A Randomized Controlled, Phase 2 Trial of the Viral Serpin Serp-1 in Patients With Acute Coronary Syndromes Undergoing Percutaneous Coronary Intervention

Jean-Claude Tardif, MD, FACC; Philippe L. L’Allier, MD, FACC; Jean Grégoire, MD, FACC; Reda Ibrahim, MD, FACC; Grant McFadden, PhD; William Kostuk, MD, FACC; Merril Knudtson, MD, FACC; Marino Labinaz, MD, FACC; Ron Waksman, MD, FACC; Carl J. Pepine, MD, FACC; Colin Macaulay, PhD; Marie-Claude Guertin, PhD; Alexandra Lucas, MD, FACC

Background—Vascular inflammation can lead to plaque instability and acute coronary syndromes (ACS). Viruses produce potent immunomodulating proteins that regulate key inflammatory pathways. A myxoma virus–derived serpin Serp-1 reduces inflammatory cell invasion and plaque growth in vascular injury models. Our objective was to evaluate the safety and efficacy of Serp-1 in patients with ACS undergoing percutaneous coronary intervention.

Methods and Results—This double-blind pilot trial included 48 ACS patients undergoing percutaneous coronary intervention randomly assigned to Serp-1 at doses of 5 μg/kg (n=19) or 15 μg/kg (n=17) or to placebo (n=12). Serp-1 was given by intravenous bolus immediately before intervention and 24 and 48 hours later. Patients were assessed for safety (primary objective) and efficacy outcomes, including biomarker analysis. In-stent neointimal hyperplasia was evaluated by intravascular ultrasound at 6 months. Key safety outcomes including coagulation parameters and adverse events did not differ between Serp-1 and placebo groups. A dose-dependent reduction in troponin I levels was observed with Serp-1 at 8, 16, 24, and 54 hours (P<0.05) and in creatine kinase-MB levels at 8, 16, and 24 hours after dose (P<0.05). The composite of death, myocardial infarction, or coronary revascularization occurred in 2 of 12 patients with placebo, 5 of 19 in the low-dose group, and none of 17 patients with the high-dose (P=0.058). Intravascular ultrasound did not detect changes in neointimal hyperplasia among groups.

Conclusions—This is the first study of a viral serpin demonstrating its safety in ACS patients. The significant reduction in myocardial damage biomarkers supports further assessment of Serp-1 in ACS patients undergoing stent deployment.

Clinical Trial Registration—URL: http://www.clinicaltrials.gov. Unique identifier: NCT00243308.

(Circ Cardiovasc Interv. 2010;3:543-548.)

Key Words: acute coronary syndrome ■ coronary angioplasty ■ stent ■ inflammation ■ serpin

Increased inflammatory cell invasion and activity in the arterial wall are characteristic of unstable atherosclerotic plaques and indicate increased risk of plaque rupture and coronary thrombosis, leading to acute coronary syndromes (ACS). Serine proteases in the thrombotic and thrombolytic cascades are upregulated at sites of plaque rupture and occlusive thrombus formation, and can also increase the activation levels of a variety of innate immune pathways. Serine protease inhibitors, termed serpins, are a widely distributed superfamily of proteinase inhibitors that regulate numerous biological pathways including inflammation, angiogenesis, complement activation, and a variety of other responses and represent 2% to 10% of circulating proteins. Serp-1 is a secreted glycoprotein of the serpin superfamily that inhibits tissue and urokinase-type plasminogen activators, plasmin, and factor Xa in vitro. Serp-1 exhibits potent antiinflammatory and antiatherogenic activity in vivo. Originally identified as a virulence factor that enables poxviruses to reduce the host’s inflammatory response to infection, purified recombinant Serp-1 has been shown to inhibit the early (24 to 72 hours) activation and infiltration of monocytes/macrophages and T lymphocytes in...
The primary objective of this study was to evaluate the safety of viral-derived Serp-1 intravenous injections in patients undergoing percutaneous coronary intervention (PCI) as treatment for ACS. Secondary objectives included serial evaluation of cardiac and inflammatory biomarkers, in-stent neointimal hyperplasia at 6 months, and the incidence of major adverse cardiac events at 6 months.

