Global Anticoagulant Effects of a Synthetic Anti-Factor Xa Inhibitor (DX-9065a): Implications for Interventional Use

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Summary: Heparin has been conventionally used as an anticoagulant for medical and surgical indications. Because factor Xa is an essential component of the prothrombinase complex and leads to the generation of thrombin, its inhibition has become a focus of newer antithrombotic drug development. The in vitro anticoagulant profile of DX-9065a, a synthetic direct factor Xa inhibitor, was studied using activated clotting time assay, thrombelastography, and global clotting tests, such as prothrombin time (PT), activated partial thromboplastin time (aPTT), dilute Russell’s viper venom time (dRVVT), thrombin time, ecarin clotting time, and amidolytic anti-Xa assay. In addition, the effect of DX-9065a on platelet aggregation and inhibition of thrombin generation markers (FPA, F1+2, and TAT) were studied. The pharmacokinetic and pharmacodynamic profiles of DX-9065a were also studied in a non-human primate (Macaca mulatta) model. DX-9065a produced a concentration-dependent increase in the Hemochron celite ACT and HemoTec ACT. Clotting times of 538 ± 19 and 401 ± 12, respectively, were reached at a concentration of 25 μg/ml, signifying that DX-9065a may be useful in interventional cardiological procedures. DX-9065a prolonged the t-time on thrombelastography. DX-9065a did not show any effect on adenosine diphosphate (ADP)-, collagen-, epinephrine-, and arachidonic acid–induced platelet aggregation at concentrations up to 10 μg/ml. DX-9065a exhibited a concentration-dependent prolongation of the PT, aPTT, Heptest, dRVVT, and reached the clotting times of 51.6, 132, 193, 47.9, 129.9 seconds, respectively, at a final concentration of 12.5 μg/ml; compared to a control value of 10.6, 30.2, 41.9, 14, 32.2 seconds, respectively. DX-9065a did not affect the ecarin clotting time and thrombin time at concentrations up to 12.5 μg/ml. Because DX-9065a prolonged the dRVVT, this may impact diagnostic screening of patients with systemic lupus erythematosus.

Key Words: Factor Xa inhibitor—Clot-based assays—Amidolytic anti-Xa assay—Thrombin generation markers—Platelet aggregation—Pharmacokinetics.

Heparin has been a conventionally used anticoagulant for medical and surgical indications. Low-molecular-weight heparins (LMWHs) obtained through chemical or enzymatic depolymerization of porcine mucosal heparin have several significant advantages over unfractionated heparin. LMWHs are characterized by a higher antithrombotic effect as a result of inhibition of factor Xa and a lower hemorrhagic potential due to a lower antithrombin effect. In the past few years, there has been a growing interest in the development of factor Xa, factor VIIa, factor VIIa-tissue factor, factor XIIIa, factor XIIa, and factor IXa inhibitors. With the exception of factor XIIIa, all these proteases augment the generation of factor Xa. Hence, it is crucial to control thrombogenesis by inhibiting factor Xa rather than inhibiting these different proteases at different sites (1). Factor Xa is an essential component of the prothrombinase complex and leads to the generation of thrombin (2). Thus, inhibition of factor Xa is an important focus for the development of newer antithrombotic drugs. Synthetic heparin pentasaccharide, fondaparinux (Arixtra®) is now indicated for the prophylaxis of deep ve-
nous thrombosis, which may lead to pulmonary embolism in patients undergoing hip fracture, hip replacement, and knee replacement surgery (3,4). While clinical development of several naturally occurring direct anti-factor Xa agents such as antistatin, tick anticoagulant peptide (TAP), and yagin is delayed, there is rapid development of newer synthetic inhibitors of factor Xa such as JTV-803, DX-9065a, and Bay 59-7939. DX-9065a is a synthetic peptidomimetic direct factor Xa inhibitor, and is in different phases of clinical trials at this time (5–7). The factor Xa inhibitors exhibit a higher margin of safety when compared to the antithrombin agents (8–10). Factor Xa inhibitors are very useful in the prophylaxis of thrombosis. DX-9065a is orally bioavailable (11–13) and excreted by urine and feces (14). However, for effective anticoagulation, they should be used as adjuncts to various antithrombotic or antiplatelet agents such as antithrombin drugs, GP IIb/IIIa inhibitors, thrombolytic drugs, and tissue factor pathway inhibitors (TFPIs).

