Recombinant Nematode Anticoagulant Protein c2, an Inhibitor of the Tissue Factor/Factor VIIa Complex, in Patients Undergoing Elective Coronary Angioplasty

Arno H. M. Moons, MD,* Ron J. G. Peters, MD, PhD, Nick R. Bijsterveld, MD,* Jan J. Piek, MD, PhD,* Martin H. Prins, MD, PhD,† George P. Vlasuk, PhD,§ William E. Rote, PhD,§ Harry R. Bülter, MD, PhD‡
Amsterdam, The Netherlands; and San Diego, California

OBJECTIVES We investigated the safety and pharmacodynamics of escalating doses of recombinant nematode anticoagulant protein c2 (rNAPc2) in patients undergoing elective coronary angioplasty.

BACKGROUND Recombinant NAPc2 is a potent inhibitor of the tissue factor/factor VIIa complex, which has the potential to reduce the risk of thrombotic complications in coronary artery disease.

METHODS In a randomized, double-blinded, dose-escalation, multicenter trial, 154 patients received placebo or rNAPc2 at doses of 3.5, 5.0, 7.5, and 10.0 μg/kg body weight as a single subcutaneous administration 2 to 6 h before angioplasty. All patients received aspirin, unfractionated heparin during angioplasty, and clopidogrel in case of stent implantation.

RESULTS Minor bleeding rates for the doses 3.5 to 7.5 μg/kg were comparable to that with placebo (6.7%), whereas a distinct increase of 26.9% was observed at the 10.0-μg/kg dose level (p < 0.01). Major bleedings occurred in the 5.0-μg/kg (n = 3) and 7.5-μg/kg (n = 1) dose groups. The three patients in the 5.0-μg/kg dose group also received a glycoprotein IIb/IIIa receptor inhibitor at the moment of major bleeding. Systemic thrombin generation, as measured by prothrombin fragment 1 + 2 (F1+2) was suppressed in all rNAPc2 dose groups to levels below pretreatment values for at least 36 h. In the placebo group, a distinct increase of F1+2 levels was observed following cessation of heparin.

CONCLUSIONS Inhibition of the tissue factor/factor VIIa complex with rNAPc2, at doses up to 7.5 μg/kg, in combination with aspirin, clopidogrel, and unfractionated heparin appears to be a safe and effective strategy to prevent thrombin generation during coronary angioplasty. (J Am Coll Cardiol 2003;41:2147–53) © 2003 by the American College of Cardiology Foundation

Tissue factor (TF) is a significant component of atherosclerotic plaques and is thought to be the primary trigger of thrombosis following plaque rupture in patients with unstable coronary syndromes (1–3). When exposed to blood, TF forms a high-affinity complex with circulating factor VIIa (TF/factor VIIa) that initiates the coagulation cascade through activation of factors IX and X, leading to the formation of thrombin and subsequent thrombosis (4). Consequently, inhibitors of TF/factor VIIa have been evaluated for their potential to reduce coronary thrombosis in experimental studies of thrombosis (5–7).

Recombinant nematode anticoagulant protein c2 (rNAPc2) is an 85-amino-acid protein that was isolated from the hemophagocytic hookworm *Ancylostoma caninum* (8). It specifically inhibits the complex TF/factor VIIa by a unique mechanism that involves the formation of a high-affinity complex with zymogen or activated factor X prior to formation of the quaternary inhibitory complex with TF/factor VIIa (9). Utilization of zymogen factor X as an inhibitory scaffold obviates the need for generating activated factor X to inhibit the TF/factor VIIa complex. Theoretically, this should result in rapid and efficient inhibition of TF/factor VIIa by rNAPc2 following a thrombogenic challenge. To this point, rNAPc2 has been shown to be very effective in reducing the incidence of deep venous thrombosis without hemostatic compromise when administered prophylactically in patients undergoing knee arthroplasty (10). In addition, the formation of a complex with factor X results in a biologic half-life of rNAPc2 of ≥50 h.

The importance of TF/factor VIIa in coronary thrombosis suggests that rNAPc2 could be effective in the treatment of patients with acute coronary syndromes. As the majority of these patients undergo percutaneous coronary interventions (PCIs), we first performed a phase II, dose-ranging study with four escalating dosages of rNAPc2 in patients undergoing elective coronary angioplasty to evaluate the safety of the drug and its ability to inhibit thrombin generation.

