Recombinant Nematode Anticoagulant Protein c2 in Patients With Non–ST-Segment Elevation Acute Coronary Syndrome

The ANTHEM–TIMI-32 Trial

Robert P. Giugliano, MD, SM, FACC,* Stephen D. Wiviott, MD,* Peter H. Stone, MD, FACC,† Daniel I. Simon, MD, PhD, FACC,‡ Marc J. Schweiger, MD, FACC,§ Alain Bouchard, MD, FACC,‖ Massoud A. Leesar, MD, FACC,∥ Michael A. Goulder, BSc,** Steven R. Deitcher, MD,†† Carolyn H. McCabe, BS,* Eugene Braunwald, MD, MACC,* for the ANTHEM–TIMI-32 Investigators

Boston and Springfield, Massachusetts; Cleveland, Ohio; Birmingham, Alabama; Louisville, Kentucky; Nottingham, United Kingdom; and San Carlos, California

Objectives
We sought to evaluate the safety and efficacy of recombinant nematode anticoagulant protein c2 (rNAPc2) in patients with non–ST-segment elevation acute coronary syndrome (nSTEMI).

Background
Recombinant NAPc2 is a potent inhibitor of the tissue factor/factor VIIa complex that has the potential to reduce ischemic complications mediated by thrombin generation.

Methods
A total of 203 patients were randomized 4:1 to double-blinded intravenous rNAPc2 or placebo every 48 h for a total of 1 to 3 doses in 8 ascending panels (1.5 to 10 \(\mu\)g/kg). All patients received aspirin, unfractionated heparin (UFH), or enoxaparin and early catheterization; clopidogrel and glycoprotein IIb/IIIa blockers were encouraged. Two subsequent open-label panels evaluated 10 \(\mu\)g/kg rNAPc2 with half-dose UFH (n = 26) and no UFH (n = 26). The primary end point was the rate of major plus minor bleeding. Pharmacokinetics, pharmacodynamics, continuous electrocardiography, and clinical events were assessed.

Results
Recombinant NAPc2 did not significantly increase major plus minor bleeding (3.7% vs. 2.5%; p = NS) despite increasing the international normalized ratio in a dose-related fashion (trend p = 0.0001). Higher-dose rNAPc2 (≥7.5 \(\mu\)g/kg) suppressed prothrombin fragment F1.2 generation compared with placebo and reduced ischemia by >50% compared to placebo and lower-dose rNAPc2. Thrombotic bailout requiring open-label anticoagulant occurred in 5 of 26 patients treated without UFH, but none in the half-dose UFH group (19% vs. 0%: p = 0.051).

Conclusions
In patients with nSTEMI managed with standard antithrombotics and an early invasive approach, additional proximal inhibition of the coagulation cascade with rNAPc2 was well tolerated. rNAPc2 doses ≥7.5 \(\mu\)g/kg suppressed F1.2 and reduced ischemia, though some heparin may be necessary to avoid procedure-related thrombus formation. (Anticoagulation With rNAPc2 to Eliminate MACE/TIMI 32; http://www.clinicaltrial.gov/ct/show/NCT00116012?order=1; NCT00116012) (J Am Coll Cardiol 2007;49:2398–407) © 2007 by the American College of Cardiology Foundation
subendothelial tissue factor (TF) and releases microparticles containing TF, a highly procoagulant material found in elevated concentration in the necrotic atherosclerotic core. This results in the rapid initiation of platelet activation and aggregation and thrombus formation. Current standard antithrombotic therapies (heparins, aspirin, clopidogrel, glycoprotein [GP] IIb/IIIa blockers) do not suppress thrombin generation and percutaneous coronary intervention (PCI) enhances it. Because proximal inhibitors of the coagulation cascade are potent inhibitors of new thrombin generation, several agents have been developed to exploit this potential advantage over drugs that act more downstream in the cascade, such as heparins.

