Coronary thrombolysis with recombinant human tissue-type plasminogen activator

HERMAN K. GOLD, M.D., JOHN T. FALLON, M.D., PH.D., TSUNEHIRO YASUDA, M.D., ROBERT C. LEINBACH, M.D., BAN AN KHAW, PH.D., JOHN B. NEWELL, B.A., J. LUIS GUERRERO, F. MICHAEL VISLOSKY, R.N., CHARLES F. HOYNG, PH.D., ELLIOTT GROSSBARD, M.D., AND DÉSIRÉ COLLEN, M.D., PH.D.

ABSTRACT The thrombolytic potency and myocardial infarct–sparing potential of recombinant tissue-type plasminogen activator (rt-PA) were studied in electrocardiographically monitored, open-chest, anesthetized dogs. Localized coronary thrombosis was produced in the left anterior descending artery by endothelial injury and instillation of thrombin and fresh blood. After 2 hr of stable thrombotic occlusion, rt-PA was infused intravenously. At a dose of 4.3 μg/kg/min, time to reperfusion was greater than 40 min (n = 3). However, at higher infusion rates a linear, dose-dependent time to coronary reperfusion was obtained (r = .88): at 10 μg/kg/min reperfusion occurred after 31 ± 2 min (n = 3), at 15 μg/kg/min it was at 26 ± 7 min (n = 4), and at 25 μg/kg/min, lysis was accomplished within 13 ± 3 min (n = 3). Thrombolysis was not associated with alterations in either plasma hemostatic factors (fibrinogen, plasminogen, and α2-antiplasmin) or in systemic blood pressures. Epicardial electrographic measurements revealed a significant reduction in ST elevation in all reperfused hearts. A randomized, blinded study was also carried out with 15 μg/kg/min of rt-PA saline in 18 dogs with 30 min of coronary thrombosis. Reperfusion in the treated group occurred after 28 ± 3 min. No evidence of thrombolysis was noted in the saline-treated group within 240 min. Size of myocardial infarction was determined by triphenyl tetrazolium chloride staining and planimetry. Infarction involved 2.5 ± 0.5% of the left ventricular wall in the group receiving rt-PA, but 16 ± 3% of the left ventricle in the saline-treated group (p = .001). It is concluded that intravenous infusion of rt-PA results in rapid, dose-dependent coronary thrombolysis without systemic fibrinolytic activation and that early lysis of coronary thrombi is associated with substantial salvage of myocardial tissue.


CORONARY THROMBOSIS, superimposed on atherosclerotic plaques, occurs in the majority of patients with acute myocardial infarction.1 Thrombolytic therapy with streptokinase can result in recanalization of occluded coronary arteries in up to 80% of patients,2–5 but is associated with systemic fibrinolytic activation predisposing to a tendency to bleeding. Although this tendency will result in clinically significant bleeding in only a minority of cases, it complicates surgical intervention within the first few hours after the infusion.

Tissue-type plasminogen activator (t-PA), which is normally found at very low concentrations in human blood, produces thrombus-specific fibrinolysis by activating plasminogen on the surface of fibrin. In the studies performed thus far it has been shown to be essentially devoid of adverse side effects on the hemostatic system.6 Although sufficient quantities of t-PA have been obtained from tissue culture media to allow investigations of its biological and thrombolytic properties in small-scale studies,7 it is both difficult and expensive to produce from this source for use in large-scale clinical investigations. Recently, Pennica et al.8 cloned and demonstrated the expression of cDNA of the mRNA for human t-PA in *Escherichia coli*. In a further development, the cDNA for t-PA was expressed in a mammalian cell system.

The present study details the ability of recombinant human t-PA (rt-PA) from this mammalian cell system to lyse coronary thrombus in an experimental canine preparation of acute myocardial infarction. We report the dose-dependent time to clot lysis, the effects on systemic clotting factors, and the ability of rt-PA to
Materials and methods

rt-PA. rt-PA was obtained by expression of cDNA coding for the entire sequence of melanoma cell–derived t-PA in a mammalian cell system. The rt-PA was highly purified to homogeneity from the cell culture fluid in the absence of protease inhibitors and consisted essentially of the two-chain form. It is indistinguishable from melanoma-derived t-PA by a number of physical and biochemical criteria.10 The rt-PA used for the present study was devoid of pyrogens.