METHODS

Patients. Five centers in the Netherlands enrolled patients between 18 and 80 years of age, with a history of stable angina and scheduled for elective PCI of one or two lesions with >50% diameter stenosis. Exclusion criteria included total occlusion, unstable angina or myocardial infarction.
(MI) within 14 days, a recent history of surgery, trauma, cardiopulmonary resuscitation, confirmed peptic ulcer, a previous history of abnormal bleeding, uncontrolled hypertension, renal or hepatic insufficiency, and treatment with or intended use of oral antithrombotic agents other than aspirin and clopidogrel during the study period. Planned use of glycoprotein (GP) IIb/IIIa receptor antagonists was excluded because there was no information available regarding the potential interaction of rNAPc2 with these agents.

The study protocol was conducted according to the International Conference on Harmonisation Good Clinical Practice Guidelines (11) and approved by the institutional review boards. Written, informed consent was obtained from all patients.

**Study design.** This study was designed as a randomized, placebo-controlled, double-blinded, multicenter study to evaluate the safety of rNAPc2 in addition to standard treatment during PCI. Patients were randomized to receive treatment with rNAPc2 or placebo in a ratio of 4:1, respectively, in each dose group. As a dose group consisted of ~30 patients, this resulted in ±24 patients per group treated with rNAPc2 and ±6 patients who received placebo. All placebo-assigned patients in each dose group were clustered into one placebo group. After randomization, patients received a single subcutaneous dose of rNAPc2 or placebo 2 to 6 h before PCI. The dosing of rNAPc2 was such that maximal levels were reached during PCI. After access to the femoral artery, unfractionated heparin (UFH) was administered as intravenous bolus injections to reach an activated clotting time 180 s was reached. Patients were hospitalized for 36 h after dosing, and follow-up assessment at two weeks. The four doses of rNAPc2 tested consisted of a telephone interview at 48 h and a clinical examination of the surface area.

Sheath (5F or 6F) removal after PCI was performed as soon as an activated clotting time <180 s was reached. Patients were hospitalized for 36 h after dosing, and follow-up consisted of a telephone interview at 48 h and a clinical assessment at two weeks. The four doses of rNAPc2 tested sequentially were 3.5, 5.0, 7.5, and 10.0 μg/kg body weight. Each increase in dose was dependent on review by an independent Data Safety Monitoring Committee. All patients received at least 80 mg/day aspirin throughout the study. If stent implantation was performed, a loading dose of 300 mg clopidogrel was administered, followed by 75 mg/day for at least three weeks. Patients receiving a stent in the initial 3.5-μg/kg rNAPc2 dose group were given clopidogrel after sheath removal as an additional safety measure at the start of the trial. After successful completion of this dose group, the protocol was amended, allowing clopidogrel administration within three days prior to the intervention to reflect current practice. Subsequently, a second 3.5-μg/kg dose group was completed. This led to a total of six treatment groups: one placebo and five rNAPc2 (3.5, 3.5, 5.0, 7.5, and 10.0 μg/kg).

**Blood collection and assays.** Six blood samples were taken from each patient during hospitalization—that is, before administration of the study drug, after access to the femoral artery but before angioplasty, at 2 and 8 h after the last bolus administration of UFH, and at 24 and 36 h after study drug administration. Blood was collected in citrated vacutainer tubes and immediately centrifuged at 4°C. Plasma samples were stored at −70°C until assayed.

Thrombin generation was quantified as prothrombin fragment 1+2 (F1+2) and thrombin/antithrombin (TAT) complexes by using the enzyme-linked immunosorbent assay (ELISA) for both measurements (Behringwerke AG, Marburg, Germany). Normal values ranged from 0.4 to 1.1 nmol/l for F1+2 and from 1.0 to 4.1 μg/l for TAT complexes. For plasma concentrations between 0.4 and 5.0 nmol/l of F1+2 and between 2.0 and 60.0 μg/l of TAT complexes, the intra-assay coefficient of variations varied between 5.0% and 7.5%, and between 4.0% and 6.0%, respectively. The plasma concentrations of rNAPc2 were also analyzed by ELISA (Corvas International, San Diego, California).