Recombinant nematode anticoagulant protein c2 (rNAPc2) is an 85-amino acid serine protease inhibitor fashioned after the original protein isolated from the hemotaphagous hookworm parasite Ancylostoma caninum. The anticoagulant activity results from inhibition of the catalytic complex of TF/tissue factor/activated factor VII (fVIIa) by a unique mechanism that requires the initial binding of rNAPc2 to zymogen or activated factor X before the formation of the final ternary inhibitory complex with TF/fVIIa. The elimination half-life in man is 50 to 60 h and is independent of dose and route of administration (intravenous or subcutaneous). Earlier studies of rNAPc2 have demonstrated that it safely and effectively inhibits thrombin generation in patients undergoing elective PCI and reduces the incidence of deep venous thrombosis when administered prophylactically in patients undergoing elective total knee arthroplasty.

The primary goal of the present phase 2 trial was to evaluate the safety and efficacy of a range of doses of rNAPc2 in patients with nSTE-ACS managed with standard therapies, including an early invasive strategy. We hypothesized that proximal inhibition of the coagulation cascade with rNAPc2 would reduce new thrombin generation and recurrent ischemia following nSTE-ACS without substantially increasing bleeding. The heparin de-escalation phase of this study was an open-label pilot experience to explore the feasibility of the stepwise withdrawal of heparin in patients treated with the highest studied dose of rNAPc2 with the goal of optimizing treatment simplicity, safety, and efficacy.

**Methods**

**Patient population.** The ANTHEM–TIMI-32 (Anticoagulation With rNAPc2 to Help Eliminate Major Adverse Cardiac Events–Thrombolysis In Myocardial Infarction–32) trial was a multicenter trial consisting of 2 parts: a randomized, double-blind, placebo-controlled, ascending rNAPc2 dose-ranging phase and a single-arm, open-label, stepwise, unfractionated heparin (UFH) de-escalation pilot study of 10 μg/kg rNAPc2. In the dose-ranging phase, approximately 25 patients in each of 8 dose panels were randomized to receive treatment with rNAPc2 or placebo in a 4:1 ratio. In the UFH de-escalation phase, 2 open-label panels of 26 patients each with half-dose or no UFH were studied.

Eligibility criteria included: age 18 to 75 years, hospitalization with moderate to high-risk nSTE-ACS with ischemic discomfort at rest lasting ≥5 min consistent with ACS within 48 h of randomization, and management with an early invasive strategy (≤72 h). In panels 1 to 3, moderate to high risk was defined as a TIMI risk score for nSTE-ACS (11) of ≥3. Subsequently, a protocol amendment also permitted patients with nSTE-ACS and either ST-segment...
deviation ≥0.5 mm (depression or transient elevation) or an elevated cardiac marker (troponin or creatinine kinase-myocardial band) to be enrolled regardless of the TIMI risk score.

Exclusion criteria were ST-segment elevation myocardial infarction, ACS due to a distinct nonatherosclerotic mechanism, increased bleeding risk, fibrinolytics within 24 h, planned coronary artery bypass graft (CABG) surgery within 7 days, creatinine >4-mg/dl, anemia, aminotransferase >3 times normal, allergy to aspirin, history of heparin-induced thrombocytopenia, pregnancy, or other conditions placing the patient at increased risk. In the heparin de-escalation phase, low-molecular-weight heparin within 12 h before randomization was not permitted.

The study was conducted according to the International Conference on Harmonization Good Clinical Practice Guidelines, approved by the institutional review boards/ethics committees of each center, and registered with the National Institutes of Health (NCT00116012). Written informed consent was obtained from all patients.

Study protocol. The study design is shown in Figure 2. A total of 255 patients (203 randomized in a 4:1 fashion to rNAPc2 or placebo in the dose-finding phase, 52 patients enrolled in the single-arm, open-label heparin de-escalation
phase) participated from 28 centers in the U.S. and Canada between November 2002 and May 2006. A central randomization system was used that involved a permuted-block design in which assignment was blocked by site (block size of 5).

