Clinical pharmacology in patients with evolving myocardial infarction of tissue-type plasminogen activator produced by recombinant DNA technology*

ALAN J. TIEFENBRUNN, M.D., ALICE K. ROBISON, PH.D., PETER B. KURNIK, M.D., PHILIP A. LUDBROOK, M.B., B.S., AND BURTON E. SOBEL, M.D.

ABSTRACT This study was performed to characterize selected pharmacologic properties and effects on the fibrinolytic system of tissue-type plasminogen activator synthesized by recombinant DNA technology (rt-PA) in 12 patients treated for coronary thrombosis. rt-PA was infused parenterally (by the intracoronary route in four patients and intravenously in eight) in doses of 8.3, 12.5, or 16.7 μg/kg/min for 30 to 60 min, yielding a total dosage of 20 to 40 mg/patient. The drug induced coronary thrombolysis in 10 of the 12 patients treated (83%), including six of the eight given rt-PA intravenously. No bleeding complications were encountered. Serial blood samples were obtained before, during, and after infusion of rt-PA and analyzed for t-PA antigen (i.e., immunoassayable rt-PA protein), functional fibrinolytic activity attributable to rt-PA, fibrinogen, plasminogen, α₁-antiplasmin, fibrinogen degradation products, prothrombin time, activated partial thromboplastin time, and protamine-corrected thrombin time. Pretreatment plasma t-PA antigen levels averaged 16.5 ± 5(SD) ng/ml. Peak plasma values were generally proportional to dose, averaging 3330 ± 1201 ng/ml. Approximately 90% of peak level was reached in 30 min, with a plateau at peak reached within 40 min. Functional t-PA activity increased monotonically in a comparable fashion. Curves for disappearance of both t-PA antigen and functional activity were monoexponential for at least two half-lives (r = .99 for both) and were concordant. The observed half-lives were similar, averaging 8.3 and 9.1 min, respectively. Changes in concentrations of fibrinogen were transient and modest (17 ± 6% of baseline). Plasminogen and α₁-antiplasmin levels declined moderately to 51 ± 6% and 32 ± 7% of pretreatment values at the end of infusion of rt-PA. Prothrombin time, protamine-corrected thrombin time, and assay of fibrinogen degradation products corroborated the lack of a lytic state. Thus, desirable levels of rt-PA can be achieved consistently with short-term infusions of appropriately selected doses without induction of a systemic lytic state predisposing to bleeding.


TISSUE-TYPE plasminogen activator (t-PA) is a naturally occurring serum protein with high affinity for fibrin. It activates plasminogen and induces fibrinolysis physiologically or pharmacologically. Since the $K_m$ (Michaelis constant) of t-PA for free plasminogen is so much greater (65 μM) than the $K_m$ for plasminogen bound to fibrin (0.14 μM), essentially no conversion of plasminogen to plasmin occurs in the circulation even when plasma t-PA levels are elevated several hundredfold. Thus, with t-PA, in contrast to the case after administration of equipotent, clot-lysing doses of streptokinase or urokinase, induction of a systemic lytic state predisposing to bleeding can be avoided.

We recently demonstrated that exogenous human t-PA harvested from spent Bowes cell line tissue culture medium promptly induced coronary thrombolysis in dogs and in patients without inducing a systemic lytic state.

Widespread clinical application of t-PA requires production methods capable of large yields of biologically active material. To this end, human tissue-type plasminogen activator has been produced in large quantities with the use of recombinant DNA technology (rt-PA).

We have recently pointed out that “clot selectivity”...
of an activator of the fibrinolytic system with a finite although low affinity for circulating plasminogen is related to the time-activity curve of the concentration of activator in plasma associated with any given dosage regimen. Markedly elevated concentrations of activator sustained for a sufficiently prolonged interval may overwhelm the low affinity of activator for circulating plasminogen, form sufficient plasmin to consume circulating α2-antiplasmin, and thereby induce depletion of fibrinogen and elaboration of fibrinogen degradation products predisposing to bleeding. Accordingly, it is necessary to characterize pharmacologic properties of rt-PA that influence plasma levels and the response of the fibrinolytic system to selected dosage regimens.

