Clinical research

Potent inhibition of thrombin with a monoclonal antibody against tissue factor (Sunol-cH36): results of the PROXIMATE-TIMI 27 trial

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Aims Exposure of tissue factor (TF) is a critical proximal step in the pathogenesis of acute coronary syndromes. Sunol-cH36, a chimaeric monoclonal antibody to TF, blocks binding of factor X to the TF:VIIa complex. This report describes the first completed trial of Sunol-cH36 in humans.

Methods and results We assessed the safety and pharmacokinetics of Sunol-cH36 in an open-label, dose-escalating trial among subjects with stable coronary artery disease. The safety analysis included all adverse events with a focus on overt or occult bleeding. Five doses of Sunol-cH36 (0.03, 0.06, 0.08, 0.1, 0.3 mg/kg) were administered as a single intravenous bolus to 26 subjects (three to eight subjects per dose tier). No major bleeding (≥2 g/dL haemoglobin decline) occurred. Spontaneous minor bleeding was observed with a dose-related pattern. Notably, the majority of spontaneous bleeding episodes were clinically consistent with platelet-mediated bleeding (e.g. gum, tongue) without thrombocytopenia. The median terminal half-life was 72.2 (25th, 75th: 28.4, 72.5) h.

Conclusion Sunol-cH36 exhibited dose-dependent anticoagulant effects. We postulate that the mucosal bleeding observed with this potent inhibitor of thrombin generation may reflect antplatelet effects resulting from networking between the coagulation cascade and platelet pathways that could prove clinically relevant with this novel class of anticoagulants.

Keywords Clinical trials; Antithrombins; Coronary artery disease; Tissue factor

Introduction

Rupture of vulnerable or high-risk atherosclerotic plaque, the most frequent inciting event for an acute coronary syndrome, exposes the highly pro-coagulant contents of the atheroma core, resulting in concomitant activation of circulating coagulation proteins and platelets. The exposure of tissue factor (TF) is the predominant contributor to activation of the extrinsic coagulation pathway and initiation of thrombus formation.1,2 When in contact with circulating blood, TF binds to Factor VII to initiate the extrinsic pathway and generate activated Factor X (Xa).3,4 Factor Xa converts pro-thrombin (PT)
to thrombin (Factor Ila), the key enzyme that generates fibrin. In addition to generating fibrin, thrombin promotes platelet activation and aggregation, and exerts positive feedback within the coagulation cascade. At each branch in this pathway, one molecule of activated enzyme is able to activate many molecules of its substrate protein, thereby amplifying each step in the cascade. In addition, extensive networking between substrate protein, thereby amplifying each step in the enzyme is able to activate many molecules of its sub- 

strate protein, thereby amplifying each step in the coagulation cascade. In addition, extensive networking between each branch in this pathway, one molecule of activated X (or Factor IX) complex (PROXIMATE)-TIMI 27 trial was designed to assess the inhibition using a Monoclonal Antibody to TissuE Factor (PROXIMATE)-TIMI 27 trial was designed to assess the safety and pharmacokinetics of Sunol-cH36 in an open-label, dose-escalating trial among subjects with stable coronary artery disease treated with aspirin.

Methods

Study population

Patient enrolment occurred between 1 April 2002 and 13 August 2003 in four centres in the United States. Eligible participants were men and non-pregnant women, aged 21–65 years, with stable coronary artery disease treated with aspirin. Patients with changes in their cardiovascular medications in the prior 2 weeks, hospitalization in the prior 2 months, coronary artery bypass grafting in the prior 3 months, or percutaneous coronary revascularization in the prior 6 months were excluded. Additional exclusion criteria included any history of cerebrovascular disease, poorly controlled hypertension (systolic pressure >160 mmHg or diastolic pressure >90 mmHg), estimated creatinine clearance <40 mL/min, known or suspected liver disease, prior treatment with murine antibodies, treatment with anticoagulant or antiplatelet medication other than aspirin, or indicators of increased risk of bleeding. The latter included known bleeding diathesis, anaemia (haemoglobin <11 g/dL), thrombocytopenia (platelet count <100 000/mm³), history of clinically significant bleeding in the previous year, recent trauma or surgery, documented peptic ulcer disease, haemorrhagic retinopathy, or intracranial pathology.

