Dissolution of Peripheral Arterial Thrombi by Ultrasound

Mehrdad Ariani, MD; Michael C. Fishbein, MD; Jang Seong Chae, MD; Hossein Sadeghi, BS; T. Anthony Don Michael, MD; Stuart B. Dubin, MD, PhD; and Robert J. Siegel, MD

Background. We have previously shown that continuous-wave ultrasound can rapidly dissolve human thrombi in vitro, with 99% of all residual particles measuring less than 10 μm in diameter. To assess the effects of pulsed-wave ultrasound energy on whole blood clots, 1) in vitro studies were performed to assess precisely the rates of clot disruption and to quantify particulate size, and 2) in vivo studies were performed to assess the efficacy and safety of catheter-delivered ultrasound for intra-arterial thrombus dissolution.

Methods and Results. In vitro, we studied 50 samples of human whole blood clots and, using an 89-cm-long wire probe, applied pulse-wave energies from 8 to 23 W. The corresponding peak-to-peak tip displacement range was 63.5–102 μm. We studied arterial thrombosis in vivo in 21 canine superficial femoral arteries. To produce an acute thrombosis, 200 units of thrombin followed by 2 ml of 72-hour-old autologous clot were injected into a 5–7-cm segment of femoral artery and left to coagulate for 2 hours. Ultrasound energy was intermittently applied at a frequency of 20 kHz with a prototype ultrasound wire ensheathed in a catheter and directed to clots by fluoroscopy. In nine cases, angioscopic guidance was used to put the probe into direct contact with the intra-arterial thromboses. In vitro clot dissolution times were inversely related to the ultrasound power output (r=0.95). All in vivo canine thromboses were disrupted in 4 minutes or less. All successful recanalizations were confirmed by angiography and in nine cases by angioscopy as well. Angioscopy demonstrated that probe activation caused rapid clot disruption. Histological studies of the vessels showed no evidence of thermal or cavitation injury, occlusive distal embolization, or perforation.

Conclusions. Our findings in this experimental canine model suggest that ultrasound clot dissolution has the potential to be an effective and safe alternative to current treatment modalities for peripheral arterial thrombosis. (Circulation 1991;84:1680–1688)

Peripheral thromboembolism is the most common cause of sudden arterial occlusion. The patients who are at risk for acute peripheral arterial occlusion are generally elderly with underlying cardiovascular disease. Without treatment, half of the cases will progress to gangrene. As a consequence, there is an estimated 40% mortality of untreated cases.1

The current modes of treatment include direct surgical embolectomy and/or arterial bypass, percutaneous balloon catheter extraction, and local or systemic fibrinolytic therapy.2–6 Unfortunately, each of these modalities has significant potential side effects and complications. Namely, in the older population (aged 75 or older) with inherent cardiac disease, patients are at increased risk for both operative morbidity and bleeding complications secondary to fibrinolytic therapy.7–10 For these reasons, Fogarty balloon embolectomy is currently the method most frequently used for peripheral thromboembolism. However, this technique is also not free of complications, namely, arterial perforation, intimal damage, avulsion of atherosclerotic plaque, arteriovenous fistulas, impaction of an embolus or thrombus into the distal arterial tree, or shifting of the thrombus from one branch to another.11–15

Percutaneous16,17 and intraoperative18 use of ultrasound for peripheral arterial recanalization has been safe and effective in preliminary human studies. Trubestein, Stumpff, and coworkers19–21 pioneered
the concept and demonstrated in vitro and in vivo ultrasound clot dissolution. Ultrasound energy has also been shown by others to be effective in dissolution of thrombi.\textsuperscript{22,23} In a prior in vitro study, we demonstrated that continuous-wave ultrasound energy delivered through a wire probe is able to disrupt blood clots 1–7 days old into microscopic debris, with more than 99\% of all particles smaller than 10 $\mu$m.\textsuperscript{24} Clot dissolution appears to be secondary to the cavitation and mechanical effects of ultrasound, without biochemical activation of the fibrinolytic cascade.\textsuperscript{24} We designed this study to assess thrombus dissolution for 1) in vitro efficacy of pulsed-wave ultrasound, 2) in vivo efficacy, and 3) safety of this application of intravascular ultrasound energy.