In the dose-ranging portion of the trial, we evaluated rNAPc2 doses (μg/kg) of 1.5, 2.0, 3.0, 4.0, 5.0, 7.5, and 10.0 (2 panels) in a double-blind fashion compared with placebo. In the heparin de-escalation phase we studied 10.0 μg/kg rNAPc2 (open label) without a concomitant control group. An independent data safety monitoring board, guided by an algorithm designed to terminate study of an unsafe dose (see Statistical analysis), reviewed data at the completion of each panel and approved the testing of the next dose. Patients received intravenous study drug (single injection over 60 s) followed by coronary angiography and revascularization (if appropriate). Additional doses of study drug every 48 h were permitted during hospitalization, provided CABG was not required and the patient did not experience clinically significant bleeding or other adverse event.

All patients received daily oral aspirin (75 to 325 mg). The use and timing of clopidogrel, GP IIb/IIIa blockers, and other standard ACS therapies were encouraged per current practice guidelines (12) but left to the investigator’s discretion.

In dose finding, either UFH (60 U/kg bolus [maximum 5,000 U], 12 U/kg/h initial infusion [maximum 1,000 U/h]) titrated to an activated partial thromboplastin time [aPTT] 1.5 to 2 times control) or enoxaparin (1.0 mg/kg subcutaneously every 12 h [every 24 h if the calculated creatinine clearance was <30 ml/min]) was required. In the first 3 dose panels, enoxaparin was the only anticoagulant permitted between randomization and 48 h after the last dose of study drug. However, a protocol amendment subsequently allowed the use of UFH after new external data (1) demonstrated similar outcomes between these agents. During PCI, dosing algorithms were used (see Appendix) to ensure adequate and uniform anticoagulation.

In the first panel of the heparin de-escalation phase, half-dose UFH (30 U/kg bolus [maximum 2,500 U]; 6 U/kg/h infusion [maximum 500 U/h] with no subsequent adjustment) was administered before and during coronary angiography/intervention. In the second panel, no additional anticoagulant was permitted between randomization and 48 h after the last dose of study drug (including during PCI), unless required for management of a thrombotic complication or other strong clinical indication (e.g., CABG).

**Blood collection and assays.** Blood specimens to assess the complete blood count (CBC), plasma drug concentration, and pharmacodynamic effect (international normalized ratio [INR], prothrombin fragment 1.2 [F1.2]) were obtained at baseline, 2 to 6 h, 24 h (CBC only), 48 h (if not discharged), and 7 days after last study drug, and 42 days after randomization. The INR and F1.2 (assessed by enzyme-linked immunoabsorbent assay [ELISA]) were measured by Icon Laboratories (Farmimgdale, New York) using a Stago Sta Analyzer and ELX800 Biotek instrument, respectively. The F1.2 concentration during dose ranging was measured using polyclonal antibodies (Enzygnost F1 +2 assay, reference range 0.40 to 1.10 nmol/l, sensitivity 0.04 nmol/l), but this methodology was replaced by a more specific assay using monoclonal antibodies during the heparin de-escalation phase (Enzygnost F1+2 [monoclonal] assay, reference range 69 to 229 pmol/l, sensitivity 20 pmol/l) by the manufacturer (Dade Behring, Deerfield, Illinois). Plasma drug concentrations were analyzed by ELISA (panels 1 to 3 by Corvas International, San Diego, California; all other panels by Alta Bioanalytical Laboratories, San Diego, California).

Blood samples were obtained at baseline and on day 42 to assess for the development of antibodies to rNAPc2 using rabbit polyclonal antibodies to NAPc2. If antibodies were present at day 42, then patients were retested again at 3 and 6 months, and antibodies were assessed for neutralizing activity. Local measurement of INR during dose finding was not permitted between randomization and 7 days after study drug to maintain the study blind, because rNAPc2 increases the INR in a dose-related fashion (8). The aPTT (which is only minimally affected by rNAPc2 [13]) was measured daily and 6 h after any change in the UFH dose. The activated clotting time was used to guide UFH dosing during PCI (see Appendix). Serial assessment of cardiac markers of necrosis were performed in the first 24 h and after recurrent ischemia or revascularization.