**Study outcomes and definitions.** The primary safety outcome of the study was the incidence of bleeding complications. A major bleeding episode was defined as clinically overt bleeding resulting in death; retroperitoneal, intracranial, or critical internal organ bleeding; a hemoglobin drop of ≥3 g/dl; or the requirement of transfusion of ≥2 units of blood. A minor bleed was any clinically significant bleeding that did not qualify as major, such as epistaxis, ecchymosis, macroscopic hematuria, puncture-site bleedings such as groin hematoma measuring >100 cm², or any other located hematoma. Groin hematoma size was determined by estimation of the surface area.

A secondary pharmacodynamic outcome was the extent of systemic thrombin generation performed by serial measurements of plasma levels of F1+2 and TAT complexes.

Myocardial infarction was defined as a creatine kinase, MB fraction concentration of more than three times the upper limit of normal.

We introduced a new quantitative safety measurement for puncture-site (surgical) hemostasis—femoral compression time (FCT), defined as the groin compression time (in minutes) between sheath removal from the femoral artery and achievement of complete hemostasis. This required manual groin compression just proximal to the puncture site by experienced personnel, using a standardized method described in the protocol. Continuous manual compression
was mandated for the first 10 min, followed by release, and if hemostasis was complete, FCT was recorded as 10 min. If not, compression was re-instituted for periods of 3 min until complete hemostasis, which was defined as no discernible bleeding externally or internally by careful inspection for at least 1 min.

**Statistical analysis.** Descriptive statistics included mean values ± SD or median values with interquartile ranges (IQR) for outcomes with or without a normal distribution, respectively. Distribution of the variables was analyzed by construction of histograms and by the Shapiro-Wilk W test. A p value <0.05 was considered significant. Differences between each rNAPc2 dose group and the placebo group were tested for all study outcomes. A comparison of baseline and procedural characteristics, clinical events, and minor and major bleeding incidences was made by chi-square test or Mann-Whitney U test for continuous variables, as appropriate. Regarding thrombin generation markers, the treatment groups were compared for each time point separately by means of one-way analyses of variance and subsequent pairwise comparisons with placebo, with Bonferroni correction. Spearman correlation was computed between FCT and rNAPc2 plasma levels at the moment of sheath removal, and between plasma levels of rNAPc2 and markers of thrombin generation after PCI. All statistical analyses were performed with SPSS version 10.1 (Chicago, Illinois).

**RESULTS**

**Patients.** A total of 154 patients were randomized to one of four rNAPc2 dosages or placebo. Baseline characteristics are shown in Table 1. No significant differences were found between each rNAPc2 treatment group and the placebo group, except for gender in the second 3.5-μg/kg dose group.

**Procedural characteristics.** Procedural characteristics are presented in Table 2. The mean time between study drug administration and access to the femoral artery was 3.4 ± 1.4 h. Clopidogrel was administered in 74% of the study population. In 28% of these patients, clopidogrel was started prior to PCI, with no differences between placebo and each rNAPc2 dose group.

Femoral compression time was evaluated in 149 patients (97%). Compression time was not measured in four patients, and one patient was excluded because PCI and sheath removal were performed 24 h after study drug administration. Introduction of the sheath into the femoral artery was uneventful in all patients except one, who required multiple punctures. As shown in Figure 1, a trend toward an increasing FCT was shown in the two highest rNAPc2 treatment groups, with a statistically significant difference between the highest dose group (10.0 μg/kg) and placebo (median 16 min [IQR 10 to 28] and median 10 min [IQR 10 to 14], respectively; p < 0.001). Spearman correlation showed a significant correlation between FCT and rNAPc2 plasma levels at the moment of sheath removal (r = 0.317, p < 0.001).