The protocol was approved by the Institutional Review Board at each participating centre and all patients provided written informed consent prior to the performance of study procedures.

Study protocol

The trial was an open-label, dose-escalation study of a single intravenous bolus of Sunol-cH36. The trial design included four planned dose tiers (0.03, 0.3, 1.0, and 3.0 mg/kg) with seven patients in each dose group and, provided that enrolment could be modified, or additional dose tiers were added, based upon review by the Safety Committee. Such decisions were based upon deliberation by the Committee after assessment of all available clinical and safety data. There was no formal stopping rule. After enrolment of seven subjects at 0.03 mg/kg and four subjects in the 0.3 mg/kg dose tier, the Safety Committee recommended termination of recruitment in the 0.3 mg/kg dose group, and modification of the planned dose tiers to eliminate doses higher than 0.3 mg/kg and to introduce additional lower dose groups (0.06, 0.1, 0.15, and 0.2 mg/kg) enrolling four patients (with an extension to seven patients at the highest dose level). After review of seven subjects at 0.1 mg/kg, the Safety Committee recommended elimination of doses higher than 0.1 mg/kg and study of one additional lower dose group (0.08 mg/kg).

All participants were treated with aspirin 81–325 mg once daily. The assigned dose of Sunol-cH36 was administered in 100 mL of normal saline delivered intravenously as a bolus over 15 min. Patients were observed for 8 h after administration of the bolus, and returned for follow-up evaluations at 24 h, 48 h, 72 h, 7 days, 10 days, 2 weeks, 4 weeks, and 7 weeks. One subject enrolled in the 0.3 mg/kg dose cohort, received 0.03 mg/kg through a dosing error, and was analysed according to the actual dose received.

Clinical procedures

Blood was sampled prior to administration of the study drug and at 15 min, 30 min, 1 h, 2 h, 4 h, 8 h, and each study visit thereafter for measurement of the plasma level of Sunol-cH36. A complete blood count and serum chemistries were performed pre-dose, at 2 h and 8 h after the bolus dose, and at each subsequent study visit. Coagulation parameters [PT, activated partial thromboplastin time (aPTT)] were determined at each of these time points, as well as at 30 min and 4 h post-dose. Bleeding times were not performed. Serum samples for detection of human antichimaeric antibodies (HACAs) were obtained pre-dose and at the 4 and 7 week follow-up visits. Testing of HACAs to Sunol-cH36 was performed using a double-antigen radiometric assay employing Sunol-cH36-coated polystyrene beads and 125I-Sunol-cH36. Surveillance for occult gastrointestinal bleeding was accomplished by guaiac testing at the 24-h, 48-h, 2-week, and 4-week visits. Urinalysis for microscopic haematuria was performed at 4 h after study drug and at each subsequent study visit.
Endpoints and statistical analysis

The principal objectives of this study were to assess the safety profile and pharmacokinetics of increasing doses of Sunol-cH36 in patients with stable coronary artery disease taking aspirin. Major bleeding was defined as clinically overt or occult bleeding resulting in a ≥2 g/dL decline in haemoglobin and requiring transfusion, or retroperitoneal, intra-cranial or intra-ocular haemorrhage, or haemorrhage associated with hypotension or death. Minor bleeding events were defined broadly, as clinically overt or occult bleeding not meeting the criteria for major bleeding.

All patients who received any dose of study drug were included in the safety analysis. Patients were categorized according to the dose received. Testing for an association between the dose of Sunol-cH36 and bleeding was performed using a χ² test for trend. Analyses were performed using STATA V7-intercooled (STATA Corp., College Station, TX, USA). P-values of <0.05 (two-sided) were considered to indicate statistical significance. Non-compartmental methods were used to determine the pharmacokinetic parameters. The maximum measured concentration (C_{max}) was evaluated for each patient. The area under the plasma concentration curve, elimination rate constant (k_e), and terminal half-life (t_{1/2} = ln 2/k_e) were calculated using WinNonlin Pro (Pharsight Inc., Mountain View, CA, USA).

