# Noninvasive, Transthoracic, Low-Frequency Ultrasound Augments Thrombolysis in a Canine Model of Acute Myocardial Infarction

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***Background***—Limitations of coronary thrombolysis include the time to reperfusion, patency rate, and bleeding. We evaluated the use of noninvasive transcutaneous ultrasound to augment coronary thrombolysis.

***Methods and Results***—In 24 dogs, a thrombotic occlusion of the left anterior descending coronary artery was induced and documented by 12-lead ECG and coronary angiography. After ≥60 minutes of occlusion, tissue-type plasminogen activator (t-PA; 1.42 mg/kg) was given intravenously over 90 minutes. A total of 12 of the 24 dogs had concomitant transcutaneous application of low-frequency ultrasound (27 kHz) over the chest. At 90 minutes, the mean TIMI grade flow in the t-PA alone group was 0.92±1.4 compared with 2.42±1.9 in the t-PA plus ultrasound group ($P$=0.006). TIMI 2 to 3 flow was present in 4 of 12 cases receiving t-PA alone compared with 10 of 12 cases receiving t-PA plus ultrasound ($P$=0.003). At 180 minutes, mean TIMI grade flow was 0.75±1.4 in the t-PA alone group versus 2.58±0.9 in the t-PA plus ultrasound group ($P$=0.001). Pathological examination confirmed the angiographic patency rate and did not reveal injury secondary to ultrasound in the skin, soft tissues, heart, or lungs.

***Conclusions***—In vivo, the noninvasive transthoracic application of low-frequency ultrasound (1) greatly augments the efficacy of t-PA–mediated thrombolysis, (2) seems safe, and (3) has substantial potential as a noninvasive adjunct to improve coronary patency without increasing the risk of bleeding. **(*Circulation*. 2000;101:2026-2029.)**

**Key Words:** thrombolysis ■ myocardial infarction ■ ultrasonics ■ fibrinolysis

Coronary thrombolysis reduces mortality and preserves ventricular function, but it results in angiographic TIMI 3 flow in only 40% to 60% of patients. The procedure is further complicated by major bleeding in 5% of patients and by hemorrhagic stroke in 0.8% to 1.8% of patients.[1] Consequently, an enhancement of thrombolysis without promoting bleeding is desirable. We evaluated the augmentation of coronary thrombolysis with intravenous tissue-type plasminogen activator (t-PA) and noninvasive, transthoracic, low-frequency (27 kHz) ultrasound.

## Methods

### Transcutaneous Ultrasound Device

The ultrasound device consisted of a generator/amplifier and a controller, which provided 33 W to a titanium, transcutaneous transducer (Cybersonics Inc). The transducer was coupled to a cooling system that maintained a temperature ≤98°F. The continuous mode, 27-kHz transducer provided 0.9 W/cm$^2$ at its surface.

This study was performed in accordance with the *Guide for the Care and Use of Laboratory Animals* of the US National Institutes of Health and approved by the Institutional Animal Care and Use Committee of Cedars-Sinai Medical Center.

### In Vivo Induction of Canine Thrombotic Coronary Occlusion

Dogs (20 to 30 kg) were anesthetized with thiopental; anesthesia was maintained by enflurane inhalation. Thrombotic coronary occlusions were induced in the midportion of the left anterior descending coronary artery (LAD) by electrical injury, as has been described previously.[2] When the ECG showed ST elevation ≥2 mm in 2 contiguous leads, angiography was performed.

### Coronary Thrombolysis Protocol

After LAD occlusion lasting ≥60 minutes, heparin (1000 U) was given. Dogs were randomized to receive either t-PA alone (n=12) or combined t-PA and transcutaneous 27-kHz ultrasound (n=12). The t-PA infusion (1.42 mg/kg) was given over 90 minutes. After TIMI 2 to 3 flow was obtained, heparin (1000 U) was given subcutaneously.

### Application of Ultrasound

The ultrasound transducer was applied (12 cases) to the left parasternal area. If TIMI 2 to 3 flow occurred, ultrasound was discontinued. In 4 dogs in the t-PA plus ultrasound group and in 3 dogs in the t-PA alone group, 120 minutes after the t-PA infusion was given, ultrasound was applied to the right hemithorax for 30 minutes to assess for ultrasound-induced lung damage.