**3-lead electrocardiographic monitoring.** All patients underwent continuous ambulatory 3-lead electrocardiographic (ECG) monitoring (Lifecard CF ambulatory ECG recorder; Reynolds Medical, Braintree, Massachusetts) for ischemia from randomization through 7 days after the last dose of study drug. Monitoring continued during and after PCI but was terminated just before CABG surgery. Continuous ECGs were assessed by a central core laboratory blinded to treatment assignment. An ischemic episode was defined as ≥1.0 mm horizontal or downsloping ST-segment depression or >1.0 mm ST-segment elevation lasting at least 1 min and separated from other episodes by at least 5 min, occurring in any of the 3 leads. Ischemic events occurring during catheterization or PCI were censored.

**Clinical follow-up.** Return visits to the clinic were made 7 days after the last dose of study drug and 42 days after randomization. Follow-up phone calls to assess for bleeding and intercurrent events were made at 3 and 14 days after the last dose and at 3 and 6 months after randomization to assess for death and ischemic complications.

**Clinical end points and definitions.** The prespecified primary end point of the study was the combined rate of adjudicated major plus minor hemorrhage using the standard TIMI criteria (14) (hereafter termed “significant” hemorrhage) from randomization through 7 days after the
last dose of study drug. A TIMI major hemorrhage was defined as an intracranial hemorrhage or overt bleeding associated with a >5 g/dl fall in hemoglobin. A TIMI minor hemorrhage was defined as a nonintracranial overt bleed associated with a 3 to 5 g/dl decrease in hemoglobin. Additional clinical safety end points included the rates of transfusions, serious adverse events, and antibody formation to rNAPc2.

Prespecified efficacy end points included pharmacokinetic (plasma drug concentration) and pharmacodynamic (INR, F1.2) measures and the percentage of patients with ischemia in the first 48 h on 3-lead continuous ECG. Additional prespecified ECG end points of interest included the average number of ischemic events per patient and the ST-product, defined as the product of the maximal extent of ST-segment deviation multiplied by the number of minutes of deviation.

Reinfarction, clinical recurrent ischemia, and stroke were defined with the standard TIMI definitions (15). Thrombotic bailout (TBO) was defined as an episode of refractory ischemia occurring after administration of the first dose through 7 days after the last dose of study drug that required the use of open-label anticoagulation to manage any one of the following complications due to thrombosis: 1) ischemia lasting ≥20 min despite management with standard therapies other than anticoagulants; 2) clinical instability (e.g., hypotension); 3) a decrement in TIMI flow grade; 4) a coronary artery dissection with decreased flow; 5) distal embolization; 6) side branch closure; or 7) abrupt closure of the culprit artery. All of the clinical end points described were adjudicated by an independent blinded clinical event committee. In suspected cases of TBO during the heparin de-escalation phase, angiographic results assessed by a blinded central angiographic core laboratory were provided to the event committee for consideration.

Biomarkers were obtained at baseline, 2 to 6 h, 48 h, and 7 days after last dose, and 42 days after randomization in the dose-ranging phase. These included cardiac troponin I, high-sensitivity C-reactive protein, brain natriuretic peptide, and matrix metalloproteinase 9, measured in the TIMI Biomarker Core Laboratory using established methods (Beckman Coulter AccuTnI and Hitachi 917 Analyzer; Roche Diagnostics, Indianapolis, Indiana; and Beckman Coulter Access 2 BNP; ELISA assay, R&D Systems, Minneapolis, Minnesota; respectively).