In this study we have examined the in vitro anticoagulant profile of DX-9065a using whole blood assays such as activated clotting time (ACT) and thrombelastography (TEG) and other clot-based assays such as prothrombin time (PT), activated partial thromboplastin time (aPTT), diluted aPTT, Heptest, Heptest-HI, dilute Russell's viper venom time (dRVVT) test, thrombin time (TT), ecarin clotting time (ECT), and amidolytic anti-Xa assay. In addition, the effects of DX-9065a on platelet aggregation and the inhibition of thrombin generation (FPA, F1.2, and TAT) were studied. The pharmacokinetic and pharmacodynamic profiles of DX-9065a were also studied in a non-human primate (Macaca mulatta) model.

**MATERIALS AND METHODS**

**Materials**

Synthetic direct factor Xa inhibitor DX-9065a, (+)-2S-2-[4-[[3S]-1-acetimidoyl-pyrrolidinyl]-oxy]-phenyl]-3-[7-amidino-2-naphthyl] propanoic acid hydrochloride pentahydrate, was supplied by Daiichi Pharmaceutical, Ltd (Tokyo, Japan). GP IIb/IIIa inhibitor; tirofiban (Aggrastat) was purchased from Merck Co. (West Point, PA). Pentasaccharide was purchased from Sanofi-Synthelabo (Toulouse, France).

**Anticoagulant Activity of DX-9065a**

**Global Anticoagulant Effect on Native Human Whole Blood**

ACT and TEG assessment were performed to determine the effects of DX-9065a on native human blood. ACT: hemochron instrument and celite-ACT tubes were obtained from International Technidyne Corporation (Edison, NJ). HemoTec instrument and cartridges were obtained from Medtronic HemoTec Inc. (Englewood, CO.) In each syringe 200 µL of the drug at respective concentrations was placed so that when blood was drawn up to 2 mL in each set of syringes, final concentrations of 25, 10, 5, and 0 µg/mL (saline control) were obtained. ACT were performed by the standard procedure, and the results were recorded. TEG (Hellige thrombelastograph-D model; Haemoscope, Skokie, IL) was used to obtain thrombelastograms. Using a double syringe technique, blood was drawn up to 1 mL in each of the respective syringes pre-filled with the test drug to obtain a final concentration of 10, 5, and 0 µg/mL (saline control). Thrombelastography was performed, and five different TEG parameters were calculated accordingly. Reaction (R) time was measured as the distance in millimeters from the start of the tracing until there was a 2-mm divergence. This was the point of reproducible clot formation. Total clotting (RK) time was the distance in millimeters from beginning of the tracing until a 20-mm divergence was reached. This was the time to standard clot firmness. K time was calculated as the difference between RK time and R time. The maximum amplitude (MA) corresponded to the maximum shear modulus of the clot. Divergence angle (alpha) was measured by drawing a tangent line from the point of initial divergence along the maximum curvature of the tracing.

**Clot-based Assays in Normal Human Pooled Plasma**

To determine the in vitro anticoagulant profile, DX-9065a was supplemented to pooled normal human plasma and then diluted serially with plasma to obtain concentrations from 12.5 to 0 µg/mL. Samples were assayed immediately using the following tests:

1. Prothrombin time (PT)
   Four different PT reagents were used in this study:
   a. Thromboplastin C (Dade International Inc., Miami, FL)
b. Innovin (Dade International Inc., Miami, FL)
c. Simplastin L (Organon Teknika Corp, Durham, NC)
d. Recombiplastin (Ortho Diagnostic Systems Inc., Raritan, NJ)

2. Activated partial thromboplastin time (aPTT)
   Three different aPTT reagents were used in this study:
   a. Plateletin (Organon Teknika Corp, Durham, NC)
   b. Actin®, activated cephaloplastin reagent (Baxter Healthcare Corp, Dade Division, Miami, FL)
   c. Stago reagent (Diagnostica Stago, Parsippany, NJ)

3. Heptest®
   a. Bovine factor Xa (Haemachem, Inc., St. Louis, MO)
   b. Recalmix reagent (Haemachem, Inc., St. Louis, MO)