Experimental preparation. Adult mongrel dogs were anesthetized with pentobarbital (30 mg/kg iv), and received additional doses as required. The dogs were intubated and placed on a respirator. The right carotid artery of each was exposed through an incision in the neck. A modified No. 7-1 Amplatz coronary angiographic catheter was placed in the ascending aorta under fluoroscopy and a blood pressure catheter was placed in the femoral artery. An intravenous catheter was placed in a foreleg vein. Prophylactic antiarrhythmic therapy consisted of 3 mg/min lidocaine (Xylocaine) after an intravenous loading dose of 75 mg. Procainamide, 1.5 g, was also given intravenously.

The chest was opened via a fifth intercostal thoracotomy. A pericardial cradle was fashioned to suspend the heart and epicardial electrocardiographic leads were placed. Blood pressure and the electrocardiogram were continuously recorded. A 1 cm long segment of the left anterior descending coronary artery (LAD) was isolated distal to the septal artery and any large diagonal branches. A 0.70 mm (inside diameter) catheter was inserted into a right ventricular side branch of the isolated LAD segment (figure 1). A selective angiogram of the left coronary artery was obtained with the use of 1 to 2 ml of meglumine diatrizoate and recorded on videotape.

Heparin was given as a 100 units/kg iv loading dose and then in 50 units/kg doses to maintain the activated partial thromboplastin time at 1.5 to 2 times baseline. The isolated LAD segment was traumatized by external compression with a blunt forceps to disrupt the endothelium. Snare occlusions were placed proximally and distally on the isolated LAD segment. Thrombin, 0.1 ml at 100 U/ml, was injected via the side-branch catheter into the isolated LAD segment. Citrated fresh canine blood, 0.3 to 0.4 ml, containing 0.05M calcium chloride was then injected into the isolated segment to form the intraluminal thrombus, which had a volume of approximately 0.3 ml. The proximal snare was released after 5 min and 2 min later the distal snare was released. Selective coronary angiography was repeated at 15 min intervals to monitor the occlusion.

Baseline blood samples for determination of levels of fibrinogen, plasminogen, and α₂-antiplasmin, of activated partial thromboplastin time, and of concentration of t-PA–related antigen were obtained from a peripheral vein just before the induction of the LAD thrombus (control sample) and at the end of the 2 hr coronary occlusion (zero sample). Subsequently, blood samples were obtained to measure these parameters at the onset of therapy, every 10 min for 60 min thereafter, and then at 30 min intervals for 3 hr. The blood samples were kept on ice until the end of the experiment, then centrifuged at room temperature for 10 min, and the plasma was stored frozen at −20°C until analyzed. The assays were performed as previously described.11

rt-PA dose time to reperfusion. After 2 hr of persistent coronary occlusion, 13 dogs were subjected to intravenous (via a foreleg vein) treatment with increasing doses of rt-PA to determine the time at which lysis occurred from the onset of intravenous administration of rt-PA. An additional seven dogs treated with intravenous saline infusions (5 ml/min for 30 min) served as controls. All intravenous infusions were given via a rear leg vein. The initial study was begun with an average dose of 4.3 μg/kg/min of rt-PA in three dogs for 35 min. Because occlusion persisted for up to 40 min, rt-PA was then administered in this group via the intracoronary route at the same rate for 30 min. Subsequent groups of dogs were treated with 10, 15, and 25 μg/kg/min of intravenous rt-PA for 30 min or until reperfusion was attained. Angiography was performed every 15 min or when evidence of reflow occurred, that is, at the onset of arrhythmias, upon a decrease in ST segment elevation on the electrocardiographic tracings, or if there were visual evidence of LAD reperfusion. Repeat angiography was performed at half-hour intervals after evident reperfusion to demonstrate that the coronary lumen remained patent until the end of the experiment.