Statistical analysis. The selection of 25 patients per panel was guided by the desire to gain experience with the use of rNAPc2 in combination with aspirin, heparins, clopidogrel, GP IIb/IIIa inhibitors, and an early invasive strategy. Safety boundaries were set to exclude rates of significant hemorrhage that were relatively 20% greater than the predicted historical control rate of 6% (i.e., absolute rate >7.2%) based on the observations from the National Investigators Collaboration with Enoxaparin 3 (NICE-3) registry (16), using a 1-sided 95% confidence interval. In the heparin de-escalation phase, a second safety boundary was set to exclude a rate of TBO that was relatively 20% greater than the historical control rate of TBO (6.2%) observed in the REPLACE-2 (Randomized Evaluation of PCI Linking Angiomax to Reduced Clinical Events) trial (17) (i.e., absolute rate >7.44%) using a 1-sided 95% confidence interval, because the risk of thrombosis with reduced-dose heparin is unknown.

The prespecified primary safety and efficacy end points were based on the intention-to-treat principle among patients who received any dose of study drug. For the prespecified continuous ECG analysis, patients were required to have at least 48 h of interpretable data. Analyses were performed using 2-sided tests at the 0.05 level of significance. No adjustments were made for multiple comparisons.

Baseline characteristics and safety and efficacy end points were summarized using descriptive statistics, including mean values ± SD, median values with interquartile ranges, and event rates, as appropriate, using SAS version 9.1 (SAS Institute, Cary, North Carolina). Differences between rNAPc2 and placebo were analyzed via Fisher exact test (binary outcomes), t tests (continuous normally distributed data), and the Wilcoxon rank sum test (continuous non-normally distributed data). Comparisons involving more than one rNAPc2 dose were performed using the chi-square test (binary outcomes), 1-way analysis of variance (continuous normally distributed data), and Kruskal-Wallis test (continuous non-normally distributed data). The Pearson correlation test was used to assess the correlation of log concentration rNAPc2 with INR.

The trial was designed as a collaborative effort between the TIMI Study Group, the Nottingham Clinical Research Limited (NCRL), and the sponsor. The data were collected by NCRL. The NCRL and the TIMI Study Group performed the prespecified and exploratory analyses independently of the sponsor.

Results

Patients. Baseline characteristics are shown in Table 1 and concomitant treatments in Table 2 (see Appendix for complete data in each dose panel). No significant differences were found between patients who received rNAPc2 or placebo.

Pharmacokinetics and pharmacodynamics. Dose-related increases in plasma rNAPc2 concentration and INR were observed at 2 to 6 and 48 h (Fig. 3). The log concentration was correlated with INR at 2 to 6 and 48 h (ρ = 0.51 and ρ = 0.49, respectively; p < 0.0001 for both). Patients who received increasing doses of rNAPc2 had progressively less thrombin generation (lower F1.2) at 2 to 6 h (trend p = 0.001) and 48 h (trend p = 0.002), with statistically significant reductions observed with doses ≥7.5 μg/kg compared with placebo (Fig. 4).

Continuous ECG. The rate of ischemia by continuous ECG through day 7 was similar in patients receiving rNAPc2 and placebo (18% vs. 21%; p = NS). At doses of
rNAPc2 that suppressed F1.2 through 48 h (≥7.5 μg/kg), ischemia was reduced by >50% using a variety of continuous ECG measures compared with low-dose rNAPc2 and placebo (Table 3).

