Clot-Selective Coronary Thrombolysis
With Pro-urokinase

Joseph Loscalzo, MD, PhD, Thomas P. Wharton, MD, James M. Kirshenbaum, MD, Herbert J. Levine, MD, John T. Flaherty, MD, Eric J. Topol, MD, K. Ramaswamy, MD, Bernard D. Kosowsky, MD, Deeb N. Salem, MD, Peter Ganz, MD, Jeffrey A. Brinker, MD, Victor Gurewich, MD, James E. Muller, MD, and the Pro-urokinase for Myocardial Infarction Study Group*

Recognition that myocardial infarction is caused by coronary thrombosis1 and that early reperfusion is beneficial2 has stimulated a search for a safe, rapidly acting, and effective thrombolytic regimen. Tissue plasminogen activator (t-PA) can provide relatively clot-selective thrombolysis, but one quarter of patients fail to achieve reperfusion, lysis speed is not optimal, and higher doses have been associated with an increased incidence of hemorrhagic stroke. We report the results of a multicenter study of pro-urokinase, a second naturally occurring plasminogen activator that has structural similarities to t-PA but has a different mechanism of action. Pro-urokinase was administered 3.9±1.1 hours after the onset of chest pain to 40 patients with acute myocardial infarction with angiographically confirmed complete coronary occlusion (TIMI grade 0). After a 90-minute intravenous infusion of pro-urokinase (4.7–9 million units, 36–69 mg) 51% (20 of 39) of the patients demonstrated reperfusion (TIMI grade 2 or 3) occurring 64.8±22.3 minutes after initiation of therapy. Fibrinogen levels fell only 10±17% from baseline, confirming the fibrin specificity of pro-urokinase. As with t-PA, however, this specificity was only relative. α2-Antiplasmin decreased to 39% and plasminogen decreased to 64% of initial values. Fibrinogen degradation products increased 63% and the fibrin-specific d-dimer increased 8.7-fold. Thus, pro-urokinase produces relatively clot-selective coronary thrombolysis similar to that produced by t-PA, but the use of either pro-urokinase or t-PA alone in higher doses would be likely to produce more nonspecific effects. (Circulation 1989;79;776–782)

The knowledge that myocardial infarction is caused by coronary thrombosis1 and that early reperfusion is beneficial2 has stimulated a search for a rapidly acting, safe, and effective thrombolytic regimen.3–5 Tissue plasminogen activator (t-PA), a naturally occurring enzyme, can provide effective lysis with only a modest effect on systemic clotting factors; however, approximately 25% of patients do not achieve lysis, approximately 20% of the patients who do achieve lysis require over 60 minutes to do so, and higher doses have been associated with central nervous system hemorrhage.6–8

Circulating blood contains not only t-PA, but also pro-urokinase, a second, more recently discovered plasminogen activator isolated from human urine in 1979.10 Both in vitro and in vivo experimental studies in animals have demonstrated that pro-urokinase (also called single-chain urokinase-type plasminogen activator, or scu-PA) can provide relatively fibrin-specific thrombolysis.10 A study in normal volunteers has shown that pro-urokinase produces less fibrinogenolysis for a given degree of fibrinolytic potential than does urokinase.11

Only limited information is available about the action of pro-urokinase in patients with acute myocardial infarction.12–18 These studies suggest that pro-urokinase can lyse coronary thrombi in humans with little change in systemic fibrinogen levels, but the small number of patients in each study precludes precise determination of reperfusion rate, time to lysis, and relative clot selectivity.

In this report, which represents the entire experience with the clinical use of pro-urokinase in the United States, we present the results of the administration of pro-urokinase to 40 patients with acute myocardial infarction. In addition to its larger size,
this study, in which seven hematologic parameters were measured before and after therapy, provides an extensive characterization of the effects of pro-urokinase on the clotting system.

Methods

The study was conducted by investigators from the following six institutions: Brigham and Women’s Hospital, Boston, Massachusetts; The Johns Hopkins Hospital, Baltimore, Maryland; Lahey Clinic, Burlington, Massachusetts; St. Elizabeth’s Hospital, Boston, Massachusetts; Tufts-New England Medical Center, Boston, Massachusetts; and the University of Michigan Medical Center, Ann Arbor, Michigan. Core laboratories were established at Brigham and Women’s Hospital for the central analysis of hematologic values and coronary angiograms.