This study was undertaken to delineate the half-life in plasma of immunoassayable rt-PA, the half-life of functional fibrinolytic activity of rt-PA, plasma levels of rt-PA associated with selected dosage regimens, and the nature of effects of rt-PA on the fibrinolytic system in patients given the drug for coronary thrombolysis. The 12 patients studied were participants in an initial, collaborative, 50-patient clinical study designed to assess the efficacy of rt-PA and performed at Washington University, Johns Hopkins University, the Massachusetts General Hospital, and the Genentech Corporation. Effects of rt-PA on clot lysis for the collaborative trial are reported elsewhere. The detailed evaluation of pharmacologic properties of rt-PA was performed only on the group of patients studied at the Washington University–Barnes Hospital Medical Center and are reported here. Since the primary purpose of this study was not to delineate thrombolytic efficacy but rather to elucidate pharmacologic properties of rt-PA, most results were considered in a combined fashion for patients given rt-PA by the intracoronary route (n = 4) and those given the agent intravenously (n = 8).

Methods

This study was approved by the Institutional Review Board of Washington University. Written informed consent was obtained from all patients for the catheterization procedures and the administration of rt-PA.

Patients presenting with symptoms of acute myocardial ischemia of less than 6 hr duration and with electrocardiographic changes consistent with evolving transmural myocardial infarction (ST segment elevation ≥2 mm) were candidates for the study. Prior myocardial infarction, age greater than 70 years, child-bearing potential, cardiogenic shock, recent (<3 weeks) surgery, or a history of a bleeding diathesis were criteria for exclusion. Immediate cardiac catheterization, left ventriculography, and coronary arteriography were performed in all patients by the percutaneous femoral approach with systemic heparinization (75 U/kg given by bolus injection).

If a completely occlusive coronary thrombus was delineated, rt-PA (provided by Genentech, Inc.) was infused parenterally for 30 to 60 min at doses of 8.3, 12.5, or 16.7 μg/kg/min without an initial loading dose (table 1). Three patients were given an additional infusion of 4.2 μg/kg/min for 1 hr after completion of an initial 1 hr infusion. The occluded vessel was visualized angiographically at 15 min intervals throughout the course of the infusion.

Blood samples were obtained before infusion of rt-PA; at the end of the initial infusion to provide an estimate of peak levels; 5, 10, and 15 min after the infusion had been completed (for determination of the half-lives of immunoassayable rt-PA and functional activity); and 30 min and 3 hr after completion of the infusion. In some patients samples were drawn 20, 30, 40, 50, and 60 min after initiation of infusion for determination of the time of occurrence of a plateau of blood level. Samples were assayed for immunoassayable t-PA, comprising endogenous t-PA and exogenous rt-PA, t-PA functional activity, α2-antiplasmin, plasminogen, fibrinogen, fibrinogen degradation products, prothrombin time, activated partial thromboplastin time, and protamine-corrected thrombin time as follows.

Collection of samples. Venous blood was acquired with a two-syringe technique to avoid contamination of samples with products within the dead space of the indwelling venous catheters used. Samples were placed immediately on ice in Vacutainer tubes containing citrate. Aliquots for determination of fibrinogen were placed in tubes of the same type supplemented with aprotinin (200 kallikrein inhibitor units [KIU]/ml blood) to inhibit fibrinogenolysis in vitro. Plasma was separated by centrifugation at 4°C and frozen at −70°C before assay.

In preliminary experiments blood samples supplemented with 42 nM rt-PA (approximating peak plasma concentrations in treated patients) with or without aprotinin were placed on ice for 0, 30, 60, 120, or 180 min before centrifugation and before freezing at −70°C. Plasma was thawed immediately before assay for t-PA antigen, t-PA functional (fibrinolytic) activity, plasminogen, and fibrinogen. Results of duplicate assays in each case showed that t-PA antigen levels did not decline throughout the 180 min interval of initial storage at 0°C to 4°C, and in fact remained within 99% of initial values. t-PA functional activity did not decline detectably and fibrinogen content did not decrease by more than 10%. However, plasminogen decreased by 50% at the end of 1 hr and by 80% at the end of the 3 hr storage interval. The observed decreases in fibrinogen and plasminogen in samples stored for 3 hr at 0°C to 4°C were reduced by 50% when samples were centrifuged at zero time and separated plasma was stored at 0°C to 4°C for up to 3 hr before freezing at −70°C. No decreases were seen when samples were supplemented with only 0.7 nM t-PA.