**Safety.** There were 9 significant hemorrhages (3.5%), including 4 major and 5 minor, in the entire trial; all were associated with a procedure. In dose ranging, there was no difference in the incidence of significant hemorrhage between rNAPc2 and placebo (4.3% for the pooled doses of 1.5 to 10 μg/kg rNAPc2 vs. 2.5% for placebo; p = NS). No statistical trend was observed across ascending doses of rNAPc2 individually (Fig. 5) or when grouped as placebo, low-dose (1.5 to 5.0 μg/kg), and high-dose (≥7.5 μg/kg; 2.5% vs. 2.9% vs. 4.5%, respectively; p = 0.77). All 4 major hemorrhages occurred at the highest dose (10 μg/kg); 3 were patients with excessive bleeding requiring transfusions after CABG. A shorter interval between the last dose of rNAPc2 and CABG surgery was associated with a higher risk of bleeding (40% vs. 10% vs. 0% for surgery performed <48 h vs. 48 to 96 h vs. >96 h, respectively, after the last dose of study drug; trend p = 0.077). Other safety outcomes, including rates of other (either major or minor) bleeding (9.8% vs. 10.0%), frequency (6.5% vs. 2.5%) and average number (2.2 vs. 2.0) of units of red blood cell transfusions, and serious adverse events (33% vs. 30%) were similar between rNAPc2 and placebo, respectively (all p = NS). No patients required treatment with FVIIa concentrate to reverse rNAPc2 anticoagulation (18). Three patients receiving rNAPc2 developed mild thrombocytopenia (93,000, 98,000, and 99,000 cells/mm³) with no clinical sequelae.

Anti-rNAPc2 antibody formation occurred in 13% of patients receiving rNAPc2. No patients with positive antibodies experienced symptoms (e.g., allergic reaction, anaphylaxis) attributable to the antibody, nor were there differences in INR or clinical outcomes among those with or without antibodies.

**Clinical events.** Clinical event rates were low and there were no differences in the rates of death, reinfarction, clinical recurrent ischemia, stroke, and late revascularization either individually or in composite between the treatment groups (Table 4). The composite event rate was 24% among patients receiving higher dose (≥7.5 μg/kg) rNAPc2 versus 38% for placebo (p = 0.15). Thrombotic bailout with an open-label anticoagulant occurred in 5 patients during heparin de-escalation, all whom received rNAPc2 10 μg/kg and no heparin (19% vs. 0% for no-heparin vs. half-dose heparin treatment groups, respectively; p = 0.051). Of the 14 patients who had PCI in the no-heparin group, 4 had TBO (new intrastent or coronary artery thrombus formation). The fifth TBO event was an episode of clinical unstable angina without new ischemic ECG changes that required an increased dose of intravenous nitroglycerin and open-label anticoagulation before PCI.

### Table 1 Patient Baseline Characteristics

<table>
<thead>
<tr>
<th></th>
<th>Placebo</th>
<th>rNAPc2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of patients</td>
<td>40</td>
<td>215</td>
</tr>
<tr>
<td>Median age, yrs</td>
<td>56</td>
<td>58</td>
</tr>
<tr>
<td>Women, %</td>
<td>20</td>
<td>34</td>
</tr>
<tr>
<td>History of myocardial infarction, %</td>
<td>35</td>
<td>33</td>
</tr>
<tr>
<td>Prior percutaneous coronary intervention, %</td>
<td>55</td>
<td>41</td>
</tr>
<tr>
<td>Prior coronary artery bypass graft surgery, %</td>
<td>15</td>
<td>20</td>
</tr>
<tr>
<td>History of congestive heart failure, %</td>
<td>8</td>
<td>9</td>
</tr>
<tr>
<td>History of hypertension, %</td>
<td>80</td>
<td>78</td>
</tr>
<tr>
<td>History of diabetes mellitus, %</td>
<td>33</td>
<td>34</td>
</tr>
<tr>
<td>History of dyslipidemia, %</td>
<td>88</td>
<td>79</td>
</tr>
<tr>
<td>Presenting non-ST-segment elevation myocardial infarction, %</td>
<td>50</td>
<td>53</td>
</tr>
<tr>
<td>ST-segment deviation ≥0.5 mm at presentation, %</td>
<td>45</td>
<td>47</td>
</tr>
<tr>
<td>Mean TIMI risk score (SD)</td>
<td>4.0 (1.2)</td>
<td>3.7 (1.3)</td>
</tr>
<tr>
<td>Low risk (1 to 2), %</td>
<td>5</td>
<td>16</td>
</tr>
<tr>
<td>Intermediate risk (3 to 4), %</td>
<td>68</td>
<td>57</td>
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<tr>
<td>High risk (5 to 7), %</td>
<td>28</td>
<td>27</td>
</tr>
<tr>
<td>Mean systolic blood pressure (mm Hg)</td>
<td>127</td>
<td>130</td>
</tr>
<tr>
<td>Median creatinine clearance (ml/min)</td>
<td>111</td>
<td>102</td>
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</table>

*There were no significant differences between treatment groups.

rNAPc2 = recombinant nematode anticoagulant protein c2; TIMI = Thrombolysis In Myocardial Infarction.