Additional experiments to demonstrate activation of the fibrinolytic system in vitro by rt-PA during storage of plasma samples. Because significant decreases in fibrinogen, plasminogen, and α₂-antiplasmin levels were observed in stored plasma samples taken during rt-PA infusion, but not in plasma samples taken towards the end of the experiment, the following additional experiments were performed to confirm that activation of the fibrinolytic system occurs in vitro in plasma containing high rt-PA levels.

In three animals rt-PA was infused intravenously at a rate of 15 μg/kg/min for 30 min, during which blood samples were taken at 10 min intervals. Subsequent blood samples were then collected at 30 min intervals for 3 hr. Blood samples were collected both into citrate (final concentration 0.01M) and into citrate containing aprotinin (Trasylol; Bayer, Leverkusen, West Germany; final concentration 100 KIU/ml). Fibrinogen levels were measured in whole blood immediately after collection, at the end of the experiment (during which the blood was kept on ice), and in plasma samples stored for 2 hr at 37°C or on ice.

Randomized blinded trial of rt-PA. This study was performed after 30 min of persistent coronary occlusion in 18 dogs subjected to a randomized, blinded trial of intravenous rt-PA at 15 μg/kg/min or saline. Angiography was performed every 10 min during the treatment period or immediately, if evidence of reflow occurred. The “blinded” therapy was infused until either reperfusion occurred and was documented angiographically or until 60 min had elapsed. After treatment was stopped, angiography was performed at 30 min intervals to determine if there

FIGURE 1. Schema of the experimental preparation of a thrombus in the LAD. For further details see text.
was continuing patency or occlusion of the coronary vessel for the 4 hr duration of the experiment.

Pathology. To determine infarct size, at 4 hr each dog was given 500 ml of 2% triphenyl tetrazolium chloride (TTC) via a left atrial injection and killed. The heart was removed and incubated in warm saline for 15 min and then fixed in 5% buffered formalin. Each heart was sliced at 1 cm intervals parallel to the atroventricular groove, photographed, and weighed. Infarct size was determined morphometrically by planimetry of the TTC-stained slices. Infarct size was expressed as a percentage of left ventricular wall volume. Histologic sections were examined to verify the presence of endothelial trauma in the thrombosed LAD section and to confirm the presence or absence of thrombus.

Statistical methods. Two random permutations of the integers 1 to 9 were performed and the first four of each of these two blocks of integer sets were defined as t-PA treatments and the remaining five as controls. This resulted in a random sequence of treatment assignments with a blocking of 9, so that five control and four t-PA experiments were completed midway through the trial with similar balance for the remainder of the trial. The treatment assignments were enclosed in numbered envelopes and used in sequence by a technician who prepared the infusions according to the instructions in the envelope, which were unknown to the angiographer or any other participants.

Results

rt-PA dose vs time to reperfusion. Angiographic results confirmed the stability of the thrombotic occlusion during the 2 hr pretreatment period. A typical coronary angiogram is shown in figure 2. There was no evidence of antegrade LAD flow in any of the 20 dogs. Furthermore, in the seven control dogs, repeated angiography showed no evidence of antegrade flow in the LAD for the entire 4 hr experimental period. Although changes in the electrocardiogram (see below) correlated with partial restoration of flow on the angiogram, the time to reperfusion was defined as the time at which the angiogram showed complete antegrade filling of the LAD.

Intravenous administration of rt-PA at a rate of 4.3 μg/kg/min in three dogs did not effect thrombolysis after 40 min. In these initial three dogs, the biological activity of the rt-PA was tested by a subsequent selective intracoronary infusion at a rate of approximately 5 μg/kg/min. This resulted in restoration of flow in the LAD in an average of 28 min.

At higher intravenous infusion rates a linear correlation between infusion rate and time to reperfusion was obtained (figure 3). With an intravenous dose of rt-PA of 10 μg/kg/min clot lysis occurred in 31 ± 2 min (n = 3). Increasing the dose of intravenous rt-PA to 15 μg/kg/min decreased lysis time to 26 ± 7 min (n = 4).
and 25 μg/kg/min of intravenous rt-PA resulted in a time to reperfusion of 13 ± 3 min (n = 3). Linear regression analysis of the infusion rate vs time to lysis in the 10 successfully reperfused dogs yielded a correlation coefficient of .88 (p < .001).