### TABLE 1

<table>
<thead>
<tr>
<th>No. of patients</th>
<th>Route of admin.</th>
<th>Dose In μg/kg/min</th>
<th>Total mg</th>
<th>Duration of infusion (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>IV</td>
<td>8.3</td>
<td>0.25</td>
<td>20</td>
</tr>
<tr>
<td>1</td>
<td>IV</td>
<td>16.7</td>
<td>0.50</td>
<td>40</td>
</tr>
<tr>
<td>6</td>
<td>IV</td>
<td>8.3</td>
<td>0.50</td>
<td>40</td>
</tr>
<tr>
<td>4</td>
<td>IC</td>
<td>12.5</td>
<td>0.375</td>
<td>30</td>
</tr>
</tbody>
</table>

IV = intravenous; IC = intracoronary.

Total dose was calculated for each patient on the basis of body weight. The value shown for total dose is for an average 80 kg patient.

As indicated in the text, the dosages used changed throughout the study in part because of its dose-finding nature and in part because of our interest in evaluating a range of blood levels.
These results indicated that assays of plasminogen in samples from patients treated with rt-PA should be performed on plasma separated promptly from red cells and frozen quickly if spurious results reflecting activation of plasminogen in vitro are to be avoided. Accordingly, the procedures used in this study included prompt centrifugation and fast freezing of all samples.

**Immunoadsorbable t-PA.** A two-site immunoradiometric assay (IRMA) developed by Rijken et al. was used to assay t-PA antigen as described previously. Thawed, citrated plasma samples were diluted serially in buffer. Duplicate determinations were performed at each dilution. The purified human melanoma t-PA used to generate the standard curve and rabbit anti-t-PA were kindly provided by Dr. D. Collen.

**t-PA functional activity.** Functional fibrinolytic activity was measured on fibrin plates exposed to euglobulin fractions from patient samples as previously described. Euglobulin fractions were prepared by dilution of citrated plasma (1:20) with distilled water, adjustment of pH to 5.6 with acetic acid, and centrifugation. Precipitates were resuspended in a volume equal to the original volume of citrated plasma with buffer containing 0.4% bovine serum albumin. Serial dilutions were spotted in duplicate on fibrin plates prepared conventionally with plasminogen obtained from Kabi Diagnostica. After plates had been incubated at 37°C for 16 hr, zones of lysis were quantified by comparison with those induced by urokinase (International Reference Standard).

**Fibrinogen.** Fibrinogen was assayed by the sodium sulfite precipitation method as previously described in plasma from samples collected in Vacutainer tubes containing citrate supplemented with aprotinin, 200 KIU/ml blood. Samples were assayed fresh or after a maximum of one freeze-thaw cycle.

**Plasminogen and α2-antiplasmin.** Plasminogen was assayed by an amidolytic method as previously described with the synthetic substrate S-2251 (Kabi Diagnostica) and a 10 min preincubation with streptokinase (Sigma) at a concentration of 3333 U/ml. Results were expressed as percentages of normal values by comparison with values from a standard curve generated with pooled, normal human plasma.

α2-Antiplasmin level was measured by the same amidolytic procedure (Kabi Diagnostica, Bulletin S-2251-antiplasmin), with S-2251 as substrate for plasmin and with added plasmin. Reductions of the amidolysis induced by the added plasmin as a result of α2-antiplasmin in the sample was expressed in terms of the percentage of normal α2-antiplasmin activity by comparing results with those obtained with corresponding dilutions of pooled, normal human plasma.

**Clinical laboratory assays.** Prothrombin time, activated partial thromboplastin time, protamine-corrected thrombin time, and fibrinogen degradation products were assayed conventionally by the Barnes Hospital clinical laboratory.