### Table 2 In-Hospital Therapies and Procedures

<table>
<thead>
<tr>
<th>Doses of study drug, %</th>
<th>Placebo</th>
<th>rNAPc2</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>83</td>
<td>85</td>
</tr>
<tr>
<td>2</td>
<td>18</td>
<td>14</td>
</tr>
<tr>
<td>3</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Enoxaparin, %</td>
<td>75</td>
<td>71</td>
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<tr>
<td>Unfractionated heparin, %</td>
<td>33</td>
<td>33</td>
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<tr>
<td>Aspirin</td>
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<td>100</td>
</tr>
<tr>
<td>Initial dose ≥300 mg, %</td>
<td>85</td>
<td>82</td>
</tr>
<tr>
<td>Maintenance dose ≥300 mg, %</td>
<td>82</td>
<td>78</td>
</tr>
<tr>
<td>Clopidogrel</td>
<td>85</td>
<td>81</td>
</tr>
<tr>
<td>Loading dose &lt;300 mg, %</td>
<td>38</td>
<td>35</td>
</tr>
<tr>
<td>Loading dose 300 to 599 mg, %</td>
<td>59</td>
<td>53</td>
</tr>
<tr>
<td>Loading dose ≥600 mg, %</td>
<td>3</td>
<td>11</td>
</tr>
<tr>
<td>Intravenous glycoprotein IIb/IIIa blocker, %</td>
<td>53</td>
<td>53</td>
</tr>
<tr>
<td>Predischarge PCI, %</td>
<td>60</td>
<td>47</td>
</tr>
<tr>
<td>Hours after randomization, median [25%, 75%]</td>
<td>5.7 [1.9, 21]</td>
<td>4.6 [1.8, 19]</td>
</tr>
<tr>
<td>Predischarge CABG surgery, %</td>
<td>3</td>
<td>11</td>
</tr>
<tr>
<td>Hours after randomization, median [25%, 75%]</td>
<td>139 [NA]</td>
<td>88 [60, 115]</td>
</tr>
</tbody>
</table>

*There were no significant differences between treatment groups. *Patients in dose ranging only

rNAPc2 = recombinant nematode anticoagulant protein c2; TIMI = Thrombolysis In Myocardial Infarction.
Discussion

This is the first randomized trial of a proximal inhibitor of the coagulation cascade that targets TF in patients with ACS. We found that intravenous rNAPc2 in doses of 1.5 to 10 μg/kg was well tolerated in patients with nSTE-ACS managed with multiple antithrombotic agents and an early invasive strategy. However, all 4 observed major hemorrhages occurred with the highest dose. The INR was increased and new thrombin generation (measured by F1.2) was suppressed in a dose-dependent fashion that correlated with plasma drug concentration. We observed 3 important issues with regard to the bleeding risk of rNAPc2. First, similar to other antithrombotic agents, the risk of bleeding is highest around the time of procedures. Indeed, all significant hemorrhages were related to the femoral access site or CABG surgery. Second, there was no statistical increase in bleeding rates with rNAPc2 (all doses pooled) compared with placebo, a result that is consistent with in vitro modeling of rNAPc2 and various antithrombotic agents that demonstrated no exaggerated effect on clotting and platelet function (13). However, in the subgroup of patients undergoing CABG, we observed a tendency toward increased bleeding rates with surgery performed <48 hours of rNAPc2 administration, particularly following the highest dose studied (10 μg/kg).