**Clinical events.** Clinical event rates were low and not apparently related to the rNAPc2 dose. Intervention-related MIs were observed in 10 patients (6% of total population). Two patients experienced an acute MI after hospital discharge. Bailout stent implantation was performed in two patients, with four experiencing transient vessel closure during the intervention. Two patients underwent coronary artery bypass grafting, one of which was an emergency procedure after PCI. There were two deaths: one due to cardiogenic shock six days after the procedure and the other due to a suspected cerebral vascular accident that occurred one day after angioplasty. The clinical diagnosis of a cerebral

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Placebo (n = 30)</th>
<th>3.5* (n = 23)</th>
<th>3.5 (n = 24)</th>
<th>5.0 (n = 24)</th>
<th>7.5 (n = 27)</th>
<th>10.0 (n = 26)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (yrs)</td>
<td>59 ± 8</td>
<td>63 ± 9</td>
<td>58 ± 6</td>
<td>60 ± 13</td>
<td>61 ± 8</td>
<td>59 ± 9</td>
</tr>
<tr>
<td>Male gender</td>
<td>67%</td>
<td>87%</td>
<td>92%†</td>
<td>79%</td>
<td>70%</td>
<td>85%</td>
</tr>
<tr>
<td>Previous MI</td>
<td>9 (30%)</td>
<td>8 (35%)</td>
<td>7 (29%)</td>
<td>8 (33%)</td>
<td>8 (30%)</td>
<td>8 (31%)</td>
</tr>
<tr>
<td>Previous PCI</td>
<td>4 (13%)</td>
<td>7 (30%)</td>
<td>3 (12%)</td>
<td>2 (8%)</td>
<td>9 (33%)</td>
<td>6 (23%)</td>
</tr>
<tr>
<td>Previous CABG</td>
<td>1 (3%)</td>
<td>2 (8%)</td>
<td>1 (4%)</td>
<td>3 (17%)</td>
<td>3 (11%)</td>
<td>1 (4%)</td>
</tr>
<tr>
<td>Single-vessel disease</td>
<td>17 (57%)</td>
<td>10 (43%)</td>
<td>14 (58%)</td>
<td>11 (46%)</td>
<td>18 (67%)</td>
<td>15 (58%)</td>
</tr>
<tr>
<td>Two-vessel disease</td>
<td>9 (30%)</td>
<td>7 (30%)</td>
<td>10 (42%)</td>
<td>12 (50%)</td>
<td>5 (19%)</td>
<td>7 (27%)</td>
</tr>
<tr>
<td>Three-vessel disease</td>
<td>4 (13%)</td>
<td>6 (26%)</td>
<td>0</td>
<td>1 (4%)</td>
<td>4 (15%)</td>
<td>4 (15%)</td>
</tr>
<tr>
<td>Body mass index (kg/m²)</td>
<td>28 ± 4</td>
<td>27 ± 3</td>
<td>28 ± 4</td>
<td>28 ± 3</td>
<td>26 ± 3</td>
<td>26 ± 2</td>
</tr>
<tr>
<td>Hypertension</td>
<td>9 (30%)</td>
<td>3 (13%)</td>
<td>6 (25%)</td>
<td>7 (29%)</td>
<td>5 (19%)</td>
<td>7 (27%)</td>
</tr>
<tr>
<td>Hypercholesterolemia</td>
<td>13 (43%)</td>
<td>10 (43%)</td>
<td>9 (37%)</td>
<td>10 (42%)</td>
<td>10 (37%)</td>
<td>9 (35%)</td>
</tr>
<tr>
<td>Diabetes</td>
<td>5 (17%)</td>
<td>1 (4%)</td>
<td>1 (4%)</td>
<td>2 (8%)</td>
<td>4 (15%)</td>
<td>4 (15%)</td>
</tr>
</tbody>
</table>

*No clopidogrel administration before PCI. †p < 0.05 vs. placebo; chi-square analysis for categorical variables and Student t test for continuous variables. Data are presented as the mean value ± SD or number (%) of patients.

CABG = coronary artery bypass grafting; MI = myocardial infarction; PCI = percutaneous coronary intervention; rNAPc2 = recombinant nematode anticoagulant protein c2.
vascular accident was not confirmed by a computed tomographic scan or autopsy. The patient had a history of ischemic stroke. There were no arteriovenous fistulae or femoral aneurysms. Differences in event rates between each rNAPc2 treatment group and the placebo group were not statistically significant.

Bleeding events. As presented in Table 3, there was no difference between the first three rNAPc2 dosage levels and placebo with respect to minor bleeding, varying from one to three bleeding episodes per patient group. This incidence of minor bleeding increased significantly to seven events in the highest rNAPc2 dose group (p < 0.01). The majority (n = 4) of these seven bleeding episodes were late rebleeds at the site of sheath removal occurring > 2 h after hemostasis.