4. Heptest®-HI
   a. Bovine factor Xa-HI (Haemachem, Inc., St. Louis, MO)
   b. Recalmix reagent-HI (Haemachem, Inc., St. Louis, MO)

5. Dilute Russell’s viper venom time (dRVVT) test
   DRVV reagent (American Diagnostica Inc. Greenwich, CT)

6. Thrombin time (TT) (thrombin 5 U/mL)
   Thrombin reagent (Enzyme Research Labs, South Bend, IN)

7. Ecarin clotting time, stock ecarin reagent was diluted in 0.025 M CaCl₂ to obtain clotting time of 40 to 45 seconds. Ecarin reagent was obtained from Pentapharm AG (Basel, Switzerland).

   These tests were performed by using standard protocols on a fibrometer (Becton Dickinson, New York) or an ACL 300 plus (Beckman-Couter, Miami, FL).

Amidolytic-based Assay in Normal Pooled Human Plasma

Plasma anti-Xa activity was determined by amidolytic assay using ACL 300 plus. Anti-Xa assay was set up using the specific chromogenic substrate for factor Xa (Spectrozyme® FXa, American Diagnostica Inc., Greenwich, CT) and bovine factor Xa was purchased from Enzyme Research Laboratories (South Bend, IN). A modified anti-Xa method was also used to determine the effect of DX-9065a on factor Xa. In this method, the reaction time was decreased to 75 seconds instead of 300 seconds.

Platelet Aggregation Assay

Platelet aggregometers (Bio/Data Corporation, Horsham, PA) were used for this assay. Platelet-rich plasma was obtained from blood collected from healthy donors into sodium citrate (3.2% final concentration) and centrifuged at 800 × g for 15 minutes at room temperature. The remaining blood was spun further at 2500 × g for 15 minutes to obtain platelet-poor plasma (PPP). PPP was used to blank the aggregometer. Aggregations were performed in the following manner. Four hundred fifty microliters of platelet-rich plasma was pipetted into aggregometer tubes and incubated at 37°C for 2 to 4 minutes, to assure that the platelets do not self-aggregate, and 50 μL of agonist was pipetted into each tube. The agonists were tested at the respective final concentrations: adenosine diphosphate (10 μM, Sigma Chemical Co., St. Louis, MO), collagen (180 μM, Bio/Data Corporation, Horsham, PA), epinephrine (10 μM, Sigma Chemical Co., St. Louis, MO), and arachidonic acid (330 μg/mL, Sigma Chemical Co., St. Louis, MO). Platelet-rich plasma was supplemented with varying concentrations of factor Xa inhibitor. The aggregation profile was analyzed according to maximum percent aggregation.

Thrombin Generation Markers

The effect of DX-9065a (0–10 μg/mL) on thrombin generation was determined by measuring thrombin/antithrombin complex (Enzygnost® TAT micro, Dade Behring Marburg, Germany), prothrombin fragment F1+2 (Enzygnost® F1+2 micro, Dade Behring Marburg, Germany), and FPA generation (Imuclone® FPA ELISA, American Diagnostica Inc., Greenwich, CT). All thrombin generation markers were measured using manufacturer provided protocols.

Pharmacokinetic Study

Six adult male or female Rhesus monkeys (Macaca mulatta) weighing 4.0 to 16.0 kg were used for this study. The animals were fed with an open formula (extruded, nonhuman primate diet) water ad libitum and were group housed in accordance with the Guide for the Care and Use of Laboratory Animals, National Research Council (National Academy Press, Washington DC,
1996). Blood samples were drawn from the femoral or saphenous vein contralateral to the site of drug injection.

DX-9065a Administration and Plasma Sampling

DX-9065a was dissolved in physiologic saline, and the drug solution was sterilized through a 0.22-mm filter. Animals were anesthetized with 20 mg/kg ketamine intramuscularly before DX-9065a administration, and they received intravenous (IV) hydration after blood sampling. DX-9065a (500 μg/kg) was administered IV. Blood samples were obtained before and after IV administration at 5, 15, 30, 60, 120, 240, 360, and 1,440 minutes. Plasma was separated immediately by centrifugation at 3000 × g for 15 minutes and frozen at −70°C until analysis. Animals were evaluated for signs of toxicity and monitored with complete blood cell count after the experiment.