Inclusion Criteria

Patients were considered for inclusion in the study if they had ischemic pain of at least 30 minutes duration and electrocardiographic evidence of salvageable ischemic myocardium (presence of an R wave in at least one lead with ST-segment elevation). It was required that the time from the onset of pain to the administration of pro-urokinase be less than 5 hours.

Exclusion Criteria

Patients were excluded for the following reasons: Killip class III or IV congestive heart failure at the time of the initial cardiac catheterization, significant valvular heart disease, cardiomyopathy, previous coronary artery bypass surgery, comatose state at the time of screening or before catheterization, use of an investigational new drug in the 4 weeks before the infarction, use of a toxic drug within the previous 3 months, severe compromise of renal or hepatic function, alcohol or drug abuse, hypertension requiring therapy with two drugs before the infarction or arterial pressure ≥160/100 at the time of screening, or cardiopulmonary resuscitation within 1 month before infarction.

Patients were also excluded if any of the following contraindications to thrombolytic therapy were present: ongoing therapy with heparin or Coumadin, venous or arterial puncture of the neck or subclavian region within the previous 10 days, cerebrovascular accident within the previous 2 months, active bleeding, peptic ulcer disease within 1 year of entry, major surgery within 10 days before entry into the study, or history of hemorrhagic diathesis or abnormal coagulation parameters or both.

Baseline Coronary Angiography

Patients who met the above criteria and gave informed consent underwent immediate coronary angiography. After venous and arterial access sites were established, heparin (5,000 units) was administered intravenously and coronary angiography performed. Only patients with total occlusion of the coronary artery supplying the presumed zone of ischemia (TIMI grade 0) were included in the study. Intracoronary nitroglycerin was administered to exclude occlusion due to reversible coronary spasm.

Study Population

Forty patients satisfied the inclusion and exclusion criteria and were enrolled in the study (34 men and six women). The first patient was enrolled on April 11, 1986, and the last on August 6, 1987. The mean age of the patients was 56±11 years; average time from onset of chest pain to the administration of pro-urokinase was 3.9±1.1 hours (range, 1.8—5.8). Thirty-eight of the patients experienced a myocardial infarction as documented by elevated plasma levels of myocardial enzymes; the remaining two patients died within 3 hours of entering the study.

Pro-urokinase Administration

Pro-urokinase was supplied by Sandoz Research Institute, Hanover, New Jersey. The biologic origin of the pro-urokinase used was the malignant human kidney cell line TCL-598. The specific activity of the pro-urokinase after plasmin activation ranged from 120,000 to 140,000 IU/mg based on the World Health Organization urokinase standard (International Reference Preparation of Human Urokinase [coded 66/46] obtained from the National Institute of Biological Standards and Control). Doses expressed in milligrams are calculated using an average specific activity of 130,000 IU/mg. The preparation included less than 1% two-chain urokinase based on amidolytic activity (S2444) before and after activation. It also contained a variable amount (from 2.6% to 12.8%) of an inactive breakdown product of pro-urokinase.

Patients received an intravenous infusion of pro-urokinase for a total duration of therapy of 90 minutes. As the trial progressed, gradually increasing doses of pro-urokinase were evaluated (Table 1).

Coronary Angiographic Studies

The Coronary Angiography Core Laboratory used the TIMI grading system to classify coronary perfusion: grade 0, no perfusion detected; grade 1, penetration of contrast without perfusion; grade 2, partial perfusion; grade 3, complete perfusion. Before pro-urokinase administration, all patients had TIMI grade 0 occlusion resistant to intracoronary nitroglycerin infusion. Repeat coronary angiograms were performed at 10, 20, 30, 40, 60, 75, and 90 minutes after initiation of therapy as in previous studies by the TIMI group. Patients whose perfusion improved to grade 2 or 3 at 90 minutes after initiation of infusion of pro-urokinase were considered to have experienced reperfusion and were classified as responders, while those with grade 0 or 1 were classified as nonresponders.