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**Coronary Angiographic Results**

| | At 20 Minutes | | At 90 Minutes | | At 180 Minutes | |
|---|---|---|---|---|---|---|
| | t-PA Alone | t-PA+USD | t-PA Alone | t-PA+USD | t-PA Alone | t-PA+USD |
| TIMI 3 | 4 | 8 | 3 | 8 | 3 | 9 |
| TIMI 2 | 1 | 2 | 1 | 2 | 0 | 2 |
| TIMI 1 | 0 | 1 | 0 | 1 | 0 | 0 |
| TIMI 0 | 7 | 1 | 8 | 1 | 9 | 1 |
| Mean±SD | 1.08±1.4 | 2.42±0.99* | 0.92±1.4 | 2.42±0.99† | 0.75±1.4 | 2.58±0.99‡ |

USD indicates ultrasound.
*$P$=0.015; †$P$=0.006; ‡$P$=0.001.

## Coronary Angiographic Protocol

Right anterior oblique, anteroposterior, and left anterior oblique angiograms were made at baseline, after occlusion, and every 30 minutes until starting treatment. After beginning t-PA or t-PA plus ultrasound treatment, ECGs and angiograms were done at 20, 40, 60, 90, 120, 150, and 180 minutes for monitoring thrombolysis. Angiograms were analyzed by an independent angiographic core laboratory that was blinded to treatment group (Cardiovascular Core Analysis Laboratory, Stanford University, Stanford, Calif).

## Measurement of Ultrasound Power Output In Vivo

A hydrophone (Bruel and Kjaer, model 8103), amplifier (Nexus, model 2692), and oscilloscope (Digital Hewlett Packard, model 54600B) measured the intrathoracic delivery of ultrasound energy in vivo in 3 additional dogs. The hydrophone was placed on the anterior surface or, alternatively, immediately beneath the posterior surface of the heart. The 27-kHz ultrasound transducer was placed on the anterior chest wall. The average peak intensity was 0.45 W/cm$^2$ on the anterior surface of the heart. The largest peak intensity on the anterior surface was 0.58 W/cm$^2$ and, on the posterior aspect beneath the heart, it was 0.55 W/cm$^2$.

## Pathological Studies

Dogs were euthanized; their hearts were then excised and fixed in formalin for 24 to 72 hours and cut transversely in 1-cm intervals from apex to base. The LAD was sectioned at 2-mm intervals. Distal arterial cross-sections were evaluated for embolization. Representative sections from the proximal, middle, and distal LAD, as well as from the skin, soft tissues, heart, and lung were submitted for microscopic examination.[2]

## Statistical Analysis

Data were expressed as mean±SD. The unpaired Student's $t$ test was used to compare the mean TIMI grade differences between the 2 groups. Fisher's exact, 2-tailed test was used to compare the percentage of TIMI grade flow between the 2 groups. $P \leq 0.05$ was considered statistically significant.

# Results

## Angiographic Results

The Table shows the TIMI grade for both groups after 20 and 90 minutes of t-PA infusion and after 90 minutes of observation. Ninety minutes after treatment in the t-PA alone group (180 minutes), 3 cases had TIMI 3 flow (25%), and 9 cases had TIMI 0 flow with persistent occlusion (75%). The group receiving t-PA plus ultrasound had a greater frequency of reperfusion: 9 dogs had TIMI 3 flow (75%), 2 had TIMI 2 flow (17%), and 1 had TIMI 0 flow (8%; $P$=0.003). The