Methods
This is a double-blind, randomized, placebo-controlled, dose-ranging phase 2 trial of 3 consecutive daily doses of Serp-1 (Viron Therapeutics, London, Canada) intravenous injection on a background of conventional ACS treatment. The study was performed at 7 sites in Canada and the United States under the direction of the Montreal Heart Institute Coordinating Center.

Patient Population
Patients were eligible for the study if they were between 18 and 80 years of age and if they had been hospitalized with a changing pattern of cardiac ischemic symptoms, including at least 1 episode of pain at rest occurring within 24 hours before admission and with at least 1 episode of angina lasting at least 5 minutes. Study patients had to have a diagnosis of ACS, including either unstable angina, or non–ST-segment elevation–myocardial infarction on presentation. Patients had to be candidates for PCI scheduled within 48 hours of the initial coronary angiogram and had to have provided written informed consent. The study was approved by the institutional review boards of the study sites.

Exclusion criteria were coronary bypass surgery within 6 months, ST-segment elevation–myocardial infarction, a culprit coronary lesion with a complete thrombotic occlusion, PCI within the previous 72 hours, thrombolytic therapy within the previous 7 days, major surgery within the previous 6 weeks, major gastrointestinal or genitourinary bleeding within 30 days, history of stroke or transient ischemic attack, coagulopathy, thrombocytopenia, neutropenia, leucopenia, or history of HIV, hepatitis A or B infection, a history of an anaphylactic reaction to any drug, previous treatment with Serp-1, or treatment with warfarin within 7 days unless prothrombin time (PT-INR and aPTT) was done 6 hours after each dose and at 14 and 28 days of follow-up. Coagulation testing (PT-INR and aPTT) was done 6 hours after each dose and at 14 and 28 days. Plasma samples obtained after Serp-1 injections were analyzed for Serp-1 levels to determine the pharmacokinetics of the drug. Plasma samples were also tested for antibodies to Serp-1 at baseline, at 14 and 28 days, and at 3 and 6 months.

At 6 months, patients underwent repeat coronary IVUS and measurements of the baseline and follow-up IVUS images were performed as previously described. In-stent neointimal volume was determined, and area measurements were also made at the PCI site with the smallest lumen area at follow-up. Major adverse cardiac events during follow-up were defined as death, myocardial infarction, and target lesion revascularization.

Statistical Analyses
The sample size of this pilot study was determined by clinical feasibility and not statistical considerations. Nevertheless, with the number of patients in our study we had an 80% power to detect an effect size of approximately 1, with an α level of 0.05 (effect size = difference between groups divided by standard deviation). Biomarker measurements were log-transformed to approximate normal distribution before analysis. Log-transformed biomarkers at different time points were analyzed through repeated-measures analysis of covariance (ANCOVA) models including a term for baseline value of the biomarker, as well as terms for treatment, time, treatment×time interaction, and drug-eluting stent use. A statistically significant treatment×time interaction indicates that the change in the biomarker level over time is different between the different groups and suggests a possible treatment effect of Serp-1 on the biomarker levels. Such a result was followed by treatment contrasts using the repeated-measures ANCOVA model, which allows for the examination of treatment effect at each time point. No further adjustment for multiple comparisons was performed. Log-transformed adjusted means and 95% confidence intervals were anti-log-transformed for descriptive purposes, yielding adjusted geometric means with their 95% confidence intervals. Changes from baseline in IVUS parameters were analyzed using ANCOVA models with terms for treatment and for baseline value of the parameter. Proportion of patients having a major adverse cardiac event during the 6-month follow-up was compared across groups using a Fisher exact test. All tests were 2-sided and conducted at the 0.05 significance level. Analyses were performed using SAS release 8.2 (SAS Institute Inc, Cary, NC).