The effects of rt-PA dose on relevant parameters of hemostasis are shown in Table 1. In samples taken toward the end of the experiments, little evidence of systemic activation of plasminogen, consumption of α2-antiplasmin, or breakdown of circulating fibrinogen was observed. Indeed, the changes in these parameters during the experiment were not significantly different from those observed in the control group. However, in samples taken during rt-PA infusion (not shown) significant alterations were observed most likely as a result of artifactual activation of the fibrinolytic system in vitro (see below). Figure 4 illustrates the rapid rise of plasma t-PA levels to measured peaks within the first 10 min of intravenous rt-PA infusion and the rapid return toward baseline after the infusion was stopped. Mean infusion times for rt-PA were 35 min at 4.3 μg/kg/min, 30 min at 10 μg/kg/min, 23 min at 15 μg/kg/min, and 13 min at 25 μg/kg/min.

Epicardial ST segment elevation averaged 2.0 ± 0.9 mV in all dogs during the 2 hr period of pretreatment occlusion. The ST segments returned to baseline in seven of the 13 dogs in which reperfusion was observed within 60 min. In the 13 dogs in which reperfusion was observed a fall of 1.9 ± 0.9 mV in the ST segment occurred when measured 1 hr after onset of rt-PA infusion. In contrast, the ST segment fell only 0.7 ± 0.4 mV on average in the seven control dogs when the segment was measured 3 hr after onset of occlusion (p = .007).

**TABLE 1**

<table>
<thead>
<tr>
<th>rt-PA dose (μg/kg/min)</th>
<th>Level before infusion</th>
<th>Level after infusion</th>
<th>Absolute change</th>
<th>p values</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fibrinogen</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>0</td>
<td>2.44</td>
<td>2.10</td>
<td>-0.34</td>
</tr>
<tr>
<td>3</td>
<td>4.3</td>
<td>1.87</td>
<td>1.71</td>
<td>-0.16</td>
</tr>
<tr>
<td>3</td>
<td>10</td>
<td>1.73</td>
<td>1.51</td>
<td>-0.22</td>
</tr>
<tr>
<td>4</td>
<td>15</td>
<td>2.85</td>
<td>2.21</td>
<td>-0.65</td>
</tr>
<tr>
<td>3</td>
<td>25</td>
<td>1.45</td>
<td>1.19</td>
<td>-0.27</td>
</tr>
<tr>
<td>Plasminogen</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>0</td>
<td>71.6</td>
<td>54.7</td>
<td>-16.9</td>
</tr>
<tr>
<td>2</td>
<td>4.3</td>
<td>106.5</td>
<td>77.0</td>
<td>-29.5</td>
</tr>
<tr>
<td>3</td>
<td>10</td>
<td>72.2</td>
<td>47.3</td>
<td>-24.8</td>
</tr>
<tr>
<td>4</td>
<td>15</td>
<td>71.9</td>
<td>59.3</td>
<td>-12.6</td>
</tr>
<tr>
<td>3</td>
<td>25</td>
<td>98.0</td>
<td>63.3</td>
<td>-34.8</td>
</tr>
<tr>
<td>α2-Antiplasmin</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>0</td>
<td>103.5</td>
<td>95.5</td>
<td>-8.0</td>
</tr>
<tr>
<td>2</td>
<td>4.3</td>
<td>107.8</td>
<td>74.8</td>
<td>-33.0</td>
</tr>
<tr>
<td>3</td>
<td>10</td>
<td>100.2</td>
<td>77.8</td>
<td>-22.3</td>
</tr>
<tr>
<td>4</td>
<td>15</td>
<td>107.5</td>
<td>90.3</td>
<td>-17.2</td>
</tr>
<tr>
<td>3</td>
<td>25</td>
<td>101.8</td>
<td>80.3</td>
<td>-21.5</td>
</tr>
</tbody>
</table>

^A Mean in control and 0 min samples taken before infusion of rt-PA.

^B Mean in samples obtained 150 and 180 min after the start of the infusion.

^C Calculated by t test comparing 0 dose group with absolute changes in each group.