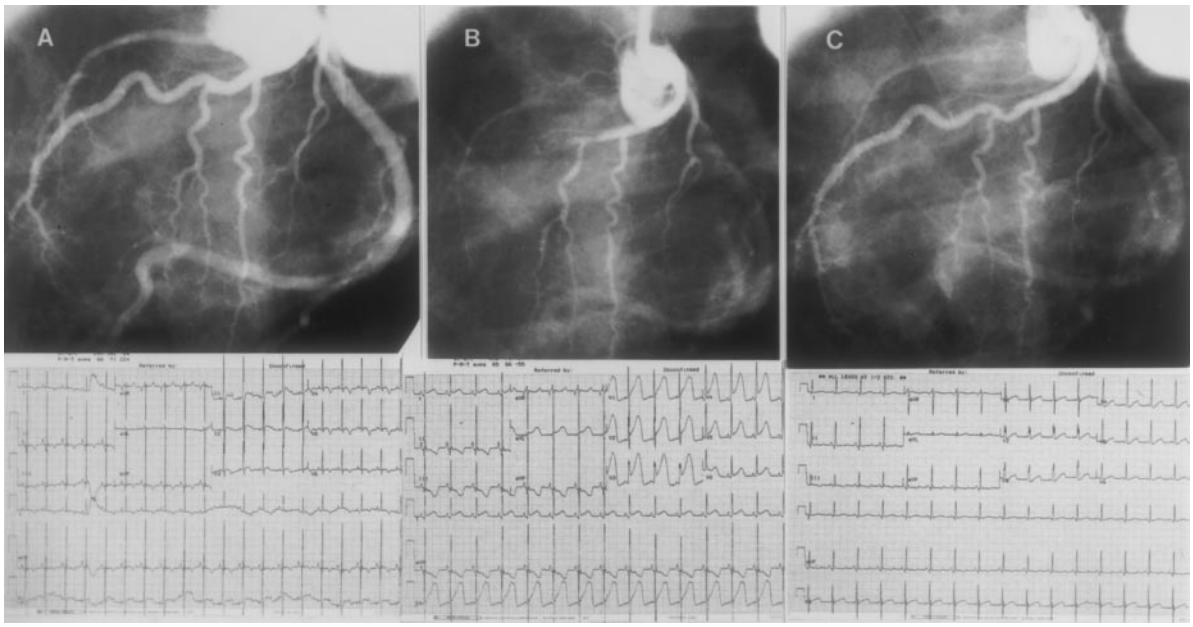


**Figure 1.** LAD thrombotic occlusion treated with combination of t-PA and ultrasound and the corresponding 12-lead ECG. A, Baseline left coronary angiogram and ECG; B, angiogram of thrombotically occluded LAD and acute ECG injury pattern; and C, angiographic patency and resolution of ECG ST segment elevation after 20 minutes of treatment.

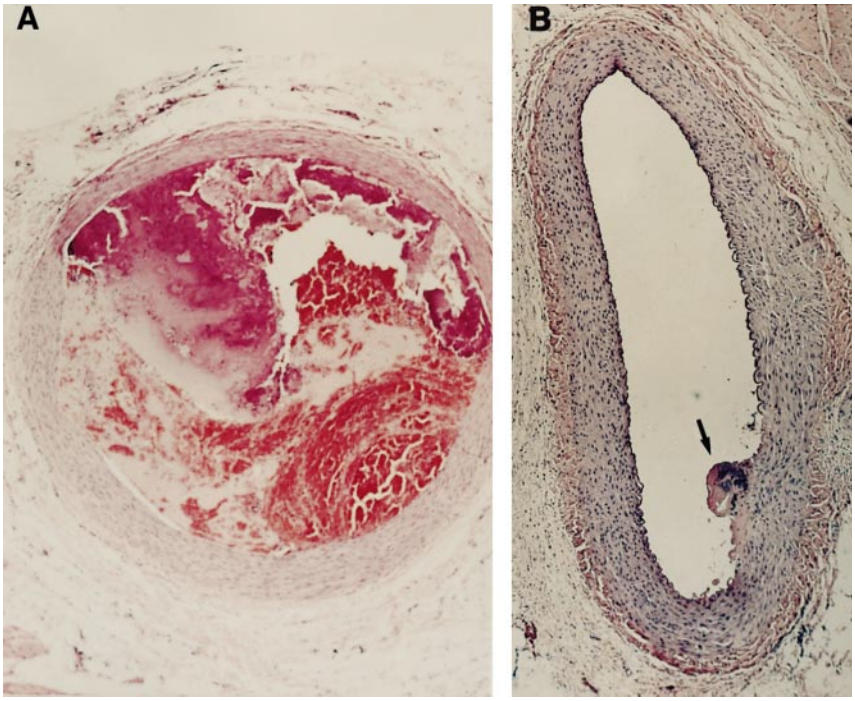


**Figure 2.** A, Example of persistent LAD occlusion after treatment with t-PA alone; B, patent LAD after treatment with t-PA and ultrasound. Arrow indicates site of intimal damage from electric thrombus induction.