Role of the Funding Source
The principal academic investigators designed the study in conjunction with the sponsor (Viron Therapeutics) and supervised its scientific conduct. Statistical analyses were performed by statisticians at the Montreal Heart Institute Coordinating Center. The manuscript was prepared by the academic researchers (who had full access to the study data) in collaboration with coauthors from the sponsor.

Results
A total of 86 patients were screened and 48 were enrolled at the 7 study sites. There were 12 patients in the placebo group, 19 in the Serp-1 5.0 μg/kg dose group and 17 in the 15 μg/kg dose group. The baseline features of the study population are summarized in Table 1 and the disposition of patients during the study is depicted in Figure 1. All patients received at least 1 dose of study treatment, but a total of 7 patients did not complete the study out to 6 months. Three patients (1 in the placebo group and 2 in the 15 μg/kg dose group) withdrew from the study before
receiving the full 3 doses of study treatment, and an additional 4 patients in the low-dose (5.0 µg/kg) group did not complete all the follow-up visits out to 6 months (3 patients withdrew after the 28-day follow-up visit and 1 patient withdrew after the 3-month follow-up visit).

Safety
The main focus of this clinical trial was to determine whether Serp-1 was safe when administered to patients with ACS. No patient died during the study and no serious treatment-related adverse events were reported. A serious adverse event was reported by 3 patients (25%) in the placebo group, 7 (37%) in the low-dose group, and 4 (24%) in the high-dose group. Only 1 patient was withdrawn from the study because of an adverse event; this patient had a stroke approximately 3 months after the last dose of study drug (5.0 µg/kg dose group). Mild or moderate adverse events were common in the 3 treatment groups and were considered unrelated to the study drug.

Serp-1 can inhibit enzymes of the thrombolytic cascade in vitro, and although no significant effects on coagulation parameters were found in whole blood in vitro or in animal studies, detecting any effect on coagulation or hematologic measurements was an important safety issue for this study. No differences were seen among the groups for hematologic or coagulation parameters or for measures of renal function. Measurements of the QT and QTc intervals did not change significantly with treatment. Serp-1 was not overly immunogenic because only 4 of the 36 treated subjects had a low level of anti–Serp-1 antibodies (titer of 1/8 to 1/256). In 1 patient, these appeared by day 28, and in 3 patients they were not measurable until 3 months. No neutralizing antibodies against Serp-1 were detected in any of the patients. No injection site reactions were noted in any of the patients.

Markers of Myocardial Necrosis
Adjusted geometric means for troponin I are listed in Table 2. In the repeated-measures ANCOVA model, a significant time × treatment group interaction was observed (P=0.019). A comparison of groups at each time point revealed that troponin levels were lower in the 15.0 µg/kg dose group compared with the placebo group at 8 hours (P=0.046), 16 hours (P=0.007), 24 hours (P=0.024), and 54 hours (P=0.015) and lower in the 15 µg/kg dose group than in the 5.0 µg/kg dose group at 8 hours (P=0.046) and 16 hours (P=0.016). The troponin values over time for the 3 groups are depicted in Figure 2.

A similar pattern was observed with CK-MB as with troponin. Adjusted geometric means for CK-MB are shown in Table 2. In the repeated-measures ANCOVA model, a significant time × treatment interaction was observed (P=0.009).
Comparison of groups at each time point revealed that CK-MB levels were lower in the 15 μg/kg dose group compared with the placebo group at 8 hours (P = 0.042), 16 hours (P = 0.007), and 24 hours (P = 0.034) and lower in the 15 μg/kg dose group compared with the 5.0 μg/kg dose group at 16 hours (P = 0.034). The CK-MB values over time for the 3 groups are shown in Figure 3.

Markers of Inflammation
Hs–C-reactive protein, myeloperoxidase, D-dimer, brain natriuretic peptide, monocyte chemotactant protein-1, and plasminogen activator inhibitor-1 levels were measured at baseline, 8, 16, 24, 48, and 54 hours and 14 and 28 days. Qualitatively, the expected rise and fall was observed over time for most of these markers; in the repeated-measures ANCOVA model, the treatment × time interaction was not statistically significant, indicating that the differences in the biomarker levels between the 3 treatment groups over time were not sufficient to be detected using this analysis.