**Methods**

**Ultrasound Device**

For disruption of thrombi, we used a previously described prototype intravascular ultrasound device.\textsuperscript{16} The power output at the acoustic horn was 8–25 W used in a pulsed mode with a 50\% duty cycle of 30 msec on and 30 msec off and a frequency of 20 kHz (model PDX-1, Blackstone Ultrasonics, Jamestown, N.Y.). The portable ultrasound generator operates on 115 V AC and weighs only 7 kg. This unit was used in both in vitro and in vivo studies. The ultrasound energy was transmitted by a flexible 0.030-in. titanium 2-mm ball-tipped solid wire probe that is 89 cm long and ensheathed in a 7F–9F guide catheter or 7F–9F angioscope. This probe has a longitudinal amplitude of vibration. At power outputs of 8, 11, 15, 18, 23, and 25 W at the acoustic horn, the corresponding peak-to-peak probe tip displacements as measured by an optical microscope are 63.5, 76, 83, 89, 102, and 111 $\mu$m. The accelerations at the probe tip range from 0 to 82,000g, varying with the power output in watts.\textsuperscript{25} During the application of ultrasonic energy, normal saline solution was infused at a rate of 20 ml/min through the catheter to prevent heating of the probe.

**In Vitro Testing**

**Human thrombi.** Blood was obtained from three healthy volunteers and allowed to clot by collection without anticoagulant. The clots were separated from serum and weighed on a scale (Mettler P1210, Princeton, N.J.). For each study, a clot (1.05±0.2 g) was transferred to a paraffin-plugged length of Tygon tubing (12-mm inner diameter) for subsequent ultrasonic disruption. Each clot (n=50) was exposed to 15-second intervals of ultrasound disruption, and incremental exposure was repeated until the clot appeared by visual inspection to be completely dissolved. Various magnitudes of power output (8–23 W as measured at the acoustic horn and peak-to-peak probe tip displacement of 63.5–102 $\mu$m) were used to determine the optimum power range and time required for clot disruption.

**Particulate size analysis.** Disrupted clots were studied with an electronic particle counter using the resistive-pulse principle (model FN, Coulter Electronics, Hialeah, Fla.). We have described the technique in detail elsewhere.\textsuperscript{24} In the configuration we use, the instrument measures particle volume (V) over a range from 7 fl to approximately $2 \times 10^8$ fl. The sensitivity in this interval is $10^2$ particles per liter. Note that this is expressed in system international (SI) units; for comparison, given in conventional units, this corresponds to only 0.001 particles per microliter. For particles smaller than 7 fl, electronic noise limits utility. Further, the instrument is not linear in response to particles with volumes exceeding $2 \times 10^8$ fl because such particles are comparable in size to the analyzing aperture. For convenience, we present our data under “Results,” assuming that the particles can be considered approximately spherical, and thus can express their diameter (D) as D=(6V/\pi)^{1/3}. By this method we can detect particles from 2.5 $\mu$m to 80 $\mu$m in diameter. The validity of this spherical approximation and the implications of other shapes is presented in “Appendix.”

**In Vivo Testing**

We applied ultrasound to 17 acute thrombotic arterial occlusions in 11 dogs. American Physiological Society guidelines were followed. Adult mongrel dogs, weighing 20–30 kg, were anesthetized with thiopental (20 mg/kg body weight i.v.), intubated with 8F endotracheal tubes, and placed on a ventilator. Anesthesia was maintained by enflurane inhalation through the ventilator. Both femoral arteries and one carotid artery were surgically exposed. In five of 22 superficial femoral arteries, however, stable thrombotic occlusions could not be induced. A 9F introducer sheath was then placed into the carotid artery. Using 7F–9F catheters, we performed contrast angiography to demonstrate the anatomy and presence of obstruction before and after induction of a thrombotic occlusion.

**Angiography protocol.** We used hand injection of a 50\% dilution of Renografin (sodium diatrizoate) and saline solution, injecting 10 ml/series. All studies were recorded on 0.75-in. videotape using images obtained from a fluoroscopy unit (Siemens Gigantos, Cine Generator). Images were recorded with a 525-line video camera (ADAC Laboratories) and stored on a 0.75-in. (1.9-cm) U-matic video tape recorder. The 7-in. (18-cm) image intensifier yields a spatial resolution of 1.6 line pairs/mm. Seven of these studies were also recorded with 35-mm cine film at 30 frames/sec at a dose of 25 microroentgen/frame and a spatial resolution of 3 line pairs/mm.