Bioanalytical Assays

Plasma concentrations of DX-9065a were determined using anti-Xa, PT, aPTT, and ACT assays. Plasma anti-Xa activity was determined with the chromogenic substrate assay as previously described using an automated coagulation laboratory (ACL) analyzer. The clot-based PT and aPTT were determined according to standard procedures. ACT assay was performed by standard procedure using whole blood.

Data Analysis

All data were analyzed by analysis of variance (one-way ANOVA) using Microsoft Excel® 2000.

RESULTS

In-vitro Effects of DX-9065a on Coagulation Parameters

Fig. 1 shows the effect of DX-9065a on the two different ACT tests. There was a concentration-dependent increase in the ACT response. Using the Hemochron instrument, activated clotting times of 310, 441, and 538 seconds were achieved at concentrations of 5, 10, and 25 μg/mL, respectively. With HemoTec instrument, clotting times of 261, 316, and 401 seconds were achieved at the same concentrations. Fig. 2 shows a concentration-dependent effect of pentasaccharide on Hemochron ACT test.

Four TEG parameters were used to demonstrate the anticoagulant effect of DX-9065a. Fig. 3 depicts the effect on R time, K time, MA, and alpha angle using two different concentrations of DX-9065a. The K time of 7.0 and 20.5 seconds were calculated at concentrations of 5 and 10 μg/mL, respectively. The R time of 29 and 47.5 seconds, the MA of 53 and 31 mm, and the alpha angles of 29 and 14 degrees were calculated at the same concentrations. R time was prolonged threefold baseline (p<0.01), K time was pro-
FIG. 2. Effect of pentasaccharide on ACT.

FIG. 3. Effect of DX-9065a on thrombelastography parameters. These results show the concentration-dependent prolongation of clot formation by DX-9065a. The statistical analysis was shown on figures (**p < 0.01, *p < 0.05).
longed 3.5-fold baseline (p<0.05) at the 10 μg/mL concentration of DX-9065a. These four parameters show the concentration-dependent prolongation of clot formation achieved with DX-9065a.

DX-9065a produced anticoagulant actions as measured by PT, aPTT, dilute aPTT, Heptest, Heptest-HI, and dRVVT tests. The TT and ECT assays were not affected.

Four different PT reagents and three different aPTT reagents were used to demonstrate the effect of DX-9065a on the coagulation system. Reagent-based variations were observed in both assays. A concentration-dependent anticoagulant effect of DX-9065a was observed with PT and aPTT tests (Figs. 4, 5, and 6) in a concentration range of 0–12.5 μg/mL. The response to PT reagents in decreasing order of activity is re-

**FIG. 4.** Effect of DX-9065a on prothrombin time. Concentration-dependent increase and reagent-dependent differences in PT results were observed.

**FIG. 5.** Anticoagulant effect of DX-9065a as measured by PT/INR. A concentration-dependent effect was observed.
combiplastin = innovin > thromboplastin C = simplastin.

The International Normalized Ratio (INR) values measured using different PT reagents in supplemented plasma samples were widely discrepant, ranging from 4.7 to 45.3. In decreasing order of activity was thromboplastin C > simplastin > innovin > combiplastin (Fig. 5).

The response of aPTT reagents in decreasing order is Stago = platelin > actin®. However, when the aPTT reagents were diluted with saline 1:1, the sensitivity was markedly increased (Fig. 7). Diluted Stago reagent showed a least sensitivity of aPTT response reaching a peak of 300 seconds at 12.5 µg/mL.

As shown in Fig. 8, DX-9065a supplemented in normal human plasma in the concentration

**FIG. 6.** Effect of DX-9065a on the aPTT. A concentration dependent effect was observed.

**FIG. 7.** Effect of DX-9065a on diluted aPTT. The sensitivity of factor Xa inhibitor was increased when aPTT reagents were diluted 1:1 with saline.
range of 0 to 12.5 μg/mL, the concentration-dependent effect demonstrated on the Heptest assay. Heptest-HI showed less sensitivity than the Heptest assay.