IVUS Results
IVUS measurements were available for 8 placebo patients, 12 in the 5.0 μg/kg dose group and 12 in the 15 μg/kg dose group. The difference among the treatment groups did not approach statistical significance for any of the IVUS measurements. Change in neointimal volume was 24.4 ± 21.0 mm³ in the placebo group, 30.5 ± 25.2 mm³ in the low-dose group, and 26.6 ± 32.4 mm³ in the high-dose group (P = 0.88).

Clinical Outcomes
During the 6-month follow-up period, a major adverse cardiac event occurred in 2 of 12 placebo patients, in 5 of 19 patients in the 5.0 μg/kg dose group, and in none of the 17 patients in the 15 μg/kg dose group (P = 0.058), as shown in Table 3. The events consisted of 5 myocardial infarctions and 4 target vessel

### Table 2. Adjusted Geometric Means and 95% Confidence Intervals for Troponin I and CK-MB Levels in the 3 Treatment Groups

<table>
<thead>
<tr>
<th></th>
<th>Placebo (n=12)</th>
<th>5.0 μg/kg (n=19)</th>
<th>15 μg/kg (n=17)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Troponin I levels</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Predose</td>
<td>0.24 (0.12–0.50)</td>
<td>0.29 (0.16–0.53)</td>
<td>0.15 (0.09–0.24)</td>
</tr>
<tr>
<td>8 h</td>
<td>0.51 (0.24–1.07)</td>
<td>0.46 (0.25–0.86)</td>
<td>0.21 (0.13–0.34)†</td>
</tr>
<tr>
<td>16 h</td>
<td>0.98 (0.47–2.05)</td>
<td>0.76 (0.41–1.39)</td>
<td>0.29 (0.18–0.47)†</td>
</tr>
<tr>
<td>24 h</td>
<td>0.87 (0.41–1.86)</td>
<td>0.58 (0.31–1.08)</td>
<td>0.31 (0.19–0.51)†</td>
</tr>
<tr>
<td>48 h</td>
<td>0.42 (0.20–0.91)</td>
<td>0.25 (0.14–0.46)</td>
<td>0.19 (0.12–0.32)</td>
</tr>
<tr>
<td>54 h</td>
<td>0.57 (0.26–1.22)</td>
<td>0.24 (0.13–0.44)</td>
<td>0.18 (0.11–0.30)†</td>
</tr>
<tr>
<td>14 d</td>
<td>0.06 (0.03–0.13)</td>
<td>0.05 (0.03–0.10)</td>
<td>0.08 (0.05–0.14)†</td>
</tr>
<tr>
<td>28 d</td>
<td>0.05 (0.02–0.10)</td>
<td>0.04 (0.02–0.08)</td>
<td>0.07 (0.04–0.12)†</td>
</tr>
<tr>
<td><strong>CK-MB levels</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Predose</td>
<td>2.48 (1.47–4.22)</td>
<td>1.99 (1.36–2.91)</td>
<td>1.84 (1.37–2.50)</td>
</tr>
<tr>
<td>8 h</td>
<td>4.26 (2.49–7.29)</td>
<td>3.60 (2.46–5.25)</td>
<td>2.25 (1.67–3.05)†</td>
</tr>
<tr>
<td>16 h</td>
<td>6.96 (4.08–11.91)</td>
<td>5.00 (3.41–7.28)</td>
<td>2.97 (2.20–4.05)†</td>
</tr>
<tr>
<td>24 h</td>
<td>5.87 (3.37–10.14)</td>
<td>4.39 (2.99–6.49)</td>
<td>2.97 (2.18–4.03)†</td>
</tr>
<tr>
<td>48 h</td>
<td>2.56 (1.46–4.45)</td>
<td>2.08 (1.43–3.05)</td>
<td>2.12 (1.55–2.91)</td>
</tr>
<tr>
<td>54 h</td>
<td>2.72 (1.56–4.77)</td>
<td>2.14 (1.47–3.14)</td>
<td>1.93 (1.40–2.67)</td>
</tr>
<tr>
<td>14 d</td>
<td>1.13 (0.65–1.97)</td>
<td>1.45 (0.99–2.11)</td>
<td>1.90 (1.37–2.62)</td>
</tr>
<tr>
<td>28 d</td>
<td>1.34 (0.76–2.31)</td>
<td>1.31 (0.90–1.92)</td>
<td>2.08 (1.49–2.91)</td>
</tr>
</tbody>
</table>

