing either DX-9065a (Daiichi Pharmaceutical, Co. Ltd., Tokyo, Japan) (0, 0.05, 0.1, 0.2, 0.4, 0.8 µg/ml), UFH (Elkin-Simm) (0, 0.5, 1.0, 2.5, 5.0 U/mL), enoxaparin (Aventis Pharmaceuticals) (0, 0.5, 1.0, 2.5, 5.0 U/mL), DX-9065a plus UFH or DX-9065a plus enoxaparin. Lower concentrations of UFH (maximum 1.0 U/mL) and enoxaparin (1.0 U/mL) were used for the combination experiments.

Several mLs of blood were transferred to tubes containing CaCl₂ (final concentration 12.5 mM) and incubated at 37°C for a total of four minutes. Immediately following incubation, 50 ul of blood was placed on a coagulation test cartridge (whole blood microcoagulation system, Hemochron Jr®, International Technidyne Corporation, Princeton, NJ). Prothrombin time (PT), (upper limit 100 seconds) and Activated Clotting Time (ACT) (upper limit 400 seconds) measurements were performed according to the manufacturers specifications. Anti-Xa measurements were made using a chromogenic assay (Rotachrome; Diagnostica Stago Inc.).

Patients scheduled to undergo elective percutaneous coronary intervention (PCI) were approached for study participation. Blood samples were obtained either before (control) or 15 minutes after intravenous administration of UFH (average bolus dose 3000 U). Some patients received a platelet glycoprotein (GP) IIb/IIIa receptor antagonist and all had been given aspirin (325 mg) within the prior 6 hours.

0.45 mL of blood was added to tubes containing DX-9065a (0, 0.05, 0.1, 0.2, 0.4, 0.8 µg/ml). After gentle mixing, 50 ul of blood was placed on coagulation test cartridges for measurement of PT, INR and ACT (Microcoagulation System Hemochron Jr®, International Technidyne Corporation, Princeton, NJ). All coagulation testing was performed at the “point of care” using a separate coagulation monitor for each DX-9065a concentration (to minimize the effect of measurement delay on test results)

To examine the sensitivity of each coagulation measurement to DX-9065a alone and in combination with either UFH or enoxaparin, the PT was mathematically transformed using the mean of the highest ACT value as a reference. The transformed PT was defined as mean ACT X PT (8). The slope, Y value, of the regression line represents a reproducible and comparable dose response parameter (9).

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Unfractionated heparin at concentrations ranging from 0.5 to 5.0 U/mL prolonged the whole blood PT ($r=0.87$, $Y=35.1 \pm 13.3$) and ACT ($r=0.92$, $Y=134.0 \pm 6.4$). Similar observations were made for enoxaparin; however, the slopes were lower for each coagulation measurement.

DX-9065a in concentrations up to 0.8 µg/mL prolonged the whole blood PT ($r=0.94$, $Y=114.8 \pm 4.53$) and ACT ($r=0.93$, $Y=143.6 \pm 9.7$). Transforming the PT yielded a $Y$ value of 280 $\pm$ 11.1, suggesting that the whole blood PT is a more sensitive coagulation measure than ACT for determining the anticoagulant effects of DX-9065a. For each concentration of DX-9065a there was a corresponding whole blood PT and ACT value within the devices range of reportable results.

UFH in relatively low concentrations (0.5 U/ml) added to DX-9065a prolonged the whole blood PT ($Y=322.1 \pm 27.9$) and ACT ($Y=355.1 \pm 32.9$). The transformed PT slope value was $786.0 \pm 65.8$. Concentrations of DX-9065a above 0.2 µg/mL prolonged both the PT and ACT measures beyond reportable limits. UFH, in moderate concentrations (1.0 U/mL), added to DX-9065a caused marked prolongation of the PT ($Y=537.0 \pm 49.8$) and ACT ($Y=654.3 \pm 60.5$), surpassing the upper limits of reportable results at concentrations of DX-9065 approaching 0.2 µg/mL. The coagulation measurement responses for enoxaparin in combination with DX-9065a were similar to those observed with UFH; however, the slopes were lower.

Anti-Xa measurements using a chromogenic assay in response to DX-9065a, UFH, enoxaparin and their combination are shown in Figure 1. There was a concentration-dependent increase in anti-Xa activity with DX-9065a. Approximately 0.2 µg/mL DX-9065a produced a similar degree of factor Xa inhibition.
inhibition (based on a chromogenic assay) to UFH and enoxaparin at 0.5 U/mL. The anticoagulant effects of DX-9065a (≤0.2 µg/mL) combined with either UFH or enoxaparin (0.5 U/mL) were additive. At higher concentrations (DX-9065a > 0.2 µg/mL and UFH or enoxaparin 1.0 U/mL) the response was more robust. Similar observations were made in our ex vivo experiments.

The development of new anticoagulants for thrombotic disorders of the cardiovascular system must carefully consider their mechanism of action, pharmacodynamic profile and full spectrum of coagulation protease inhibiting properties when used alone or in combination with other drugs. DX-9065a, a direct and selective factor Xa inhibitor prolongs whole blood coagulation measures and, in combination with either UFH or the LMWH preparation, enoxaparin (antithrombin III-dependent, indirect inhibitors), provokes additive anticoagulant effects. The work of Rezaie and colleagues (10) suggests that 7 of the 11 basic residues of the heparin-binding exosite of thrombin are conserved at similar three dimensional locations in fXa; they are Arg123, Lys126, Arg129, Arg135, Lys160, Lys166, and Arg180. In addition, their findings support overlapping binding sites on fXa for fVα and heparin, providing insights regarding the relative contribution of heparin and the heparin-antithrombin III complex neutralization of free fXa and fXa existing within the prothrombinase complex (which is not accessible for binding to AT). In contrast, DX-9065a inhibits fXa in both the plasma phase and prothrombinase complex in the presence or absence of prothrombin (11).

The unique binding characteristics for heparin and DX-9065a to fXa provide distinct targets for maximal pharmacologic inhibition and a mechanistic explanation for our findings both in vitro and ex vivo (12, 13).

DX-9065a in combination with either UFH or enoxaparin provoked a rapid anticoagulant response, as reflected in whole blood coagulation measures and anti-Xa activity. This effect has not been reported previously. Although the overall clinical significance of these observations is unknown, our in vitro and ex vivo experiments suggest that a systemic state of moderate- to-high intensity anticoagulation should be anticipated in clinical settings where heparin concentrations in excess of 0.5 U/ml coexist with DX-9065a levels of 0.2 µg/ml or greater. The common practice of combined pharmacotherapy in acute coronary syndromes with use of aspirin, clopidogrel and GP IIb/IIIa receptor antagonists could potentially “shift” the balance of safety even further with lower concentrations of either or both anticoagulants particularly in the setting of invasive procedures.

The potential influence of combined and rapidly sequenced anticoagulant pharmacotherapy on clinical outcome should not be underestimated and may be highly relevant for drug development, clinical trial design, and patient care.

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References