Hematologic Studies

To characterize the effects of pro-urokinase on the hemostatic system, seven hematologic parame-
TABLE 1. Dosage Schedule of Pro-urokinase, Reperfusion Rate, and Outcome

<table>
<thead>
<tr>
<th>Patients (n)</th>
<th>Pro-urokinase (millions of IU)</th>
<th>Bolus</th>
<th>Infusion</th>
<th>Total IU</th>
<th>mg</th>
<th>mg/kg</th>
<th>Reperfusion n</th>
<th>%</th>
<th>Recurrent MI</th>
<th>Death</th>
<th>PTCA</th>
<th>CABG</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>0.68</td>
<td>4.05</td>
<td></td>
<td>4.73</td>
<td>36</td>
<td>0.35</td>
<td>0</td>
<td>0</td>
<td>—</td>
<td>1</td>
<td>—</td>
<td>1</td>
</tr>
<tr>
<td>11</td>
<td>...</td>
<td>6.25</td>
<td></td>
<td>6.25</td>
<td>48</td>
<td>0.57</td>
<td>6</td>
<td>54</td>
<td>—</td>
<td>—</td>
<td>5</td>
<td>2</td>
</tr>
<tr>
<td>6</td>
<td>4.75</td>
<td>2.0</td>
<td></td>
<td>6.75</td>
<td>52</td>
<td>0.68</td>
<td>2</td>
<td>33</td>
<td>—</td>
<td>—</td>
<td>4</td>
<td>—</td>
</tr>
<tr>
<td>4</td>
<td>2.5</td>
<td>5.0</td>
<td></td>
<td>7.5</td>
<td>58</td>
<td>0.89</td>
<td>1</td>
<td>25</td>
<td>—</td>
<td>1</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>5</td>
<td>2.5</td>
<td>6.0</td>
<td></td>
<td>8.5</td>
<td>65</td>
<td>1.82</td>
<td>3</td>
<td>60</td>
<td>—</td>
<td>—</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>12</td>
<td>2.5</td>
<td>6.5</td>
<td></td>
<td>9.0</td>
<td>69</td>
<td>1.78</td>
<td>8</td>
<td>67</td>
<td>—</td>
<td>—</td>
<td>4</td>
<td>1</td>
</tr>
<tr>
<td>Total 40</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>20</td>
<td>51</td>
<td>(of 39*)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

MI, myocardial infarction; PTCA, percutaneous transluminal coronary artery; CABG, coronary artery bypass graft.

Patients receiving the lowest one third of doses received ≤6.25 million IU. Patients receiving the highest one third received ≥8.5 million IU.

Milligram dose calculated on the basis of specific activity of 130,000 IU/mg.

*One of the 40 patients died before the 90-minute angiogram.

Statistical Methods

Values are expressed as mean±SD. Statistical comparisons of hematologic results before and after administration of pro-urokinase were carried out by the Student’s t test. A p value ≤0.05 was considered significant.

Results

Coronary Artery Reperfusion

Reperfusion after pro-urokinase administration was observed in 20 of the 39 patients (51%) who had angiograms performed 90 minutes after initiation of therapy. (Of the initial 40 patients, one died before the 90-minute study.) As shown in Table 1, there was no apparent correlation between dose and reperfusion rate, but the number of patients studied at each dose level was small. The reperfusion rate for the one third of patients receiving the lower doses (less than or equal to 6.25 million IU) was 50% (six of 12) compared with 65% (11 of 17) for the one third receiving the higher doses (greater than or equal to 8.5 million IU), p=NS.

The average time to reperfusion was 64.8±22.3 minutes. There was no significant relation between the interval from the onset of pain to the initiation of pro-urokinase and reperfusion. There was also no relation between body weight and reperfusion.