There were four episodes of major bleeding, three of which occurred in patients treated with 5.0 μg/kg rNAPc2 (Table 3). The three incidents were classified as excessive drainage after emergency bypass grafting, sustained oral oozing after tracheal intubation, and a suspected cerebral vascular accident. In addition to aspirin, UFH, and clopidogrel, each of these three patients also received a GP IIb/IIIa antagonist. Two received abciximab and one was treated with tirofiban. It should be noted that two patients, one each in the 3.5- and 7.5-μg/kg dose groups, also received a GP IIb/IIIa antagonist (both treated with epti-

Figure 1. Femoral compression time in minutes (median value and interquartile range [IQR]) after sheath removal from the femoral artery. *No clopidogrel administration before percutaneous coronary intervention. The IQR of the 3.5* and 5.0 recombinant nematode anticoagulant protein c2 (rNAPc2) dose groups is from 10 to 10 min. A comparison between the placebo group and each rNAPc2 dose group was made by the Mann-Whitney U test.
Figure 2, F$_{1+2}$ plasma levels were lower in all treatment groups during the procedure, coincident with UFH administration. A gradual 1.4-fold increase above preprocedural levels of F$_{1+2}$ was observed over the 30-h observation period after the intervention in the placebo group only. There was continued suppression of thrombin generation in all rNAPc2 dose groups, which differed significantly from the placebo group at 24 h to 36 h following a single subcutaneous administration. The extended duration of PCI in the first 3.5-g/kg dose group did not affect these results.

The F$_{1+2}$ results were mirrored by the levels of TAT complexes, although this was statistically significant only at the two highest doses at 36 h, most probably due to the wide variation between the subjects (data not shown).

There was a dose-dependent increase in rNAPc2 plasma levels. A weak inverse correlation was shown after PCI, at 12 and 24 h between plasma levels of rNAPc2 and the thrombin generation markers F$_{1+2}$ and TAT complexes. However, this became statistically significant for both markers at 36 h ($r = -0.300$ and $r = -0.439$, respectively; $p < 0.01$). Figure 3 presents the inverse relation between rNAPc2 plasma levels and thrombin generation, as measured by F$_{1+2}$, at 36 h.

**DISCUSSION**

In patients with stable angina undergoing planned coronary stent implantation, the composite of death, MI, urgent target vessel revascularization, and bailout GP IIb/IIIa inhibitor therapy has been reported to occur in ~7% of cases within 48 h (12). The composite of death, MI, and urgent target vessel revascularization increases to 9% to 13% within 48 h after PCI in patients with unstable coronary syndromes (13,14). The TF/factor VIIa complex plays a pivotal role in initiating the thrombotic response to vascular injury (3), which makes this enzymatic complex a target for the development of anticoagulant strategies for patients with coronary thrombosis. Our study demonstrates that inhibition of the TF/factor VIIa complex by a single subcutaneous dose of rNAPc2, administered before elective coronary angioplasty, is safe in combination with aspirin, clopidogrel, and UFH and produces a prolonged and significant suppression of thrombin generation.

**Bleeding events.** Overall, rNAPc2 was well tolerated with respect to the incidence of major and minor bleeding episodes. However, of four major bleedings, three occurred in patients in the 5.0-μg/kg dose group at the time they also

![Figure 2](image-url)
suggest a dose-dependent prolongation, as well as an apparent effect on clinical hemostasis at the two highest doses of rNAPc2. In addition, there was a significant correlation between FCT and rNAPc2 plasma levels at the time of sheath removal. Systemic F$_{1+2}$ and TAT complex plasma levels were already maximally suppressed at the lowest dose of rNAPc2, and there was no correlation between these plasma markers and FCT. Clearly, hemostasis involves contributions of the arterial wall, platelets, and coagulation, and systemic coagulation marker plasma levels may not completely reflect this process. Thus, based on our findings, the FCT may provide clinically relevant quantitative information. Indeed, this must be confirmed in future trials.