**Results**

**Clinical observations.** Twelve patients were studied: seven men and five women 38 to 69 years of age (mean 56 yr). Six had complete occlusion of the right coronary artery (RCA), five had complete occlusion of the left anterior descending coronary artery (LAD), and one had a complete occlusion of the circumflex coronary artery. None manifested systemic bleeding or bleeding from arteriotomy sites in association with infusion of rt-PA (i.e., within 12 hr of infusion). Coronary thrombolysis was induced with rt-PA in 10 of the 12 patients (83%) (four of four given rt-PA by the intracoronary route and six of eight given the agent intravenously) by an average of 7 hr after the onset of symptoms (range = 4 hr 20 min to 10 hr 30 min) and was documented angiographically (figure 1). In nine patients, the onset of lysis was demonstrated within 15 to 30 min after the onset of the infusion of rt-PA, and lysis was complete within 45 min. In the tenth patient lysis was evident within 90 min after the onset of infusion.

![FIGURE 1](http://circ.ahajournals.org/)

Serial angiograms of the right coronary artery (right anterior oblique projection) from a representative patient given intravenous rt-PA. Initially, the vessel is totally obstructed proximally (A). Forty-five minutes after the onset of infusion of rt-PA patency is restored but a residual high-grade stenosis is evident (arrow in B), which persisted unchanged for 2 weeks. At that time angioplasty was performed with a good result (C).
Clot lysis was not induced by rt-PA in two patients. rt-PA levels in these two patients were not dissimilar to those in the patients in whom lysis was observed. In one of these patients who had an LAD occlusion, 250,000 IU intracoronary streptokinase was given after rt-PA. The clot was refractory to streptokinase as well as to rt-PA. In the other, who died in cardiogenic shock 48 hr after admission, an autopsy was performed. A very severe stenosis was demonstrated proximal to an RCA thrombus and this had probably limited access of rt-PA to the clot. The high incidence of lysis induced by rt-PA is consistent with results we reported previously in a pilot study of coronary thrombolysis induced with human t-PA harvested from Bowes cell tissue culture media, and with results of studies of coronary thrombolysis with streptokinase and urokinase.

**Pharmacologic properties of rt-PA.** Baseline plasma t-PA antigen assayed before infusion of rt-PA averaged 16.5 ± 5.1(SD) ng/ml. A marked increase occurred in all patients treated. Peak levels of t-PA antigen averaged 3330 ± 1201(SD) ng/ml. For the dosage regimens used, selected on the basis of our previous experience in experimental animals and in a pilot clinical study of t-PA from tissue culture media, plasma levels were generally proportional to dose (figure 2). However, variation from individual to individual was substantial. Patients given rt-PA at a dose of 8.3 μg/kg/min exhibited peak plasma levels of 2012 to 4479 ng/ml. Those given 12.5 μg/kg/min exhibited modestly higher peak levels that ranged from 2502 to 4791 ng/ml. Judging from t-PA antigen levels measured throughout the course of the infusion, approximately 90% of peak level was reached in 30 min, with a plateau at peak being reached within 40 min. The observed time to peak was consistent with the independently determined half-life of rt-PA (approximately 3.3 half-lives are required for elevation to 90% peak plateau levels in plasma, with constant infusions of moieties distributed in one compartment and cleared from plasma monoexponentially).

The increase in functional t-PA activity generally paralleled immunoreasayable t-PA (t-PA antigen). Baseline values before infusion of rt-PA averaged 11.5 ± 19.8(SD) U/ml urokinase. In contrast to t-PA antigen values at baseline, the functional activity at this time is attributable not only to t-PA but also to other circulating activators and proactivators of the fibrinolytic system, including plasminogen and prekallikrein. Thus, the percentage increase in functional activity attributable to t-PA is actually larger than the observed percentage increase in fibrinolytic activity. Functional activity after infusion of rt-PA increased to 1069 ± 641 U/ml urokinase for the group as a whole.

Disappearance curves for t-PA antigen and t-PA functional activity were monoeponential (r values for both = .99) for over 15 min and virtually concordant with half-lives of 8.3 min for t-PA antigen and 9.1 min for t-PA functional activity. Functional activity in the two patients in whom lysis was not observed was within the range of values in the 10 patients treated successfully. These results indicate that infusion of rt-PA leads to high circulating levels of t-PA antigen and functional activity generally proportional to the concentrations of t-PA in the infusate. Furthermore, infusions do not lead to detectable circulating levels of immunoreactive but functionally inactive t-PA.