Analysis of clot formation in minimally altered whole blood

In a separate study, two experiments were performed on blood from healthy volunteers. In the first experiment, blood samples (n = 5 subjects) were drawn and treated with corn trypsin inhibitor (Haemalogic Technologies Inc., Essex Junction, VT, USA) and immediately pre-incubated with or without 13 nM (2 μg/mL) final concentration of Sunol-cH36 antibody for 1 min. Recombinant human TF was added to a final concentration of 40 pM and samples incubated at 37°C. Clotting was stopped at 0, 3, 6, and 9 min by the addition of the anticoagulant solution provided in the FPA ELISA kit. Samples were then placed on ice and centrifuged at 2000 g for 15 min. Plasma samples were analysed for Fibrinopeptide A levels using ELISA kits (DiaPharma Group Inc., West Chester, OH, USA). In the second experiment, subjects (n = 5) had blood drawn on Day 1 and were given 325 mg aspirin orally on Days 1 and 2 and on Day 3 blood was again drawn. Samples were incubated with or without Sunol-cH36.

Results

A total of 26 patients were enrolled in the trial. Follow-up through 7 weeks was complete for all patients enrolled. The baseline characteristics of the study population are described in Table 1. By design, the median age was lower than that expected for a community-based population with established coronary artery disease. The proportion of patients receiving aspirin 325 mg daily was 75, 50, 75, 83, and 0% in the 0.03, 0.06, 0.08, 0.1, and 0.3 mg/kg dose tiers, respectively. The remainder received 81 mg daily with the exception of one patient in the 0.06 mg/kg dose tier (162 mg qd).

Table 1 Baseline characteristics (n = 26)

<table>
<thead>
<tr>
<th>Demographics</th>
<th></th>
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</thead>
<tbody>
<tr>
<td>Age, years</td>
<td>55 (53, 60)</td>
</tr>
<tr>
<td>Female</td>
<td>7 (26.9)</td>
</tr>
<tr>
<td>Caucasian</td>
<td>15 (57.7)</td>
</tr>
<tr>
<td>Medical history</td>
<td></td>
</tr>
<tr>
<td>Tobacco use</td>
<td></td>
</tr>
<tr>
<td>Active</td>
<td>4 (15.4)</td>
</tr>
<tr>
<td>Past</td>
<td>15 (60.0)</td>
</tr>
<tr>
<td>Diabetes</td>
<td>8 (30.8)</td>
</tr>
<tr>
<td>History of hypertension</td>
<td>22 (84.6)</td>
</tr>
<tr>
<td>Prior acute coronary syndrome</td>
<td>20 (76.9)</td>
</tr>
<tr>
<td>Prior revascularization</td>
<td>20 (76.9)</td>
</tr>
</tbody>
</table>

Data are shown as n (%) for dichotomous variables and median (25th, 75th percentile) for continuous variables.

Bleeding and other safety events

The doses administered and incidence of bleeding are detailed in Table 2. There were no major bleeding events. Spontaneous minor bleeding occurred in 14 patients, with a graded rise in the frequency of bleeding with increasing dose of study drug (P = 0.03). The majority (66.7%) of the bleeding events occurred within the first 3 days after receiving study drug, with all of the events occurring by Day 12 of follow-up. Notably, 8 of the 14 patients with minor haemorrhage experienced oral mucosal bleeding, spontaneous tongue haematomas, or cutaneous bleeding consistent with platelet-mediated bleeding (Table 3). These minor bleeding episodes occurred in the absence of significant changes in the platelet count or prolongation of the aPTT (Figure 2A and B). The PT in the highest dose group was modestly prolonged as anticipated due to the recognized interaction between Sunol-cH36 and human TF-based assays for measurement of PT (Figure 2C).