**Angioscopy protocol.** We used a Baxter Edwards LIS Division (Irvine, Calif.) 7F–9F prototype angioscope with a 2-mm lumen containing the ball-tipped ultrasonic wire probe. The angioscope was interfaced directly to a video camera, and all angioscopic data were recorded on videotape. A 300-W xenon light source was used for illumination. Saline flush was continuously
infused at a rate rapid enough to displace blood and allow continuous visualization of the 3–4-cm area of interest. The appropriate placement of the angioscope was ascertained by means of fluoroscopy.

*Induction of thrombotic occlusions.* A 5–7-cm segment of femoral artery was chosen. The side branches were sutured with 4-0 silk, and the proximal and distal ends temporarily ligated with vascular loops. The segments were subjected to crush injury by forceps, as described by Gold et al. Two hundred units of thrombin followed by 2 ml of 72-hour-old autologous clot was then injected into the crushed segment and allowed to coagulate for 2 hours. The ties were then released and arterial occlusions were documented by angiography using hand injections of contrast material recorded on 0.75-in. video and 35-mm cine film. There were 15 vessels with TIMI grade flow of 0 and two vessels with TIMI grade flow of 1.27

*Ultrasound thrombus dissolution protocol.* The energized ultrasound probe was then applied to the 17 acute thrombi (2–6 hours old). In eight cases, using a 9F introducer placed in the left carotid artery, the ultrasonic wire probe ensheathed in a 7F–9F angiography catheter was guided under fluoroscopy to the site of thrombotic occlusion. Sonication was then performed under angiographic guidance alone. In nine additional cases, the ultrasound wire probe was contained in the angioscope guide wire lumen. The 7F–9F angioscope was inserted through an introducer in the superficial femoral artery proximal to the thrombotic occlusion. Angioscopy was used not only to direct the probe but also to elucidate the mechanism of thrombus disruption. In between or during the application of ultrasound, the patency of the vessels was intermittently assessed by angiography (n=17) and by angioscopy (n=9) until the vessel was patent. Angiographic grading was performed according to the TIMI study guidelines.27 Each angiogram was assessed by the consensus of three observers for the presence of luminal patency, intraluminal thrombus, arterial dissection, distal embolization, or perforation. For analyzing angioscopic results, three observers assessed the luminal patency and presence or absence of angioscopically detected thrombus during and immediately after the procedure as well as on later review of the videotapes.

*Histology.* Postmortem histological examination was then performed on the arterial segments exposed to the ultrasound probe. The arteries were fixed in 10% neutral buffered formalin. The arteries were serially sectioned at 2–3-mm intervals, and all tissue segments were embedded in paraffin. Histological sections were subsequently stained with hematoxylin and eosin. A planimeter (Nikon Microplan II) was used to measure luminal and thrombus cross-sectional areas to calculate percent residual luminal thrombus.

*Control studies.* Four superficial femoral arteries served as controls. Intra-arterial thromboses were induced by the above-described method. The presence of the stable clots was confirmed by angiography and angioscopy. Angiograms were recorded on 1/4-in. videotape and 35 mm cine film. An unactivated 56-cm titanium ultrasound wire probe with a 2-mm ball tip was then passed through the clot under fluoroscopic guidance. Thrombi were angioscopically imaged for 5 minutes, which required continuous saline irrigation. To assess further the effects of the angioscope on the intra-arterial thrombi, the angioscope was pushed through the femoral arterial thrombotic occlusions and advanced to the popliteal artery.

*Statistical analysis.* Linear regression analysis was used to examine the relation between thrombus dissolution time and power output (watts). Student's t test was used to compare percent microscopic residual luminal thrombus in the studies performed solely with angiography versus those in which angioscopy was also used.

*Results*

*In Vitro Testing*

Figure 1 is a graph demonstrating the average disruption time on the vertical axis and the power output at the transducer on the horizontal axis. As expected, the disruption time decreases with increasing power (r=0.95). The minimum power output studied was 8 W power output; it had an average disruption time of 2.4 minutes. With 23 W power output, 1 g of thrombus was dissolved in 15 seconds or less in each case (n=10). In Figure 2 we display the particulate size distribution for a series of clots disrupted by ultrasound at power levels of 8, 11, 15, 18, and 23 W with corresponding peak-to-peak probe tip displacement of 63.5, 76, 83, 89, and 102 μm. In each case, 15-second applications of ultrasound were applied sequentially until the visual impression of complete liquefaction was obtained. At each power level, more than 99% of the resulting particulates are
smaller than 10 μm in diameter. Here we have assumed that the particles can be approximated as spheres, as indicated under "Methods." Expressed in this method, our approach detects particles in the diameter range of 2.5–80 μm. We discuss the validity of this assumption and the implications of other shapes in "Appendix."