Vol. 70, No. 4, October 1984
Blood pressure monitoring during rt-PA infusion showed no change in mean blood pressure either during or after infusion of rt-PA as compared with before treatment.

**Activation of the fibrinolytic system in vitro during storage of blood.** Figure 5 summarizes the effect of storage of blood in vitro on the fibrinogen level in one of the three animals. Whereas no changes in fibrinogen levels were observed in the blood samples collected in aprotinin, a progressive drop in level was found in the samples containing a high concentration of rt-PA (during the infusion). Degradation of fibrinogen was not observed when levels were measured immediately after collection of blood samples, but was significant in blood kept on ice until the end of the experiment (3 to 4 hr). In blood samples taken toward the end of the rt-PA infusion (at a time when the plasma rt-PA was greatly reduced as a result of clearance of rt-PA from plasma) no fibrinogen breakdown was observed during storage.

**Randomized blinded trial of rt-PA.** None of the 10 control dogs receiving saline infusions showed evidence of thrombolysis during the 4 hr experimental period. All of the eight dogs given 15 μg/kg/min of rt-PA showed lysis of the LAD thrombus, with a mean time to reperfusion of 28 ± 3 min. Thus, the total time of LAD occlusion in the rt-PA–treated dogs was approximately 1 hr.

Size of myocardial infarction in the control dogs was 16 ± 2% (mean ± SD) of the left ventricle, whereas the mean size of the infarcts in the rt-PA–treated dogs was 2.5 ± 0.5% (p = .001). All infarcts were in the anteroseptal region. Except in one instance, infarcts in the control hearts involved more than half of the thickness of the left ventricular wall and generally showed focal transmural involvement. In contrast, infarcts in the reperfused hearts were most often patchy, subendocardial, and did not show transmural involvement (figure 6). Reperfusion with rt-PA was not associated with significant hemorrhage or edema in the infarct zone.

Blood pressure and electrocardiographic findings were in keeping with results of the dose-response studies.

**Discussion**

This study was undertaken to investigate the thrombolytic potency of intravenously administered rt-PA in a canine preparation of coronary thrombosis and acute myocardial infarction. The preparation was designed...
to permit evaluation of thrombolysis of coronary clots without the use of intracoronary thrombogenic foreign materials. In addition it simulated two features of coronary thrombosis that approximate more closely the human situation: endothelial damage and adherence of the thrombus to the arterial wall. The preparation allowed for monitoring of coronary anatomy by repeated angiographic examinations and continuous monitoring of electrical and hemodynamic parameters and for repeated measurements of levels of plasma clotting factors. Finally, the preparation allowed for the pathologic evaluation of thrombolysis by t-PA and the effect of early reperfusion on subsequent size of myocardial infarct.

At an intravenous infusion rate of 4.3 μg/kg/min no reperfusion was obtained after 40 min. However, in a
dose range of between 10 and 25 μg/kg/min a linear relationship between the rate of intravenous rt-PA infusion and the time to reperfusion was observed. The thrombolytic potential of rt-PA thus is comparable to that of natural t-PA. 15

The measurements of rt-PA levels in serum that have been obtained confirm the short half-life (3 to 5 min) of t-PA in the circulation. 14 This means that once infusion of rt-PA is discontinued, fibrinolysis ceases within 25 min (five half-lives), thereby restoring a normal hemostatic system. Therefore, thrombolysis with rt-PA is rapidly and easily controlled.

There is no evidence from our data of systemic fibrinolysis. We did observe very significant changes in fibrinogen, plasminogen, and α2-antiplasmin levels in stored plasma samples obtained from blood taken during the infusion of rt-PA, but not in plasma samples taken toward the end of the experiment. The transient nature of the changes, however, were suggestive of artifacts in vitro, because decreases in the fibrinogen level induced by systemic activation of the fibrinolytic system with, e.g., urokinase, persist for several hours after the end of the infusion. 11 In additional experiments in vitro, in which rt-PA was added to plasma in concentrations of up to 1 or 2 μg/ml, activation of the fibrinolytic system did occur, as evidenced by a progressive decrease in the levels of fibrinogen, plasminogen, and α2-antiplasmin. The same phenomenon was observed in dog, baboon, and human plasma, but could be prevented by collection of the blood in aprotinin. The artifactual nature of these transient changes was finally demonstrated in additional experiments in vivo in which rt-PA was infused at a rate of 15 μg/kg/min (figure 5).