**Effects of rt-PA on the fibrinolytic and hemostatic systems.** Depletion of circulating fibrinogen was absent or modest in most patients. Maximal diminution (to 83 ± 6% of pretreatment values) of circulating fibrinogen was found in samples obtained 5 to 10 min after cessation of the infusions of rt-PA, at a time when t-PA levels were high potentially giving rise to fibrinolysis in vitro (figure 3). Values in subsequent samples were generally higher. Fibrinogen levels 3 hr after the infusion had been terminated averaged 95 ± 5% of

---

**FIGURE 2.** Peak plasma concentrations of t-PA antigen (mean ± SE) reached with infusions of selected doses (table 1) of rt-PA (closed circles). Four patients were given intracoronary rt-PA at a dose of 12.5 μg/kg/min. Eight others were given intravenous rt-PA. The data are considered in aggregate because plasma concentrations of an agent that is not selectively extracted by myocardium are the same after intracoronary compared with intravenous administration in view of the brevity of the circulation time. For purposes of comparison, peak plasma t-PA levels, which have not been reported previously, are also shown for the three patients we have treated recently with t-PA derived from the Bowes melanoma cell line (Δ). Values are consistent with those elicited by rt-PA, suggesting that the pharmacokinetics of the two proteins are similar. The solid line is the regression line (least squares method) derived for the rt-PA values only.

Vol. 71, No. 1, January 1985
Patients, averaging of consumed. Nevertheless, maintained and production with 5).

These baseline values returned shortly after the end of infusion of rt-PA in the sample. In samples drawn subsequently, values returned toward pretreatment levels (285 ± 19[SD] mg/dl). baseline (figure 3). Thus, infusion of t-PA did not lead to substantial fibrinogenolysis in vivo.

Plasminogen depletion was evident, as was the case in a previous study of corresponding doses of t-PA in experimental animals.3 The lowest values were those in samples obtained immediately after completion of the infusion (figure 4). A decrease to 51 ± 6% of pretreatment values was seen followed by subsequent increase to 66 ± 3% of pretreatment values 3 hr after completion of the infusion. Thus, the maximal decrease probably reflected, in part, some activation of plasminogen in vitro by exogenous t-PA in the sample. A reduction of α2-antiplasmin activity paralleling the decrease in plasminogen level was observed as well (figure 5). Values in samples obtained immediately after completion of the infusion of rt-PA averaged 32 ± 7%. Subsequent values increased to 48 ± 4% of baseline 3 hr after completion of the infusion (figure 5). These changes are not indicative of a systemic lytic state since circulating levels of fibrinogen were well maintained and since α2-antiplasmin was only partly consumed. Nevertheless, they are indicative of production of some plasmin in the circulation and underscore the need for titration of dose to avoid loss of clot selectivity with high doses of rt-PA.8

Prothrombin time was slightly increased in all patients, averaging 14.1 sec 90 min after the onset of infusion of rt-PA and 13.7 sec 3 hr after completion of the infusion. Patients given higher doses of rt-PA or an additional infusion in the hour after the initial infusion exhibited values comparable to those in the group as a whole. The effect of infusion of rt-PA on partial thromboplastin time could not be ascertained because of prolongation resulting from concomitant treatment of

FIGURE 3. Concentrations of fibrinogen (mean ± SE) in response to infusion of rt-PA. A modest decline was noted particularly in samples obtained shortly after the end of infusion of rt-PA at a time when rt-PA in the sample may have elicited some unavoidable fibrinogenolysis in vitro despite the presence of aprotinin. In samples drawn subsequently, values returned toward pretreatment levels (285 ± 19[SD] mg/dl).

FIGURE 4. Concentrations of plasminogen (mean ± SE) in response to infusion of rt-PA. A decline, noted particularly in samples obtained shortly after cessation of infusion of rt-PA, may reflect in part some unavoidable conversion in vitro of plasminogen attributable to high concentrations of rt-PA in these plasma samples (see text).