In Vivo Testing

Angiography. In all 17 vessels, thrombi were successfully dissolved. Angiographically, the arteries appeared widely patent after being subjected to the ultrasound energy, and there was no angiographic evidence of distal embolization. In three cases, proximal small intraluminal arterial (nonocclusive) filling defects were identified by angiography. However, no distal arterial occlusions were detected and the distal runoff appeared brisk by contrast angiography (TIMI grade 3).27 The duration of ultrasound application was 4 minutes or less, that is, sixteen 15-second applications of ultrasound energy with power outputs ranging from 15 to 25 W. Figure 3 demonstrates an acute (2-hour-old) thrombotic occlusion of the superficial femoral artery. The ultrasound wire probe was then applied directly to the thrombus (Figure 3B) for 2 minutes of intermittent sonication with the energized ultrasound probe (eight 15-second applications). Figure 3C demonstrates the angiographic recanalization of the superficial femoral artery.

Figure 4A shows an angiogram of the superficial femoral artery, which is filled with intraluminal clot and had slow flow with minimal runoff. In Figure 4B, after 3 minutes of ultrasound the large intraluminal filling defects have resolved and arterial distal runoff was brisk.

Angioscopy. Figure 5A is an angioscopic example of a thrombotic arterial occlusion as evidenced by a raised red intraluminal mass. The tip of the ultrasonic probe is also evident. Figures 5B, 5C, and 5D demonstrate the effects of application of ultrasound to the thrombus after 15, 60, and 90 seconds, respectively, with the probe held stationary. Note that after 90 seconds of application of ultrasound, the clot was totally dissolved (Figure 5D). The intimal surface of the artery appears smooth after thrombus disruption, and there is no evidence of intimal tears, dissection, or residual thrombus. Luminal patency was found in all nine cases in which ultrasonic thrombus disruption was performed under angiographic guidance and monitoring. On pullback after passage of the angioscope, however, intimal disruption was present in four cases. This was not found during the initial passage of the ball-tipped ultrasound probe.

Histology. Figure 6 is a histological section from a recanalized, previously thrombosed artery. The intimal layer is intact and no dissection, cavitation (vacuolization), or thermal injury (increased basophilic staining with loss of architectural detail) is detected. Figure 7 demonstrates a histological section after ultrasound that shows residual nonocclusive thrombus within the artery. Histological data were available in 12 of the 17 arteries treated with ultrasound (Table 1). In the cases with the ultrasound probe guided by angiography alone, there were three nonocclusive focal thrombi as well as three vessels with microscopic fibrin deposition. None of these vessels had histological evidence of significant intimal or medial damage. In the arteries

| TABLE 1: Abnormal Pathological Findings After Ultrasound Clot Dissolution Using Angiographic or Combined Angiographic/Angioscopic Guidance |
|-----------------------------|-----------------|------------------|
|                             | Angiography (n=8) | Angioscopy* (n=9) |
| Focal fibrin deposition     | 3               | 1                |
| Microscopic thrombi (nonocclusive) | 3               | 0                |
| Focal intimal disruption    | 0               | 2                |
| Small medial dissection     | 0               | 3                |

*Combined angiographic/angioscopic guidance.
In three other cases, the clots did not appear to be affected by the passage of the unactivated wire probe as assessed by angiographic and angioscopic imaging. These control vessels were also imaged with angioscopy. Irrigation at a rate sufficient to permit angioscopic visualization did not cause clot dissolution. Passage of the angioscope through the clot caused distal embolization in all four cases. Histological examination revealed that residual thrombi were present in three, subintimal or medial hemorrhage in three, and focal intimal or medial dissection in two of the four control arteries.