Troponin and CK-MB values were log-transformed to meet distributional assumptions before performing the repeated-measures ANCOVA and anti-log-transformed to produce the geometric means and 95% confidence intervals shown here. The P values are for comparisons at each time point of the adjusted geometric means derived from the ANCOVA model. The number of patients who had measurements of biomarkers is the same for CK-MB as what is listed for troponin I.

*P < 0.05 versus placebo; †P < 0.05 versus 5.0 μg/kg dose group.
revascularizations; all 5 infarctions occurred within the first month of follow-up, and the repeat PCI procedures were at 6, 19, 90, and 120 days. Coronary artery bypass graft was not required, and no cardiovascular deaths occurred.

Discussion

This randomized, placebo-controlled, multicenter pilot study tested viral-derived Serp-1, a first-in-class, antiinflammatory protein therapeutic, given as 3 daily injections at the time of stent placement for ACS. The primary purpose of the study was to assess safety, and no safety concerns were uncovered. In terms of biomarker analysis, Serp-1 treatment was found to significantly reduce troponin I and CK-MB levels after PCI, both being markers of cardiac damage. Serp-1 was not found to have a statistically significant effect on circulating markers of inflammation. Finally, no major cardiac events were observed during follow-up in the high-dose group, although 2 patients in this study arm did not complete their 6-month follow-up and no statistically significant differences in event rates were noted between the 3 groups. These are remarkably encouraging findings given the small size of this trial, and these results must be confirmed in a larger trial. These findings are consistent with the very good safety profile and lack of adverse events seen in the prior phase 1 trial performed using single doses of Serp-1 in 16 normal volunteers.9 Further study is also warranted to assess the safety and efficacy of higher doses of Serp-1.

Markers of Myocardial Injury After PCI

The long-term prognosis after PCI is worse for patients who have elevated levels of troponin or CK-MB immediately after the procedure. In a meta-analysis involving 20 studies and 15,581 patients undergoing elective PCI, 32.9% had an elevated troponin level after the procedure.10 During a mean follow-up of 16 months, mortality rate was increased by 35% (P=0.001) and the risk of death or myocardial infarction was increased by 59% (P<0.001) in this subgroup. A similar increase in risk has been documented for patients with elevated levels of CK-MB after PCI.11,12

In small placebo-controlled clinical trials, statins have been shown to reduce the levels of troponin release after PCI, both in patients with stable coronary disease and after ACS.13–16 This benefit occurs before LDL-cholesterol has been reduced and has been attributed to the antiinflammatory activity of the statin. Both ACS17 and stent implantation18 are associated with inflammatory cell infiltrates at the site of the culprit lesion. Serp-1 reduces these inflammatory cell infiltrates in animal models of angioplasty injury.6 This effect may also reduce the risk of microembolization during PCI and account for the reduction in markers of myocardial necrosis seen in Serp-1–treated patients in this study. Reductions in inflammatory cell infiltrates and in myocardial damage would be expected to improve long-term outcome, based on the observational data cited above.10–12 On the other hand, no data exist as to whether Serp-1 actually reduces inflammatory infiltrates in human plaque, and the notion that this might influence microembolization during PCI is speculative. The beneficial effects of Serp-1 might also be attributable to a direct antiinflammatory effect on the myocardium.