mean TIMI flow grade for the t-PA group alone was 0.75, compared with 2.58 for the dogs receiving t-PA plus ultrasound ($P<0.001$). Figure 1 shows successful reperfusion of a LAD thrombotic occlusion treated with the combination of t-PA plus ultrasound, and the corresponding ECGs. During angiographic monitoring, 2 dogs in the t-PA alone group reoccluded (40%), whereas no dogs in the t-PA plus ultrasound group had coronary reocclusion. No dogs with TIMI 0 flow at 90 minutes improved their TIMI flow grade after 180 minutes of observation. No arterial dissection, disruption, or side branch occlusion secondary to ultrasound was detected.

### Pathological Studies

Angiographic findings were confirmed by histopathology (Figure 2). Eleven cases treated with t-PA plus ultrasound had minimal (occupying <25% of lumen) thrombus or no thrombus, and one case had an occlusive thrombus. No ultrasound-mediated injury to the skin, soft tissue, myocardium, coronary arteries, cardiac conduction system, or lungs occurred. In the t-PA alone group, 9 dogs had occlusive thrombi in the LAD, and 1 had distal embolization on angiography, which was confirmed by pathologic examination. Distal embolization was not detected in any dog receiving t-PA and ultrasound.

## Discussion

This is the first in vivo study to demonstrate that noninvasive ultrasound can be used to enhance coronary arterial thrombolysis. Twice as many dogs receiving adjunctive transthoracic ultrasound had TIMI 3 flow at 20 minutes compared with those receiving t-PA alone (8 versus 4 dogs). On the follow-up angiograms taken 90 minutes after completion of the t-PA infusion, a 3-fold greater incidence of TIMI 3 flow existed in the ultrasound group (9 versus 3 dogs; $P=0.039$). The low incidence of reperfusion with t-PA alone is consistent with the findings of Cercek et al,[3] who found in a canine model that the concomitant use of full-dose heparin was necessary for effective clot lysis with t-PA. The low incidence of reocclusion in the ultrasound-treated group may reflect more complete thrombolysis and/or the absence of downstream embolization in this group. Gross and histopathologic studies confirmed the angiographic patency of vessels after treatment in each group.

Data from this and previous studies using similar transcutaneous, low-frequency ultrasound devices[4–6] suggest that this method is safe and without acute adverse effects. The absence of damage is consistent with the use of peak intensity levels $\leq 0.6$ W/cm$^2$. This peak intensity is below the 1 to 2 W/cm$^2$ value described by Suchkova et al[6] as being potentially hazardous.

Other authors have shown that low-frequency ultrasound accelerates enzymatic thrombolysis at intensities similar to those used in our study.[6–9] Low-frequency ultrasound has excellent tissue penetration for effective clot lysis.[4–6] Ultrasound enhancement of t-PA fibrinolysis occurs in the absence of heating and with minimal mechanical effect on the clot.[6–8] This effect, which is not fibrinolytic-specific, occurs with t-PA, streptokinase, and urokinase. The multiple mechanisms for the ultrasound augmentation of fibrinolysis have been primarily elucidated by the laboratory run by Francis.[6–9] One primary effect of ultrasound is an increase in the transport of the fibrinolytic enzyme into the clot.[7–10] Ultrasound increases t-PA uptake and its depth of penetration into clots. Ultrasound exposure enhances fluid permeation through fibrin gels.[10] Electron microscopy has shown that ultrasound exposure reversibly disaggregates fibrin fibers.[9] Such changes are thought to promote an increase in fibrin binding sites for fibrinolytic agents.