**Technical limitations.** In two cases, in which the probe was used at maximal power output for 4 minutes, wire fractures occurred. These two probes were used in more than one study, increasing the total usage time to 6 minutes or more. In each case, the wire fracture occurred within the guiding catheter system. The 0.030-in. ultrasound wire has limited flexibility and steerability. In three of nine cases, small residual nonocclusive thrombi were detected by angioscopy, not angiography. These nonocclusive thrombi locally covered the arterial surface, and because of limitations in steerability of the system, they could not be accessed and disrupted.

**Discussion**

In this study we demonstrated that pulsed-wave ultrasonic energy is effective for in vitro and in vivo dissolution of thrombi. In vitro, an 11-W power output (76 μm peak-to-peak tip displacement) is able to disrupt 1 g of thrombus in 90 seconds or less, and with a 23-W power output (102 μm peak-to-peak tip displacement), thrombi were liquefied in 15 seconds or less with approximately 80% of all particles in the range of 2.5–5.0 μm. Of the particulates measured, 99% were smaller than 10 μm in diameter. However, particulates greater than 80 μm cannot be measured by the technique we used. In vivo, all thrombotic occlusions with angiographic or angioscopic guidance were recanalized in 2–4 minutes of intermittent ultrasound, without angiographic or angioscopic evidence of distal embolization. We found that angioscopic visualization could be used to direct intraluminal thrombus dissolution. Angioscopic guidance may be important for two reasons: 1) angiography is insensitive for thrombus detection,28–31 and 2) angioscopy allows guidance of the probe into contact with intraluminal thrombus. In addition, angioscopy allows visualizing the recanalization of thrombotic occlusions with our ball-tipped probe system. We were concerned that recanalization could have been related to a purely mechanical effect of pushing the probe through the thrombus to open the artery. In each case with angioscopic evaluation it was demonstrated that with the probe held stationary but in contact with the thrombus, activation of the probe led to visible and rapid thrombus dissolution. However, there was no statistical difference (p=0.3) between percent microscopic residual thrombus whether dissolution was guided by angioscopy.
(6.5±8%) or angiography (15.5±18%). This may be a result of the small sample size in this study.

Ritchie et al. assessed the efficacy of a rotational thrombectomy device in a canine model of femoral arterial occlusion that is similar to the model we used. Using a rotational thrombectomy device, they studied 14 thrombotic occlusions and had one arterial perforation. Their data demonstrated that evaluation by angiography was insensitive for the presence of residual thrombus after thrombectomy. Six of 14 arteries had normal angiograms after rotational thrombectomy, whereas evaluation by angioscopy demonstrated residual thrombus and intimal flaps in all cases and partially occlusive thrombi in six cases. In this study we identified intimal trauma in five cases by angioscopy. Histologically, there were three vessels with small medial dissections, two cases with small intimal tears, and another three in which distal arterial segments contained microscopic residual mural thrombus. As with our previous in vivo studies of therapeutic intravascular ultrasound, we found no microscopic evidence of thermal or cavitation injury to the arterial wall, significant intimal damage, or perforation. The data from our study suggest a favorable potential for ultrasound thrombus dissolution with less associated intimal trauma and more complete dissolution of thrombi than with rotational thrombectomy as assessed by angiography and angioscopy. However, these two studies are not directly comparable because we do not have angioscopic data on eight of our cases, and when angioscopic visualization was obtained, it was used not only for observation but also to put the ultrasound wire probe in direct contact with the thrombus to induce disruption.

The small intimal flaps discussed earlier have also been reported by other authors. Grundfest et al. and Rees and coworkers in prior studies have also documented the occurrence of intimal tears after angioscopy. In 12 of 17 arteries undergoing ultrasonic clot dissolution in which histological data were available, the source of the intimal damage or focal medial dissection may have been from the method of the crush injury, from the angioscope, or from the method of ultrasonic clot lysis. Intimal tears were not detected in those cases in which angioscopy was not used. Of the four control vessels, subintimal or medial hemorrhage was present in three and focal intimal or medial dissection in two arteries. These control data suggest that the microscopic damage is likely to have been in part a result of the trauma from the method of inducing the thrombotic occlusions.

Current limitations with the ultrasound probe system for peripheral clot lysis include the potential for
wire fracture, probe stiffness, and relative lack of steerable. Since the commencement of this study, improvement in wire probe durability has been demonstrated by in vitro testing (personal communication with R. Pflueger, Baxter Health Care Corp., Edwards Division, Irvine, Calif.). However, improved steerable flexibility will still be required to access more tortuous segments of the arterialvasculature as well as to allow probe contact with thrombi on each aspect of the intra-arterial surface.