Hematologic Effects

 Adequate plasma samples for assessment of hematologic response were available in 36 of the 40 patients. The 51% reperfusion rate was associated with only minimal disturbance of the coagulation system. The levels of the seven hematologic variables before infusion of pro-urokinase are presented in Table 2. Figure 1 shows the changes in these variables 90 minutes after the start of pro-urokinase therapy, expressed as a percentage of baseline values. Fibrinogen fell only 10±17% for the group, and in no patient did it fall below 100 mg%. Importantly, the method used to measure fibrinogen may
underestimate the actual fibrinogen reduction. Plasminogen and α2-antiplasmin fell substantially more than did fibrinogen. FDP and B-β1-42, markers of degradation of both fibrin and fibrinogen, increased, while B-β15-42 and XDP, markers of selective lysis of fibrin, showed the greatest increases. The decreases in fibrinogen, plasminogen, and α2-antiplasmin, and the increases in XDPs and B-β15-42 were all significant at the p<0.05 level (see Figure 1).

Although no clear dose-response relation was observed between pro-urokinase and depression of the hemostatic values, the fibrinogen decreased by 7±11% in the one third of patients receiving the lowest dose versus 14±24% in the one third of patients receiving the highest dose (p=NS). There was also no clear dose-response relation noted between pro-urokinase on a milligrams per kilogram basis and depression of the hemostatic values or hemorrhagic complications.

Hemorrhagic and Other Complications

Only one patient experienced a significant hemorrhagic event after pro-urokinase administration. In this patient, a retroperitoneal hemorrhage requiring 3 units of transfused blood was detected over 10 hours after femoral artery catheterization and pro-urokinase administration. At the time of the hemorrhage, the patient was receiving heparin therapy and the partial thromboplastin time was longer than 80 seconds. The mean hematocrit for all patients included in the analysis was 42.4% before pro-urokinase and 35.6% within the first 24 hours after pro-urokinase. Two patients died within the 24 hours after pro-urokinase therapy. One died of cardiogenic shock 80 minutes after initiation of pro-urokinase therapy; the second died during balloon inflation for coronary angioplasty performed 3 hours after pro-urokinase administration. No cerebrovascular events occurred during the first 24 hours after therapy nor were any allergic reactions observed.

Relation of Hematologic Effects to Reperfusion

The hematologic responses of the 18 patients who achieved reperfusion and had hematologic data available, termed responders, were compared with those of the 18 nonresponders with data available in an attempt to determine if development of a systemic lytic state was more likely to lead to thrombolysis. Before treatment, there were no significant differences between responders and nonresponders in any of the seven hemostatic values. After treatment, the decreases of fibrinogen and plasminogen, and the increases of FDP, XDP, B-β1-42, and B-β15-42 were not significantly different between groups.

Discussion

In this study of patients with acute myocardial infarction, pro-urokinase produced effective, relatively fibrin-specific lysis of coronary thrombi similar to that achievable with t-PA as monotherapy. Fifty-one percent of the 39 patients achieved reperfusion in response to a range of doses. Plasma fibrinogen values were not markedly depressed, and only more sensitive hematologic measures indicated the presence of a mild systemic lytic state. One patient experienced a significant hemorrhage 10 hours after pro-urokinase, while receiving heparin therapy.

Pro-urokinase was discovered by Husain, Gurewich, and Lipinski who isolated it from human urine in 1979.6–11 Although pro-urokinase has been less extensively studied than t-PA, sufficient data are now available to indicate its promise as a thrombolytic agent for myocardial infarction.
Pro-urokinase is a 55,000-Da protein composed of 411 amino acids\textsuperscript{26} that, like t-PA, contains a serine protease active site, a “kringle” domain, and an epidermal growth factor-like domain. It differs from t-PA, however, in that, despite the presence of its kringle domain, it does not bind strongly to fibrin and is stable when exposed to plasma.\textsuperscript{30}

Two theories have been advanced to explain its relative fibrin-specificity, which has been well-documented with in vitro plasma and in vivo animal studies. Pannell et al.\textsuperscript{30,31} have proposed that pro-urokinase selectively activates fibrin-bound Glu-plasminogen due to a specific conformational change induced in the Glu-plasminogen when the latter proenzyme binds to certain lysine residues on partially digested fibrin. It is postulated that pro-urokinase is converted to its active form, high molecular weight two-chain urokinase, by plasmin generated on the fibrin surface.\textsuperscript{30,31} An alternative theory is that the clot-selective action of pro-urokinase results from its inhibition in plasma by an inhibitor that is not active in the presence of fibrin.\textsuperscript{32}

Pro-urokinase was first administered to patients by Van der Werf et al.\textsuperscript{13} who reported lysis of coronary thrombi in four of six patients in 1986. A number of studies reported relatively fibrin-specific thrombolysis.\textsuperscript{14–18} Diefenbach et al.\textsuperscript{14} showed that a higher dose produced effective reperfusion but depleted fibrinogen.