The effect of DX-9065a supplemented in normal human plasma in the concentration range of 0 to 12.5 μg/mL was studied using dilute Russell's viper venom time test. A concentration-dependent anticoagulant effect was observed (Fig. 9).

Fig. 10 demonstrates that DX-9065a did not have any effect on the thrombin time and ECT tests. The effect of DX-9065a supplemented in normal human plasma over a concentration range of 0 to 12.5 μg/mL was also studied using

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**FIG. 8.** Effect of DX-9065a on Heptest and Heptest-HI tests in the concentration of 0–12.5 μg/mL. The concentration-dependent effect of DX-9065a on Heptest assay was observed. Heptest-HI assay showed less sensitivity than the heptest assay.

**FIG. 9.** This figure shows the effect of DX-9065a on dRVVT test. This test is extremely sensitive to this factor Xa inhibitor.
the amidolytic anti-Xa assay and a modified anti-Xa method. The modified assay showed a stronger factor Xa inhibition than that in the conventional anti-Xa assay (Fig. 11).

Fig. 12 shows the synthetic factor Xa inhibitor is capable of inhibiting ADP-induced platelet aggregation at concentrations greater than 100 µg/mL. At therapeutic blood levels of DX-9065a (0–10 µg/mL), there was no effect on agonist-induced platelet aggregation (Fig. 13).

The inhibitory effect of DX-9065a on thrombin generation markers was also studied over a concentration range of 0–10 µg/mL. Fig. 14 de-
picts the concentration-dependent inhibition of thrombin generation markers.

The effect of a GP IIb/IIIa inhibitor, aggrastat, on the anticoagulant effects of DX-9065a supplemented in normal human whole blood in the concentration range of 0 to 10 μg/mL was studied using the ACT assay. Simultaneous addition of aggrastat (50 μg/mL) increased the ACT levels (Fig. 15).

**Pharmacodynamic Profile of DX-9065a**

As measured by the anti-factor Xa activity, the plasma concentration of DX-9065a decreased
FIG. 14. DX-9065a inhibits thrombin generation at a concentration as low as 1 μg/mL.

FIG. 15. This figure shows DX-9065a increased ACT levels (p < 0.05) when combined with a GP IIb/IIIa inhibitor, Aggrastat.

from 10.1 ± 0.2 at 5 minutes, to 3.6 ± 0.8 μg/mL 2 hours after the intravenous injection at a dose of 500 μg/kg (Fig. 16, Table 1). The pharmacokinetic parameters derived from anti-Xa activity are listed in Table 2. The mean activated partial thromboplastin time (APTT) and prothrombin time following the single iv administration of DX-9065a are presented in Figs. 17 and 18, respectively. Following the injection, peak APTT and PT were 54.0 ± 15.9 and 18.5 ±
This figure shows the inhibitory effect of DX-9065a on factor Xa following intravenous administration to nonhuman primates.

**FIG. 16.**

**TABLE 1.** Pharmacokinetics of DX-9065a Based on Anti-factor Xa Activity After an Intravenous Administration at 500 μg/kg in Primates

<table>
<thead>
<tr>
<th>Time (hr)</th>
<th>Plasma Concentration of DX-9065a (μg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0.0 ± 0.0</td>
</tr>
<tr>
<td>0.083</td>
<td>10.1 ± 0.2</td>
</tr>
<tr>
<td>0.25</td>
<td>6.8 ± 1.0</td>
</tr>
<tr>
<td>0.5</td>
<td>5.6 ± 1.0</td>
</tr>
<tr>
<td>1.0</td>
<td>4.4 ± 0.7</td>
</tr>
<tr>
<td>2.0</td>
<td>3.6 ± 0.8</td>
</tr>
</tbody>
</table>

0.4 seconds (5 mins. post), respectively. Both APTT and PT returned to baseline by 2 hours after DX-9065a injection. The mean ACT increased from baseline to an Emax of 199 ± 28 seconds at 5 minutes after the bolus injection, and returned to baseline after 2 hours (Fig. 19). In fibrinopeptide A (FPA) generation assay, FPA level decreased from 102 ± 16 ng/mL to 37 ± 9 ng/mL at 5 minutes after the bolus injection, and returned to baseline after 2 hours (Fig. 20, Table 3).