There were no serious adverse events judged as related to study medication by the local investigators. Five patients experienced non-serious (non-bleeding) adverse events reported as possibly or probably related to the study drug (Table 4). These non-serious adverse events included headache, fatigue, and asthenia. No clinically significant abnormalities of liver chemistry, creatinine, or electrolytes were observed. Nine patients developed detectable HACAs by 7 weeks of follow-up.

Pharmacokinetics

Maximum plasma concentrations were observed after the intravenous bolus with subsequent decline in an apparently bi-exponential manner (Figure 3). Median C_{max} (0.69, 1.52, 2.13, 3.0, 10.1 μg/mL) and AUC_{last} values increased in nearly linear fashion over the five doses studied. The median apparent terminal half-life (t_{1/2})
was 72.2 (25th, 75th: 28.4–72.5) h over the 7 weeks covered by this study.

Effects on coagulation in whole blood

The potency of Sunol-cH36 was evaluated using the minimally altered whole blood assay. Whole blood was drawn from five healthy subjects before and after administration of aspirin. Clot formation was initiated with 40 pM of recombinant human TF. As shown in Figure 4, the addition of Sunol-cH36 at a final concentration of 13 nM (2 μg/mL), similar to the Cmax achieved with the 0.08 mg/kg infusion of Sunol-cH36 in PROXIMATE, delayed clot formation. When added to the whole blood assay in the absence of aspirin, Sunol-cH36 was associated with significant inhibition of thrombin generation (Figure 5A, P < 0.03). Moreover, when tested in combination with aspirin, both the antithrombotic effect of Sunol-cH36 measured by clotting time (Figure 4) and the degree of inhibition of thrombin generation (Figure 5B, P < 0.03) were more pronounced compared with Sunol-cH36 alone.

Discussion

In this first completed trial of Sunol-cH36 in humans, this monoclonal antibody against TF exhibited dose-dependent anticoagulant effects. The safety profile from this preliminary experience is encouraging for future investigation. The observation that the bleeding episodes were minor but clinically consistent with platelet-mediated bleeding, including tongue haematomas characteristic of disorders of primary haemostasis such as Glanzmann’s thrombasthenia [absence of the glycoprotein (GP) IIb/IIIa receptor], is intriguing.

Human TF is a 263 amino acid transmembrane glycoprotein with an extracellular domain that binds to Factor VIIa as well as its inactive zymogen, Factor VII. This cell surface protein is expressed by endothelial cells, monocytes, and smooth muscle cells and is upregulated in response to a variety of stimuli, including vascular endothelial injury. Although TF is primarily a membrane bound protein, it is detectable in circulating plasma at low concentrations (0.0067 nM) in healthy individuals and at modestly but detectably higher concentrations in patients presenting with unstable angina (0.01 nM), as well as among patients with angiographic no-reflow during percutaneous coronary intervention. TF is also found more abundantly in atherosclerotic plaque from patients with unstable compared with stable angina. In addition to initiating the extrinsic and intrinsic (via IXa) coagulation pathways, TF has been implicated in short- and long-term adverse effects mediated through inflammatory pathways. Inhibitors of TF thus have the potential to modulate both thrombotic and inflammatory effects of the protein. Moreover, by virtue of the critical initiating role of TF in the coagulation cascade, upstream of multiple pathways of forward and feedback amplification, inhibitors of this protein may impede thrombin generation and activity more effectively than antithrombins acting primarily downstream at the level of Factor Xa or thrombin itself.