Suppression of ischemia on continuous ECG has been associated with a reduction in clinical ischemic events in studies with enoxaparin (19) and GP IIb/IIIa blockers (20), including patients who underwent PCI (21). A novel finding in the ANTHEM–TIMI-32 trial was that the doses of rNAPc2 that suppressed F1.2 through 48 h were also associated with a ≥50% reduction in ischemia on continu-
ous ECG. This provides a link between the mechanism of action of this drug and the hypothesized benefit in patients with plaque rupture and vessel wall injury. A larger trial would be required to test the hypothesis that rNAPc2 reduces clinical events, because the current trial was not powered for such an assessment. Because rNAPc2 has a half-life of 50 to 60 h and can be administered subcutaneously, prolonged inhibition of new thrombin generation with rNAPc2 for several weeks has the potential to improve the recommended discharge regimen after ACS, which currently does not include routine use of an anticoagulant.

Although several small studies have explored the use of reduced-dose UFH (22) or no heparin (23) during PCI, the risk of intraprocedural thrombosis remains a concern (24). In the ANTHEM–TIMI-32 trial, we observed thrombotic complications in 5 of 26 patients (19%) who were treated with 10 μg/kg rNAPc2 without UFH. However, no thrombotic complications occurred when half-dose UFH was administered. In 4 out of 5 cases of TBO, new intracoronary thrombus formed during PCI. Catheter-related thrombosis has been reported more frequently with the parenteral Xa inhibitor fondaparinux than UFH despite flushing catheters with heparin (25). Similarly, thrombus formation on the guiding catheter and wire has been reported with enoxaparin despite concomitant therapy with aspirin, clopidogrel, and GP IIb/IIIa blockers (26).

These observations, although small in number, raise the possibility that inhibitors of the terminal portion of the coagulation cascade (i.e., “distal protection”) may be necessary during intravascular procedures to inhibit thrombin. Distally acting agents may be more effective in inhibiting the contact pathway that is activated when catheters are introduced into the bloodstream (27). Although proximal inhibitors of the clotting cascade may be more effective in reducing thrombin generation, even with high doses of a proximal inhibitor, thrombus formation can still occur when foreign bodies are present and heparin is withheld. The present data are consistent with emerging clinical (25,26) and experimental (27) data which suggest that concomitant inhibitors of the contact pathway that act on formed thrombus may be necessary during intracoronary procedures. Therefore, we believe that reduced-dose distal-acting
antithrombins should be tested in combination with proximal inhibitors currently in development.

Study limitations. This phase 2 trial was not designed with sufficient power to exclude small differences in safety or clinical efficacy. The trial enrolled moderate- to high-risk patients in whom an early invasive strategy was planned and CABG was not intended; therefore, the observed results may not apply to other patients. We did not control for prior therapies and concurrent treatments (other than anti-thrombotics), which may have affected the biomarkers and outcomes. Too few patients (on average 3 per panel) received more than 1 dose of rNAPc2 to draw any meaningful conclusions from the administration of multiple doses. The heparin de-escalation phase was open label without concurrent controls.

Conclusions

In patients with nSTE-ACS managed with standard therapies including an early invasive approach, addition of rNAPc2, a potent inhibitor of the TF/fVIIa complex, at doses up to 10 μg/kg, was well tolerated. The observation that doses ≥7.5 μg/kg suppressed F1.2 (a marker of thrombin generation) may explain the observed >50% reduction in ischemia detected by continuous ECG. Some heparin may be necessary to prevent catheter-based thrombosis. Coronary artery bypass graft surgery should be avoided within 48 hours of dosing. Future trials of rNAPc2 in nSTE-ACS are needed to assess the potential clinical role of this novel agent.

Reprint requests and correspondence: Dr. Robert P. Giugliano, TIMI Study Group, 350 Longwood Avenue, First Floor Offices, Boston, Massachusetts 02115. E-mail: rgiugliano@partners.org.

REFERENCES