**DISCUSSION**

**Effect on ACT System**

DX-9065a exhibited a concentration-dependent effect on the Hemochron (celite) and

**TABLE 2.** Pharmacokinetic Parameters of DX-9065a After Single-dose Intravenous Administration

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>PK parameters based on Anti-Xa</td>
<td></td>
</tr>
<tr>
<td>Basal (μg/mL)</td>
<td>0.0 ± 0.0</td>
</tr>
<tr>
<td>Cmax (μg/mL)</td>
<td>10.1 ± 1.0</td>
</tr>
<tr>
<td>Tmax (hr)</td>
<td>0.083</td>
</tr>
<tr>
<td>AUC (μg/mL•hr)</td>
<td>9.5 ± 1.5</td>
</tr>
<tr>
<td>t1/2 (hr)</td>
<td>0.2 ± 0.1</td>
</tr>
<tr>
<td>Ke (1/h)</td>
<td>3.8 ± 1.1</td>
</tr>
<tr>
<td>APTT</td>
<td></td>
</tr>
<tr>
<td>Basal (s)</td>
<td>36.6 ± 10.7</td>
</tr>
<tr>
<td>Emax (s)</td>
<td>54.0 ± 15.9</td>
</tr>
<tr>
<td>Tmax (hr)</td>
<td>0.083</td>
</tr>
<tr>
<td>AUC (s•hr)</td>
<td>10.4 ± 6.6</td>
</tr>
<tr>
<td>PT</td>
<td></td>
</tr>
<tr>
<td>Basal (s)</td>
<td>10.1 ± 0.5</td>
</tr>
<tr>
<td>Emax (s)</td>
<td>18.5 ± 0.4</td>
</tr>
<tr>
<td>Tmax (hr)</td>
<td>0.083</td>
</tr>
<tr>
<td>AUC (s•hr)</td>
<td>7.9 ± 2.2</td>
</tr>
<tr>
<td>ACT</td>
<td></td>
</tr>
<tr>
<td>Basal (s)</td>
<td>132 ± 13</td>
</tr>
<tr>
<td>Emax (s)</td>
<td>199 ± 28</td>
</tr>
<tr>
<td>Tmax (hr)</td>
<td>0.083</td>
</tr>
<tr>
<td>AUC (s•hr)</td>
<td>37.0 ± 20.0</td>
</tr>
</tbody>
</table>

*Data are presented as mean ± SD; dose: 500 μg/kg.

Cmax, Maximum plasma concentration; Emax, maximum plasma effect; tmax, time to reach Emax; AUC, area under the effect-time curve; t1/2, half-life; ke, elimination constant.
HemoTec (cartridge) ACT systems. Even at a final concentration of 5 µg/mL, DX-9065a increased both the ACTs to approximately 250 seconds. At a final concentration of 10 µg/mL, the ACTs increased to more than 300 seconds. This suggests that DX-9065a at these concentrations may be a useful anticoagulant during interventional cardiologic procedures such as PTCA, atherectomy, and rotablation. Other synthetic factor Xa inhibitors such as fondaparinux (Arixtra®), which requires AT for its anticoagulant effects does not increase the celite ACT to
the same extent as DX-9065a. Fondaparinux at a final concentration of 100 μg/mL exhibited a modest increase of ACT to approximately 200 seconds. This suggests that this agent may not be useful for interventional cardiologic procedures.

Even if it increases the ACTs at higher concentrations, one would be cautious to achieve such high concentrations for fear of bleeding complications. The reason why DX-9065a increases the ACT in contrast to fondaparinux may be due to...
Table 3. Effect of DX-9065a on Fibrinopeptide A (FPA) Generations After an Intravenous Administration at 500 µg/kg in Primates

<table>
<thead>
<tr>
<th>Time (hr)</th>
<th>FPA Generation (ng/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>102 ± 16</td>
</tr>
<tr>
<td>0.083</td>
<td>37 ± 9</td>
</tr>
<tr>
<td>0.25</td>
<td>51 ± 10</td>
</tr>
<tr>
<td>0.5</td>
<td>78 ± 9</td>
</tr>
<tr>
<td>1.0</td>
<td>98 ± 9</td>
</tr>
<tr>
<td>2.0</td>
<td>106 ± 10</td>
</tr>
<tr>
<td>AUC (ng/mL•hr)</td>
<td>33 ± 4</td>
</tr>
</tbody>
</table>