The clinical observation from PROXIMATE-TIMI 27 of an unanticipated, dose-related incidence of mucosal
bleeding characteristic of disruption of primary haemostasis, points towards possible important effects of Sunol-cH36 on platelets. Confirmatory measurements of platelet function are not available from the PROXIMATE trial; however, the results raise a plausible hypothesis to be addressed in future investigations. Moreover, the results of our testing in whole blood point towards possible synergistic effects between Sunol-cH36 and aspirin on inhibition of clot formation and thrombin generation. Interactions between the coagulation and platelet pathways are multifaceted and central to maintaining haemostasis. The links between these two pathways, and the impact of therapies directed at one pathway or the other have taken on particular importance as the combined clinical use of more potent antithrombins and antiplatelet agents has increased in the management of acute coronary syndromes. The influence of platelets on thrombin generation has attracted intense interest, as agents which bind to the GP IIb/IIIa receptor on platelets have been shown to inhibit production of thrombin, possibly by interrupting binding of PT to platelet surface integrins and reducing the platelet surface area acting as a platform for the pro-thrombinase complex.\textsuperscript{5,14} In turn, thrombin promotes platelet activation and aggregation, both through binding of its byproduct fibrin to integrins on the platelet surface, and
as a direct agonist via the G-protein-coupled receptor family known as protease-activated receptors (PARs), and possibly GP Ib. Through G-protein-coupled signalling, thrombin activates phospholipase C, thereby increasing the cytosolic concentration of Ca2+ and inducing changes in platelet shape.15 As such, thrombin is believed to play an important role in extension of the platelet plug during primary haemostasis and to contribute to initiation of platelet activation. TF and/or the TF:VIIa complex may also play a direct role in platelet activation and/or aggregation.17,18 Specifically, TF has been shown to act as a cofactor for the activation of both PAR2 (endothelial cells) and PAR1 (platelets).17,19 Moreover, TF accumulation in the developing thrombus through a mechanism involving P-selectin on the platelet surface has been demonstrated.18,20 We postulate that possibly through interruption of key TF–platelet interactions and/or ultra-potent inhibition of thrombin generation, Sunol-ch36 may modify their contributions to platelet activation and aggregation, and thus act synergistically with aspirin to exert significant direct antiplatelet effects.

Notably, studies of other antithrombins have also demonstrated inhibition of thrombin-induced platelet activation.21 At least one direct antithrombin, bivalirudin, has been proposed to reduce or obviate the need for GP IIb/IIIa antagonists in selected groups of patients.22 Should future mechanistic studies in humans support relevant antiplatelet actions of Sunol-ch36, study of the agent as an alternative to combined use of anticoagulant and antiplatelet drugs may be appropriate.

There are limitations to this study. By virtue of the small sample size, the confidence limits around our estimates of rates of minor bleeding are necessarily wide. By design, the trial excluded patients receiving other antithrombins or antiplatelet therapy more potent than aspirin. As such, study in larger trials and in combination with such agents will be important in defining the safety of Sunol-ch36 in the context of contemporary care for patients with unstable coronary syndromes. Also important to the future study of this agent will be the assessment of biochemical measures of the clinical effect in humans. As demonstrated in this study, standard measures such as the PT using human TF are subject to ‘interference’ by binding of Sunol-ch36 to the test reagent and modest elevation of the PT at concentrations that manifest a clinical effect. In the absence of comprehensive testing of platelet function, our observations must be viewed as hypothesis generating and as a basis for additional investigation.

Conclusion

Administration of a chimaeric antibody against TF offers a novel approach to anticoagulation, with the potential to be more effective than agents acting principally at the level of Factors Xa and IIa. We postulate that the mucosal bleeding observed with this potent inhibitor of thrombin generation may reflect antiplatelet effects.
resulting from important networking between the coagulation cascade and platelet pathways that could prove clinically relevant with this novel class of anticoagulants.

Acknowledgement

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Appendix

Enrolling centres (number enrolled)

Brigham and Women’s Hospital, Boston, MA, USA (4); Investigators: David A. Morrow, Howard A. Cooper, Benjamin S. Scirica.

Minneapolis Heart Institute Foundation, Minneapolis, MN, USA (5); Investigator: Timothy D. Henry.

University of Miami/Jackson Memorial Hospital, Miami, FL, USA (7); Investigators: Raphael Sequeira, S. Medrano.

SFBC International, Miami, FL, USA (10); Investigator: Lawrence Galitz.

References