While the in vitro work of Williams and coworkers demonstrated the potential for platelet aggregation and a procoagulant effect of ultrasound, prior studies by Trubstein and Stumpf and Rosenzweig in accord with our findings that catheter-delivered ultrasound energy disrupts thrombotic occlusions. Trubstein, Stumpf, and coworkers pioneered the concept of ultrasound thrombolysis. They were able to disrupt femoral arterial thrombi in 19 dogs, using an inflexible 2-mm hollow rigid metal probe. The ultrasonic frequency they used was 26.5 kHz. The thrombi were destroyed and aspirated in 2.5–5 minutes. However, no detailed angiographic or histological data were presented. More recently Rosenzweig and colleagues reported on ultrasonic thrombus disruption in seven canine femoral arteries. They found that the percent angiographic stenosis fell from 93±11% to 18±7%. In their study, however, no data regarding the extent of residual thrombi or the presence of distal emboli were presented. Further, as shown in Figure 4, thrombotic occlusions frequently permit contrast material to pass and result in intraluminal filling defects with slow runoff but without total occlusion. Thus, percent stenosis on angiography is not an optimal method to assess clots or an adequate measure of the degree of vascular occlusion. Our study, in addition to angiographic assessment of distal embolization and histological evaluation of residual thrombus, is the first to demonstrate the potential for angiographic guidance for ultrasonic disruption of thrombotic occlusions.

In summary, the advantages of ultrasound thrombus recanalization that portend a successful outcome are minimal intimal damage, patent distal arterial tree, and the potential for angiographic guidance to optimize clot dissolution. These advantages, in addition to the rapidity of ultrasound thrombus dissolution, could make this modality particularly suitable as an adjunct or alternative for treating peripheral arterial and venous thromboses. If more steerable and flexible ultrasound probes can be developed and if ultrasound clot dissolution proves to be safe and effective in the peripheral vasculature of humans, the same technique could potentially be used in the pulmonary and coronary arterial circulations.

Acknowledgments

The authors gratefully acknowledge the contributions of Eugene De Castro, AAS, in the coinvention and development of the ultrasound catheter prototype; Russell Pflueger in assisting in the coupling of the angioscopy/ultrasound units; Jun Park, MD, for technical assistance; and James S. Forrester, MD, for his continued support and guidance.

Appendix

It should be noted that the characteristic dimension of the particle size distribution in the present...
work and our earlier study\textsuperscript{24} is not strongly dependent on the spherical model we have chosen for simplicity. For example, if we regard the particles as large flat disks (i.e., “flakes”), the diameter of which is 10 times the thickness, then it is straightforward to demonstrate that $D=(40V/\pi)^{1/3}$. For a given volume, then, the diameter deduced in this disk model is $(40/6)^{1/3}$, or about 1.9 times as large as the spherical approximation. Taking the opposite position, if we model the particles as long rods (i.e., “fibers”), whose length $L$ is 10 times their diameter $D$, we obtain $L=10D=(400V/\pi)^{1/3}$. Thus, the length of such particles for a given volume is $(400/6)^{1/3}$, or 4.1 times the diameter given by the spherical model. Since our goal is simply to obtain a reasonable idea of the characteristic dimension of the particles, the spherical model fulfills this need. Further, as particles become more asymmetric (either as long rods or flat disks), they become more flexible as well, and thus perhaps less likely to be of significant clinical concern. Finally, particles larger than about $2\times10^3$ fl in volume are not detected by our approach. Such particles would have a characteristic dimension greater than 80 $\mu$m (spherical model), 150 $\mu$m (disk model), or 330 $\mu$m.
(rod model). In an earlier study, we showed by direct microscopic visualization that particles of this size range produced by ultrasonic disruption are present in numbers on the order of 10^11.

References


KEY WORDS • ultrasound • clot disruption • lysis
Dissolution of peripheral arterial thrombi by ultrasound.
M Ariani, M C Fishbein, J S Chae, H Sadeghi, A D Michael, S B Dubin and R J Siegel

Circulation. 1991;84:1680-1688
doi: 10.1161/01.CIR.84.4.1680
Circulation is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 1991 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/84/4/1680

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/