**Thrombin generation.** Although the patients enrolled in this study were not considered to be at high risk, in the placebo group, we observed a marked and sustained elevation of thrombin generation, as measured by plasma levels of F$_{1+2}$, to above preprocedural levels up to at least 30 h after PCI. In contrast, thrombin generation continued to be suppressed in all of the rNAPc2 treatment groups, which was sustained through the last sampling time point at 36 h. These data suggest that the ongoing thrombin generation observed in the placebo group following the procedure was effectively suppressed by rNAPc2. Plasma levels of TAT complexes, used as another measure of thrombin generation, showed, as expected, a similar response as that found for F$_{1+2}$. Thus, TF/factor VIIa may play an important role in the process of ongoing thrombosis after PCI, as suggested recently in high-risk patients with unstable coronary syndromes (17), and rNAPc2 appears to effectively suppress this process.

Unfractionated heparin and low-molecular-weight heparins are widely used for the treatment of patients with acute coronary syndromes or those undergoing PCI. A synthetic pentasaccharide (fondaparinux), which may serve as an alternative for both drugs, is currently undergoing clinical development in these patient populations (18,19). However, it should be noted that although these agents should theoretically attenuate thrombin generation via the inhibition of factor Xa assembled in the prothrombinase complex (i.e., factor Va, negatively charged membrane surfaces, and calcium ions), the dependence on antithrombin III may limit their effectiveness in situations where there is a high level of prothrombinase activity, such as at the site of an atherosclerotic plaque rupture, because it has been shown that antithrombin III–dependent inhibitors, including heparins of any molecular size, do not effectively inhibit factor Xa assembled in the prothrombinase complex (20). Therefore, this may explain the reduced antithrombotic potential of these agents in suppressing the thrombogenicity of coronary lesions leading to clinical events during or shortly after early therapy (21,22). In contrast, the inhibition of TF/factor VIIa complex-mediated generation of factor Xa and subsequently thrombin by rNAPc2 is enhanced following the assembly of the TF/factor VIIa complex (9). Therefore, rNAPc2 has the potential advantage of atten-
ating the highly amplified formation of thrombin by inhibi-
ting the coagulation cascade at a more proximal site relative
to direct inhibitors of factor Xa and thrombin, and thus may
offer a more effective approach to reducing thrombosis in
patients with coronary artery disease.

Optimal rNAPc2 dose. As mentioned, the suppression of
plasma levels of F1+2 and TAT complexes by rNAPc2 was
already maximal at the lowest dose (3.5 µg/kg). The
significant inverse correlations between plasma levels of
rNAPc2 and each of these two markers at 36 h indicate that
the two highest doses of rNAPc2 result in a more sustained
suppression of both markers, compared with the lower doses
(Figs. 2 and 3). However, the usefulness of the systemic
markers of thrombin generation in assessing the clinical
efficacy of rNAPc2 could not be determined in this study.

The effect of rNAPc2 on the observed clinical bleeding
complications suggests that the optimal and sufficiently safe
dose of rNAPc2, when combined with aspirin, clopidogrel,
and UFH, is likely to be <10.0 µg/kg. This is consistent
with the significant prolongation of FCT at the highest
dose.

Conclusions. Our study indicates that inhibition of the
TF/factor VIIa complex at doses up to 7.5 µg/kg of
rNAPc2, in combination with aspirin, clopidogrel,
and UFH, appears to be a safe and effective strategy to prevent
thrombin generation in patients undergoing a catheter-
based coronary intervention. These results are supportive of
advancing rNAPc2 into a phase II, dose-ranging trial in
patients with unstable angina or non–Q-wave MI.

Reprint requests and correspondence: Dr. Arno H. M. Moons,
Department of Cardiology, Academic Medical Center, Room
F3-241, Meibergdreef 9, 1105 AZ Amsterdam, The Neth-erlands.
E-mail: A.H.Moons@amc.uva.nl.