Prior studies have shown the feasibility of using ultrasound to facilitate thrombolysis in vitro[6,7,11,12] and in vivo.[4–6,8,11–13] In vivo studies have been primarily performed in thrombosed

iliofemoral arteries and have shown substantial enhancement of chemical thrombolysis, namely, a ≥4-fold increase in reperfusion.[4,8,12] Initial studies using high-power ultrasound outputs were complicated by thermal damage.[8] Subsequent studies, however, have shown an elimination of tissue damage while still achieving reperfusion rates that are greater than those achieved with the thrombolytic agent alone.[4,5,8] In summary, the in vivo studies to date are consistent with the vitro finding that ultrasound augments chemical thrombolysis.[4,6,8,11–13]

### Limitations

In this study, we used a prototype of a therapeutic ultrasound device. In the one case in this study in which there was no facilitation of t-PA–mediated coronary thrombolysis, a subsequent evaluation of the ultrasound unit by the manufacturer revealed that the device was not delivering energy sufficient for clot lysis. Although this finding reflects a current technological limitation, it also suggests that the lack of augmentation of t-PA thrombolysis by ultrasound (1 of 12 cases) was due to mechanical failure. The development of an energy feedback system is requisite before clinical application. Further, chronic survival studies in animals are needed to assess the long-term effects of ultrasound. Because neither aspirin nor other antiplatelet agents were given in this study, future experiments should be done to assess their interaction with ultrasound.

### Conclusions

This study demonstrates that a nonpharmacological, noninvasive approach is feasible to facilitate coronary thrombolysis. We found that transcutaneous, transthoracic, low-frequency ultrasound significantly augments t-PA–mediated coronary thrombolysis in a dog model of acute myocardial infarction. The theoretical advantages of this approach include the following. (1) The efficacy of the thrombolytic drug is enhanced. (2) The enhancement of the thrombolytic effect is localized to the ultrasound treatment area. (3) The addition of ultrasound does not increase the risk of systemic bleeding. (4) No additional adverse interactions seem to exist, such as those that may occur with adjunctive pharmacotherapy for thrombolysis.

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## References

1. White HD, Von de Werf F. Thrombolysis for acute myocardial infarction. *Circulation.* 1998;97:1632–1646.
2. Steffen W, Fishbein MC, Luo H, et al. High intensity, low frequency catheter-delivered ultrasound dissolution of occlusive coronary artery thrombi: an in vitro and in vivo study. *J Am Coll Cardiol*. 1994;24: 1571–1579.
3. Cercek B, Lew A, Hod H, et al. Enhancement of thrombolysis with tissue-type plasminogen activator by pretreatment with heparin. *Circulation*. 1986;74:583–587.
4. Luo H, Birnbaum Y, Fishbein MC, et al. Enhancement of thrombolysis in vivo without skin and soft tissue damage by transcutaneous ultrasound. *Thromb Res*. 1998;89:171–177.
5. Birnbaum Y, Luo H, Nagai T, et al. Recanalization of thrombosed iliofemoral arteries by transcutaneous ultrasound combined with intravenous infusion of microbubbles. *Circulation*. 1998;97:130–134.
6. Suchkova V, Siddiqi FN, Carstensen EL, et al. Enhancement of fibrinolysis with 40-kHz ultrasound. *Circulation*. 1998;98:1030–1035.
7. Francis CW, Önundarson PT, Carstensen EL, et al. Enhancement of fibrinolysis in vitro by ultrasound. *J Clin Invest*. 1992;90:2063–2068.
8. Riggs PN, Francis CW, Bartos SR, et al. Ultrasound enhancement of rabbit femoral artery thrombolysis. *Cardiovasc Surg*. 1997;5:201–207.
9. Braaten J, Goss RA, Francis CW. Ultrasound reversibly disaggregates fibrin fibers. *Thromb Haemost*. 1997;78:1063–1068.
10. Siddiqi FN, Blinc A, Braaten J, et al. Ultrasound increases flow through fibrin gels. *Thromb Haemost*. 1995;73:495–498.
11. Lauer CG, Burge R, Tang DB, et al. Effect of ultrasound on tissue-type plasminogen activator-induced thrombolysis. *Circulation*. 1992;86: 1257–1264.
12. Luo H, Nishioka T, Fishbein MC, et al. Transcutaneous ultrasound augments lysis of arterial thrombi in vivo. *Circulation*. 1996;94:775–778.
13. Luo H, Steffen W, Cercek B, et al. Enhancement of thrombolysis by external ultrasound. *Am Heart J*. 1993;125:1564–1569.