FIGURE 5. Concentrations of α2-antiplasmin (mean ± SE) in response to infusion of rt-PA. The decline noted particularly in samples obtained immediately after cessation of infusion of rt-PA may partly reflect some unavoidable consumption of α2-antiplasmin in vitro attributable to high concentrations of rt-PA in the plasma samples obtained early after cessation of infusion of rt-PA (see text).
all patients with heparin at the time of cardiac catheterization. However, protamine-corrected thrombin time averaged 15.7 sec 90 min after the onset of infusion of rt-PA and 15.1 sec 3 hr after the completion of infusion (normal range ≤18.5 sec). Furthermore, fibrinogen degradation product titers determined 15 to 30 min after infusion of rt-PA averaged 1:16 (range 1:1 to 1:64). Thus, infusion of rt-PA did not impair the integrity of the coagulation system as assessed in vitro.

Discussion

With the use of computer simulations we have previously estimated the ranges of blood levels of t-PA likely to be effective yet safe. However, several factors may give rise to considerable variability in the response of the fibrinolytic system to markedly elevated blood levels of t-PA, including differences in pre-treatment levels of α2-antiplasmin and other inhibitors of plasmin, prevailing levels of inhibitors of t-PA, and concomitant activation of plasminogen in vivo through alternative pathways such as the factor XII-dependent pathway among others. Thus, although reasonable hypothetical limits for the upper bound of blood levels of t-PA likely to be desirable have been identified, direct measurements of changes in blood levels of t-PA and of the response of the fibrinolytic system to infusion of rt-PA were believed to be needed.

The results obtained in this study have several potentially practical implications. They indicate that pharmacologic blood levels of rt-PA within selected ranges can be achieved and sustained in a dose-dependent fashion in patients treated for coronary thrombolysis with parenteral infusions of the agent. The relatively short half-life demonstrable for t-PA antigen is paralleled by the half-life of functional activity. Taken together, the disappearance curves of immunoassayable and functional t-PA activity suggest that administration of rt-PA does not give rise to immunoreactive denatured products on the one hand or to functionally active but immunounreactive fragments on the other. The short half-life of rt-PA and the lack of elaboration of functionally active fragments during the course of infusions of rt-PA probably contributed to the absence of prolonged adverse effects on the hemostatic system, in contrast to the case after infusion of conventional activators of the fibrinolytic system such as streptokinase.

Overall, in the group of patients given rt-PA, depletion of circulating fibrinogen was modest, depletion of circulating plasminogen was incomplete, and prolongation of the prothrombin time and/or protamine-corrected thrombin time was clinically insignificant.

However, as anticipated from consideration of the kinetics of the multiple reactions involved in fibrinolysis and conditions affecting their rates and interactions, conversion of circulating plasminogen to plasmin can be induced by high doses of rt-PA. Thus, it is not surprising that some consumption of α2-antiplasmin and some depletion of circulating plasminogen occurred.

This study evaluated a relatively small number of patients exposed to a limited range of doses of rt-PA. Effects of even higher blood levels of t-PA activity or more prolonged elevations cannot be extrapolated beyond the data available. Since t-PA is cleared by the liver, blood levels in patients with marked impairment of liver function can be anticipated to differ from those in patients without such impairment.

Because of the higher affinity of t-PA for plasminogen bound to fibrin compared with its affinity for free, circulating plasminogen, infusion of rt-PA offers promise for coronary thrombolysis without concomitant induction of a systemic bleeding diathesis. The short half-life of rt-PA delineated in this study should facilitate prompt achievement of a desired steady-state plasma level with a given dose regimen. In addition, it should provide protection by facilitating a prompt decrease of t-PA activity to normal in blood relatively soon after cessation of an infusion of rt-PA. Such reductions are likely to be advantageous in patients who may require emergency surgery or other invasive procedures soon after treatment with rt-PA.

References


Clinical pharmacology in patients with evolving myocardial infarction of tissue-type plasminogen activator produced by recombinant DNA technology.
A J Tiefenbrunn, A K Robinson, P B Kurnik, P A Ludbrook and B E Sobel

Circulation. 1985;71:110-116
doi: 10.1161/01.CIR.71.1.110

Circulation is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 1985 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/71/1/110

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/