REFERENCES

1. Marmur JD, Thrivikraman SV, Fyfe BS, et al. Identification of active
32.
2. Toschi V, Gallo R, Lettino M, et al. Tissue factor modulates the
thrombogenicity of human atherosclerotic plaques. Circulation 1997;
4. Rapaport SI, Rao VM. Initiation and regulation of tissue factor-
factor factor reduces the thrombogenicity of disrupted human atherosclerotic
plaques: effects of tissue factor pathway inhibitor on plaque thrombo-
of coronary patency after fibrinolysis with tissue factor pathway
7. Atsuchi N, Nishida T, Marutsuka K, et al. Combination of a brief
irrigation with tissue factor pathway inhibitor (TFPI) and adenovirus-
mediated local TFPI gene transfer additively reduces neointima
formation in balloon-injured rabbit carotid arteries. Circulation 2001;
103:570–5.
of the hookworm Angiastoma caninum. Proc Natl Acad Sci USA
Role of zymogen and activated factor X as scaffolds for the inhibition of
the blood coagulation factor VIIa–tissue factor complex by recom-
binitant nematode anticoagulant protein c2. J Biol Chem 2001;276:
10063–71.
factor VIIa/tissue factor inhibitor recombinant nematode anticoagu-
ulant protein c2 in prevention of postoperative venous thromboem-
bolism in patients undergoing total knee replacement. Circulation
11. International Conference on Harmonisation. Good Clinical Practice:
Consolidated Guideline. Published in the Federal Register. Washing-
709.
12. The ESFRIT Investigators. Novel dosing regimen of epifibatide in
planned coronary stent implantation (ESFRIT): a randomised,
 corona ry intervention with antibody against platelet IIIb/IIIa integrin
for reduction of clinical restenosis: results at six months. Lancet
14. The RESTORE Investigators. Effects of platelet glycoprotein IIb/IIIa
blockade with tirofiban on adverse cardiac events in patients with
unstable angina or acute myocardial infarction undergoing coronary
VIIa to generate thrombin during inhibition of tissue factor in human
16. Mielke CH, Rodvien R. Bleeding time procedures. In: Seligsohn D,
Schmidt RM, editors. Clinical Laboratory Science. Section 1: Hema-
17. Ardissino D, Merlino PA, Bauer KA, et al. Thrombogenic potential of
pentasaccharide, a pure factor Xa inhibitor, as an antithrombotic agent:
a pilot study in the setting of coronary angioplasty. Thromb Haemost 
inhibitor (Org35154/S9R017A) as an adjunct to fibrinolysis in acute
myocardial infarction: the PENTALYSE study. Eur Heart J 2001;22:
1716–24.
20. Rezaie AR. Prothrombin protects factor Xa in the prothrombinase
complex from inhibition by the heparin–antithrombin complex. Blood
22. Becker RC, Spencer FA, Li Y, et al. Thrombin generation after the
abrupt cessation of intravenous unfractionated heparin among patients
with acute coronary syndromes: potential mechanisms for heightened

APPENDIX 2

Steering Committee. H. Büsser, MD, A. Moons, MD, G.
Vlasuk, PhD, W. Rote, PhD, R. Peters, MD (Chair).

Coordinating and Method Center, Academic Medical
Center, Amsterdam, The Netherlands. Coordinating
Clinical Group: A. Moons, MD, R. Peters, MD; Clinical
Trial Unit: M. Prins, MD, R. Koolma, N. Fleitour, T. van
Leeuwen, R. Breed, M. Roskam, MSc, Y. Graafisma;
Treatment Allocation Center: P. Friederich, MD, B.-J.
Sanson, MD, B. van den Blink, MD; Central Laboratory: J.
Meijers, PhD, H. Jansen.
Clinical Centers in the Netherlands. Academic Medical Center, Amsterdam: J. Piek, MD, K. Koch, MD, R. de Winter, MD, C. Schotborgh, MD, M. Bax, MD, G. Sianos, MD, R. Peters, MD, A. Moons, MD, N. Bijjsterveld, MD; Catharina Hospital, Eindhoven: J. Koolen, MD, P. Huinink, MD, P. Tonino, MD, P. Rademaker, MD; Academic Hospital Dijkzigt, Rotterdam: M. van den Brand, MD, M. Knook, MD, A. Wardeh, MD; Maastricht Academic Hospital, Maastricht: V. van Ommen, MD, A. Lousberg; Ignatius Hospital, Breda: J. te Riele, MD, P. Schelfhout, J. Franssen.

Data Safety Monitoring Committee. Academic Medical Center: M. Prins, MD; Slotervaart Hospital: D. Brandjes, MD; University Hospital VU: G. Veen, MD.