These studies suggested the importance of pro-urokinase as a relatively fibrin-specific thrombolytic agent, but each consisted of too small a number of patients to narrow the confidence intervals for reperfusion rate, time to lysis, and fibrinogen degradation, and none reported as extensive data describing the effects on the hemostatic system as the present study.

The results of the present study of pro-urokinase are similar to those obtained in the initial TIMI study of t-PA. In phase I of the TIMI trial, 62% of patients who received t-PA achieved patency at 90 minutes. The hematologic results indicate that pro-urokinase is at least as fibrin specific as t-PA.

The hematologic parameters measured in the present study permit thorough characterization of the fibrin specificity of pro-urokinase. The minimal depression of fibrinogen (a 10% decrease), despite effective lysis, clearly places pro-urokinase in a class with t-PA as a relatively fibrin-specific thrombolytic agent, and distinguishes it from streptokinase, urokinase, acylated streptokinase-plasminogen activator complex (APSAC), and other nonselective thrombolytic agents. The potential underestimation of the degree of fibrinogen reduction by the sulfite method notwithstanding, these results indicate only a modest reduction of fibrinogen that compares favorably with studies of other plasminogen activators in which a similar fibrinogen assay was used.

The complete hematologic profile obtained indicates that the fibrin-specificity of pro-urokinase is not absolute. As has been observed with t-PA, pro-urokinase produced marked decreases in both plasminogen and $\alpha_2$-antiplasmin.

It has been proposed that a systemic lytic state, such as that generally observed after treatment with the nonselective plasminogen activators, may have a beneficial effect on myocardial perfusion unrelated to lysis of the occlusive coronary thrombus.\textsuperscript{33,34} It has been suggested that the systemic depletion of fibrinogen produced by streptokinase may improve blood flow within the infarcted area by decreasing blood viscosity.\textsuperscript{35} Although a selective effect on the offending thrombus would appear to be the most desirable for reasons of safety, the question of the potential beneficial effect of nonspecific lysis remains unresolved because studies of the nonselective agent streptokinase have demonstrated a mortality benefit, while similar studies have not yet been conducted with pro-urokinase. An answer to this question may be obtained from ongoing mortality trials comparing t-PA with streptokinase.

Whatever the outcome of such comparisons, it is essential to continue the search for the most effective, safe, and clot-selective regimen. Such a regimen would provide the physician with the flexibility to attack the clot alone if desirable (or necessary because of bleeding risks) or to add a systemic lytic effect if such an effect is proven to provide benefits beyond those achieved with selective coronary thrombolysis. Although both t-PA and pro-urokinase originally offered promise as ideal thrombolytic agents, neither appears to be completely capable of achieving the triple goals of safe, rapid, and effective clot lysis when used as monotherapy. In the present study, pro-urokinase failed to produce reperfusion in 49% of the patients, and a mean of 64.8 minutes of crucial time was lost from initiation of therapy to reperfusion. There is a suggestion in the data, supported by a previous study,\textsuperscript{14} that higher doses would provide higher reperfusion rates, but the price would be a greater depletion of systemic clotting factors and a possible higher risk of hemorrhagic complications.

Such potential problems with higher doses of pro-urokinase have already been experienced with t-PA. The 150-mg t-PA dose, which produced the most rapid lysis and a 76% reperfusion rate,\textsuperscript{6–8} created a significant systemic lytic effect and an unacceptable rate of hemorrhagic stroke (1.6%).\textsuperscript{8} Experience with the 150-mg dose thus defined an upper limit on the dosage of t-PA. The 100-mg dose now recommended for use has a favorable risk-benefit ratio in appropriately selected patients, but one fourth of the patients treated will not achieve reperfusion and those who do must in some cases experience continued myocardial necrosis for up to 90 minutes before lysis occurs.