AUC, Area under the concentration-time curve.

the antithrombin-sparing effect with DX-9065a. Because DX-9065a does not require antithrombin for its anticoagulant actions, this antithrombin-sparing effect may contribute to an additional anticoagulant effect. The implications of this difference for the mechanism of action of DX-9065a and fondaparinux enable DX-9065a to be used in patients with antithrombin deficiency. DX-9065a also has a significant advantage over fondaparinux in terms of its ability to inhibit the clot-bound factor Xa. Fondaparinux being a heparinomimetic agent requires endogenous AT for its effects and hence upon its complexation with AT is not capable of inhibiting the clot-bound factor Xa. Similarly DX-9065a is capable of inhibiting the prothrombinase-bound factor Xa in contrast to Fondaparinux which has limited inhibition of clot-bound and prothrombinase-bound factor Xa. Inhibition of the prothrombinase-bound factor Xa and clot-bound factor Xa are important for any anticoagulant drug, since its sparing effect would enable further modulation of thrombin resulting in thrombosis.

Effect of DX-9065a on Thrombelastography

DX-9065a exhibited a potent anticoagulant effect by thrombelastography causing the R-time to increase approximately threefold at a final concentration of 10 µg/mL. There is a corresponding decrease in other thrombelastographic parameters such as maximum amplitude (MA) and angle (α).

Effect of DX-9065a on Platelet Aggregation

DX-9065a did not show any effect on ADP-induced platelet aggregation at a final concentration of 100 µg/mL. Similarly, there was no effect on collagen-, epinephrine-, and arachidonic acid–induced platelet aggregations. Although there was a significant increase in the inhibition of ADP-induced platelet aggregation at a final concentration of 225 and 450 µg/mL, these are far beyond the therapeutic or surgical plasma concentrations at which toxic and adverse complications might be manifested. The lack of antithrombotic effect of DX-9065a at therapeutic plasma levels would be advantageous in terms of decreased bleeding complications. However, in situations such as stenting, atherectomy, and rotablation procedures, where one would expect increased platelet activation, DX-9065a may be used in combination with antithrombotic agents such as aspirin, thienopyridine derivatives such as ticlopidine and clopidogrel, or GP IIb/IIIa receptor antagonists.

Effect of DX-9065a on Global Coagulation Profile

DX-9065a exhibited a concentration-dependent increase in the prothrombin time using four different PT reagents. Thromboplastin-C and Simplastin exhibited a relatively lower increase in the PT values (up to 45 seconds) compared to recombiplastin and innovin reagents (up to 70 seconds). However, the calculated INR values, Thromboplastin-C (45.3) and simplastin (20.7) were surprisingly higher than those with recombiplastin (4.7) and innovin (6.6). These significant findings have therapeutic implications when DX-9065a is used in combination with oral anticoagulant drugs such as coumadin. Because of these false increases of INR values with DX-9065a, patients who are on concomitant coumadin therapy should be carefully evaluated for their corresponding INR values and dosage adjustment. Because the PT reagents recombiplastin and innovin exhibit a minimum increase in the INR values with DX-9065a, these reagents may be used with caution to calculate the INR values in patients who are on DX-9065a and coumadin combination therapy. The significant alterations of INR with DX-9065a also have implications in the treatment of patients who are initially on DX-9065a therapy during the hospital stay and are going to be switched over to coumadin therapy on discharge.