Inflammatory Biomarkers

In several preclinical models of vascular injury caused by balloon angioplasty and stent placement, Serp-1 administration was found to reduce both monocyte infiltration into the sites of injury and neointimal expansion in response to the injury.6,9 A reduction in the levels of circulating inflammatory biomarkers was not measured in these preclinical models. In this small clinical trial, a statistically significant effect on the levels of the measured inflammatory biomarkers was not observed. The 3 daily injections of Serp-1 might be sufficient to reduce cellular infiltrates in culprit and stented lesions and affect myocardial necrosis but insufficient to reduce circulating levels of inflammatory biomarkers. Inflammatory biomarkers generally rise in the hours after an acute coronary event and fall over the ensuing days and weeks, with great variability among different biomarkers and between different subjects as the result of differences in their rates of production and elimination. These changes may obscure any drug effect, particularly when sample size is small and the dose levels limited, as in this study.

Limitations of the Study

The main limitation of this study is its small size, which limits the evaluation of both efficacy and safety; one cannot infer from the absence of any serious adverse events in a small pilot trial that the treatment is harmless.19 Minor imbalances among the treatment groups in baseline levels of troponin and CK-MB (Table 2) may have had a slight effect on the results; however, the baseline values of these biomarkers were included as covariates in the statistical models, attenuating the effects of any imbalance. The study was halted before the highest dose (50 µg/kg) in the series of dose escalations was tested because of slow patient recruitment. The 15 µg/kg dose appears to be both safe and more efficacious than the 5.0 µg/kg dose, but data are needed for higher doses. The rate of major adverse cardiac events was numerically higher in the low-dose group compared with placebo, but the difference was not statistically significant and not in accordance with the decrease in troponin levels. The relevance of that result is unknown.

In conclusion, this study represents the first use of a virus-derived protein therapeutic in patients. The results support the safety of Serp-1 when given in three intravenous doses of 15 µg/kg started at the time of stent placement for ACS. The reduction in markers of myocardial damage observed in the

Table 3. Incidence of Major Adverse Cardiac Events in the 3 Treatment Groups During Follow-Up

<table>
<thead>
<tr>
<th></th>
<th>Serp-1</th>
<th>Placebo (n=12)</th>
<th></th>
<th>5.0 µg/kg (n=19)</th>
<th>15 µg/kg (n=17)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cardiovascular death</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Myocardial infarction (No. of events)</td>
<td>1</td>
<td>4</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Target vessel revascularization (No. of events)</td>
<td>1</td>
<td>3</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Total MACE* (No. of patients)</td>
<td>2 (17%)</td>
<td>5 (26%)</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
<td></td>
</tr>
</tbody>
</table>

*Fisher exact test, P=0.058. MACE indicates major adverse cardiac events.
higher dose treated group indicates that Serp-1 may become a clinically useful therapeutic agent in this setting.

Sources of Funding
The study was funded by Viron Therapeutics, London, Ontario, Canada.

Disclosures
Drs Lucas and McFadden founded Viron Therapeutics and hold stock in the company. Dr Macaulay is an employee of Viron Therapeutics.

References
A Randomized Controlled, Phase 2 Trial of the Viral Serpin Serp-1 in Patients With Acute Coronary Syndromes Undergoing Percutaneous Coronary Intervention

Jean-Claude Tardif, Philippe L. L'Allier, Jean Grégoire, Reda Ibrahim, Grant McFadden, William Kostuk, Merril Knudtson, Marino Labinaz, Ron Waksman, Carl J. Pepine, Colin Macaulay, Marie-Claude Guertin and Alexandra Lucas

Circ Cardiovasc Interv. 2010;3:543-548; originally published online November 9, 2010; doi: 10.1161/CIRCINTERVENTIONS.110.953885

Circulation: Cardiovascular Interventions is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2010 American Heart Association, Inc. All rights reserved.
Print ISSN: 1941-7640. Online ISSN: 1941-7632

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circinterventions.ahajournals.org/content/3/6/543

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Circulation: Cardiovascular Interventions can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Circulation: Cardiovascular Interventions is online at:
http://circinterventions.ahajournals.org//subscriptions/