It is possible that the limitations of both pro-urokinase and t-PA as individual agents can be overcome by their combined use. Such a combination is suggested by the natural state in which both activators are found in human plasma, by their
distinct yet complementary mechanisms of action, and by laboratory and clinical studies suggesting that their combination has a synergistic effect. The combined regimen of t-PA and pro-urokinase must now be compared with t-PA monotherapy in a study with a larger number of patients in which there is no delay for a pretreatment angiogram.

Acknowledgment

We are grateful for the assistance of Ms. Kathleen Carney in the preparation of the manuscript.

Appendix

The Pro-urokinase for Myocardial Infarction Study Group

Study Personnel

Clinical Centers

Brigham and Women’s Hospital, Boston, Massachusetts: James M. Kirshenbaum, MD, Principal Investigator; Peter Ganz, MD, Coinvestigator; Elliott Antman, MD, Coinvestigator; Peter H. Stone, MD, Coinvestigator; Gail Aylmer, Coordinator; Noreen McGowan, RN; and Kathy O’Halloran, RN.

The Johns Hopkins Hospital, Baltimore, Maryland: John Flaherty, MD, Principal Investigator; Carol J. Dreyer, RN, BSN; Jeffrey A. Brinker, MD, Coinvestigator; and Howard Grill, MD, Coinvestigator.

Lahey Clinic, Burlington, Massachusetts: Robert Malacoff, MD, Principal Investigator; and Sidney Alexander, MD, Coinvestigator.

St. Elizabeth’s Hospital, Boston, Massachusetts: Thomas Wharton, MD, Principal Investigator; Victor Gurewich, MD, Coinvestigator; Bernard Kosowsky, MD, Coinvestigator; Nancy Otovic, RN, BSN, Coordinator. Participating Investigators: John O. Pastore, MD; K. Ramaswamy, MD, Director, Cardiac Catheterization Laboratory.

Tufts New England Medical Center, Boston, Massachusetts: Herbert J. Levine, MD, Principal Investigator; Deeb N. Salem, MD, Coinvestigator; Carolyn Henry RN, Coordinator. Participating Physicians: Jeffrey M. Isner, MD; Marvin A. Konstand, MD; N.A. Mark Estes, III, MD.

University of Michigan Medical Center, Ann Arbor, Michigan: Eric Topol, MD, Principal Investigator; Eva Kline, RN, BS, Coinvestigator and Coordinator; William W. O’Neill, MD, Coinvestigator; Joseph A. Walton, MD, Coinvestigator; and Stephen G. Ellis, MD, Coinvestigator.

Coordinating Center

Harvard Medical School, Boston, Massachusetts: James E. Muller, MD, Director; Victor Gurewich, MD, Consultant; Peter H. Stone, MD, Consultant; Gail Aylmer; and Kathy Carney.

Hematology Core Laboratory

Brigham and Women’s Hospital, Boston, Massachusetts: Joseph Loscalzo, MD, PhD, Director.

Angiography Core Laboratory

Brigham and Women’s Hospital, Boston, Massachusetts: James Kirshenbaum, MD, Director; Peter Ganz, MD, Coinvestigator; Thomas Wharton, MD, Coinvestigator; and Deeb Salem, MD, Coinvestigator.

References


38. Colleen D, van der Werf F: Coronary arterial thrombolysis with low-dose synergistic combinations of recombinant tissue-type plasminogen activator (rt-PA) and recombinant single chain urokinase-type plasminogen activator (rscu-PA) for acute myocardial infarction. *Am J Cardiol* 1987;60:431–434

**KEY WORDS** • coronary thrombosis • thrombolytic therapy • fibrinolysis • fibrinogenolysis
Clot-selective coronary thrombolysis with pro-urokinase.

Circulation. 1989;79:776-782
doi: 10.1161/01.CIR.79.4.776
Circulation is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 1989 American Heart Association, Inc. All rights reserved.
Print ISSN: 0009-7322. Online ISSN: 1524-4539

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circ.ahajournals.org/content/79/4/776

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Circulation 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 is online at:
http://circ.ahajournals.org//subscriptions/