The occurrence of activation of the fibrinolytic system in vitro in plasma does not reflect systemic fibrinolysis in vivo. Its origin is unclear but may be related to protein denaturation during storage of the blood. 15 It can largely be prevented by collecting blood in aprotinin. Because a similar activation of the fibrinolytic system was observed in human plasma in vitro, assays of blood samples to determine parameters of the fibrinolytic system in patients receiving infusions of rt-PA will have to be interpreted with caution unless the blood is collected in aprotinin or the assays are performed immediately.

Even though the experimental model used was an open-chest canine preparation, at no time during infusion of rt-PA did we observe an effect on normal hemostasis. Bleeding did not occur from the multiple instrumentation sites or the operative site. These observations are in agreement with the findings of Buchanan et al., 16 who quantitated bleeding after infusion of t-PA in rabbits and observed no difference in bleeding compared with that in control animals.

Angiographic examination during the time of thrombotic occlusion never revealed either spontaneous thrombolysis or evidence of movement, dislodgement, or embolization of the LAD clot. The onset of reflow was heralded by either a decrease in epicardial ST segment elevation or onset of ventricular arrhythmias, including ventricular premature beats, idioventricular rhythm, and ventricular tachycardia. In this study, reflow induced by rt-PA thrombolysis did not result in induction of ventricular fibrillation. Angiography performed at the time of these electrocardiographic changes often revealed partial clot lysis and incomplete reflow of the distal LAD and, when repeated 5 to 10 min later, demonstrated complete antegrade filling of the LAD with either minimal or no residual thrombus evident. In no instance was fragmentation or embolization of thrombus visualized angiographically during infusion of rt-PA. These findings suggest that rt-PA results in progressive fibrinolysis, perhaps diminishing the potential for reflow-induced ventricular fibrillation. In this preparation, rethrombosis was not observed. Pathologic examination of the LAD confirmed the angiographic findings of either complete thrombosis in control animals or thrombolysis in rt-PA–treated groups. Although histologic sections of the LAD segment from rt-PA–treated hearts revealed superficial mural thrombus on the injured intimal surface, in no case was there evidence of the presence of significant intraluminal thrombus, thus confirming the thrombolytic potency of short-term, intravenous infusion of rt-PA.

The randomized, blinded study confirmed the specific thrombolytic properties of rt-PA and in addition confirmed the earlier findings of Reimer and his colleagues 17, 18 that early reperfusion results in a significant reduction of infarct size. Thus, although the observed infarct-sparing effect is not specific for rt-PA, our results show no specific deleterious effects of reperfusion induced by the drug.

In summary, the biological properties of rt-PA permit rapid and predictable coronary thrombolysis without systemic fibrinolysis when the drug is given intravenously. Extrapolation of the results of this study to the clinical setting suggests that the potential of rt-PA therapy to effectively interrupt the process of myocardial infarction will not exclusively depend on its thrombolytic potency but will largely be determined by the time to reperfusion after the onset of coronary thrombolysis.
We are grateful to Dr. Richard Harper for his expert advice, to Diane Wathen for aiding in the pathologic evaluations, to Dr. H. R. Lijnen for the performance of the clotting factor and t-PA assays, and to Lovely Templeton for preparing this manuscript.

Addendum
The conclusions of the present study are in agreement with those of a study published recently in Circulation while this manuscript was in review.19

References
18. Reimer KA, Jennings RB: The “wavefront phenomenon” of myocardial ischemic cell death. II. Transmural progression of necrosis within the framework of ischemic bed size (myocardium at risk) and collateral flow. Lab Invest 40: 633, 1979
Coronary thrombolysis with recombinant human tissue-type plasminogen activator.

Circulation. 1984;70:700-707
doi: 10.1161/01.CIR.70.4.700
Circulation is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 1984 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/70/4/700

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/