DX-9065a exhibited a concentration-dependent effect on the aPTT assay performed using three different reagents reaching up to 110 seconds at a final concentration of 12.5 µg/mL. However, the diluted aPTT assay using the three different reagents exhibited a marked increase in
the aPTT reaching up to more than 300 seconds with Stago reagent, 225 seconds with Actin reagent, and 193 seconds with Platelin reagent at a final concentration of 12.5 μg/mL. This suggests that in addition to the amidolytic anti-Xa assay, aPTT or diluted aPTT assays may be used to monitor the anticoagulant effects of DX-9065a. DX-9065a has a moderate effect on Heptest assay reaching up to 50 seconds at a final concentration of 12.5 μg/mL. Thrombin time and ecarin clotting time were not affected with DX-9065a. DX-9065a being a direct factor Xa inhibitor has a significant effect on the amidolytic anti-Xa assay reaching a maximum of 68.5% inhibition at a final concentration of 12.5 μg/mL. This assay may be used to monitor the therapeutic levels of DX-9065a. Prolongation of the dRVVT is a sensitive measure of factor Xa inhibitor levels. DX-9065a significantly prolonged the dRVVT up to 130 seconds at a final concentration of 12.5 μg/mL and could be used to monitor the anticoagulant effects of this agent. This has significant diagnostic implications in patients with systemic lupus erythematosus (SLE) who are on concomitant therapy with DX-9065a as normal individuals on diagnostic workup for SLE who are on DX-9065a therapy may have an abnormal false increase of dRVVT and are at risk of a false diagnosis of SLE. Patients with SLE who are on DX-9065a therapy may have an abnormally higher elevation of dRVVT values interfering with the interpretation of the severity of SLE.

Effect of DX-9065a on Thrombin Generation Markers

DX-9065a produced a marked reduction in thrombin generation as assessed by measuring levels of markers such as FPA, F1.2, and TAT complex. Even at a final concentration of 1 μg/mL, there was a significant inhibition of thrombin generation. Because DX-9065a is capable of inhibiting not only factor Xa, but also markers of thrombin generation, clot-bound and prothrombinase-bound factor Xa, it may be a relatively better anticoagulant drug when compared to others.

Pharmacokinetic and Pharmacodynamic Profile of DX-9065a in Nonhuman Primates

The anti-factor Xa activity of DX-9065a in plasma decreased from 10.1 ± 0.2 (5 minutes post) to 3.6 ± 0.8 μg/mL 2 hours after the intravenous administration at a dose of 500 μg/kg. The calculated half-life based on anti-Xa assay of DX-9065a administered intravenously to non-human primates at a dose of 500 μg/kg was 0.2 ± 0.1 hours. Due to the lack of an effective antagonist to DX-9065a, its relative shorter half-life (1.5 hours) in humans, when compared to fondaparinux (14 hours) is a significant advantage when used in acute surgical procedures. The dosages can be titrated and adjusted depending on the length of the surgical procedure. Because of its short half-life, limited bleeding side effects, and a predictable dose response, monitoring of the anticoagulant effects of DX-9065a may not be required. Patients who are receiving intravenous DX-9065a during the hospital stay for any surgical procedure may be discharged on oral DX-9065a, because of its oral bioavailability. Because of the predictable dose response with DX-9065a, such a transition would be easier, unlike coumadin, which would require time for initiation of its anticoagulant effect. DX-9065a is mainly eliminated through urine and feces. In patients with renal failure, the dosage of this drug would be adjusted to avoid accumulation of the drug in the body or perhaps bleeding complications.

CONCLUSIONS

1. DX-9065a produced a concentration-dependent increase in the Hemochron celite ACT and HemoTec ACT and may be used in interventional cardiac procedures.
2. DX-9065a significantly prolonged the R time on thrombelastography.
3. DX-9065a did not show any effect on ADP-induced, collagen-induced, epinephrine-induced, and arachidonic acid–induced platelet aggregation at therapeutic concentrations. Most probably, this contributes to fewer bleeding effects with DX-9065a.
4. DX-9065a exhibited a concentration-dependent prolongation of the PT values, using four different PT reagents. However, the calculated INR values were markedly increased. Hence, INR values are of no help when coumadin is concomitantly administered to the patient.
5. DX-9065a exhibited a concentration-dependent effect on aPTT test using three different aPTT reagents. The diluted APTT assays showed greater sensitivity than the regular aPTT assay.
6. DX-9065a, being a direct factor Xa inhibitor, showed significant effect on the amidolytic anti-Xa assay.
7. DX-9065a prolonged the dRVVT and hence this test could be used to monitor its anticoagulant effect. This will have an impact on diagnostic interpretation in patients with SLE.
8. DX-9065a produced a marked reduction in thrombin generation as measured by decreases in the levels of FPA, F 1.2, and TAT complex.

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