Coronary thrombolysis with recombinant human tissue-type plasminogen activator: a prospective, randomized, placebo-controlled trial*

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ABSTRACT Forty-five patients with acute transmural myocardial infarction and angiographically confirmed complete coronary occlusion were prospectively randomized, two for one, to treatment of acute coronary thrombosis with intravenous recombinant human tissue-type plasminogen activator (rt-PA) or placebo. Each of five additional consecutive patients was treated with a high dose of rt-PA for 2 hr. Twenty-five of 33 patients (75%) receiving 0.5 to 0.75 mg/kg of rt-PA over 30 to 120 min had angiographically proven recanalization within 90 min of initiation of therapy. Only one of 14 patients given placebo had spontaneous recanalization within 45 min ($p < .001$). Thirteen placebo-treated patients were crossed over to the intracoronary rt-PA group. Nine (69%) exhibited subsequent recanalization within 45 min. Levels of circulating fibrinogen decreased after treatment with rt-PA by an average of only 8% of baseline values. None of the patients manifested a depletion of fibrinogen level to below 100 mg/dl. Six patients who were completely unresponsive to rt-PA were subsequently treated with intracoronary streptokinase and none responded. Thus, either intravenous or intracoronary rt-PA induced coronary thrombolysis without eliciting clinically significant fibrinogenolysis in patients with evolving myocardial infarction due to thrombotic coronary occlusion.
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MYOCARDIAL INFARCTION is often associated with thrombotic occlusion of an atherosclerotic coronary artery.[1] Reperfusion induced by intracoronary or intravenous administration of thrombolytic agents may preserve myocardial function[2–5] and reduce mortality.[6] However, conventionally used activators of the fibrinolytic system deplete levels of circulating fibrinogen and may induce a systemic lytic state predisposing to a bleeding diathesis.

Although most clinical experience has been obtained with intracoronary and intravenous streptokinase,[2–6] other thrombolytic agents such as urokinase,[7] acylated streptokinase–plasminogen complex,[8] and tissue-type plasminogen activator (t-PA)[9] have been evaluated with a view toward achieving therapeutic coronary thrombolysis with clot selectivity and hence more safety.[9–13]

Human t-PA exhibits considerable fibrin specificity.[14] It has been shown to induce thrombolysis without systemic fibrinogenolysis in experimental animals with thrombosis[10, 12, 15, 16] and in pilot studies in patients.[9, 17] However, extensive studies of its potential clinical utility have been limited by the lack of widespread availability of the agent from natural sources, despite its presence in cells in tissue culture.[18] This obstacle has been overcome by the recent successful cloning and expression of the human t-PA gene,[19] which has facilitated synthesis of sufficient quantities of t-PA for more widespread clinical investigation of its potential utility for coronary thrombolysis. The present study was performed to elucidate the safety, efficacy, and therapeutically effective dose ranges of

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recombinant t-PA (rt-PA) in patients with evolving myocardial infarction.

Methods

Patients studied. This collaborative study was performed at the Johns Hopkins Medical Institutions, the Massachusetts General Hospital, and the Washington University–Barnes Hospital Medical Center. Patients with pain attributed to myocardial ischemia of less than 6 hr duration and with electrocardiographic criteria of acute transmural myocardial infarction were eligible. Exclusion criteria included a history of bleeding diathesis; major surgery, trauma, or hemorrhage within 3 weeks; significant hepatic, pulmonary, or renal disease; previous transmural myocardial infarction; or cardiogenic shock.

Written informed consent was obtained from all patients after which coronary angiography was performed in each by the Judkins technique. Heparin (75 to 100 IU/kg) was administered intravenously. Patients with complete obstruction of a major vessel that was likely to be supplying the area of infarction were entered into the study.

Overall design. The randomized trial involving the first consecutive 45 patients studied entailed a prospective two for one randomization to treatment with intravenous rt-PA or placebo with the investigator blinded to the treatment for the first 45 min of the study. Angiographic examination was performed at 15 min intervals to assess persistence of thrombotic occlusion or its resolution. After 45 min the code was broken and patients with persistent occlusion who had been given placebo were crossed over to the group receiving intracoronary rt-PA. After a total interval of 90 min, any patient with persistent or recurrent thrombotic occlusion became eligible for administration of intracoronary streptokinase (20,000 U as a bolus injection followed by 4000 U/min) for as long as 60 min.

Initially the intravenous dose of rt-PA was 0.25 or 0.5 mg/kg over 30 min and the intracoronary dose was 0.375 mg/kg over 15 min. Beginning with patient 9, the dose of intravenous rt-PA was modified to 0.5 mg/kg over 60 min and the intracoronary dose of 0.375 mg/kg was infused over 30 rather than 15 min. Starting with patient 31, the intravenous infusion dose of rt-PA was extended to include an additional 0.25 mg/kg over an additional 60 min (total dose 0.75 mg/kg over 120 min). The prospective randomization scheme was maintained for all 45 patients; changes in the protocol during the evolution of the study were only for dose and duration of assigned treatment regimens. An additional five consecutive patients not taking part in the randomization protocol were given 0.5 mg/kg of rt-PA over 60 min followed by 0.25 mg/kg over 60 min. This resulted in a total number of treated patients of 50.

Complete recanalization was defined as rapid antegrade filling of the distal portion of the previously occluded vessel. Partial recanalization was defined as delayed filling of the distal vessel such that visualization was incomplete at a time when branches proximal to the site of obstruction were completely filled with contrast medium. No response was defined as no change in the appearance of the obstruction.

Plasma was sampled before treatment and 90 min after the start of the infusion for determination of levels of fibrinogen and fibrinogen degradation products (FDPs). For assay of fibrinogen and FDPs in rt-PA–treated patients, samples were collected in tubes containing 100 IU/ml blood of aprotinin (Trasylol) to prevent fibrinogen degradation in vitro as a result of activation of the fibrinolytic system within the sample. This procedure was implemented in patients 10 through 50 after results in the first nine patients were found to be distorted by fibrinogenolysis in vitro in samples devoid of aprotinin. Blood samples for assay of coagulation system components were collected in citrate (final concentration 0.01M), cooled to 4° C, centrifuged within 60 min, and stored at −20° C. Fibrinogen level was measured by the method of Clauss[20] or Rampling and Gaffney,[21] and FDPs were assayed by the method of Merskey et al.[22] or with the Thrombo-Wellcotest kit (Wellcome; Beckenham, England).

At the conclusion of the protocol (150 min or less) the subsequent treatment of the patients was left to the discretion of the cardiologists in each center. Patients were all followed for at least 10 days after treatment with clinical and laboratory evaluations to detect signs of treatment-related morbidity or mortality.

Drug formulation. Human rt-PA was produced by expression of the cloned human t-PA gene[19] in mammalian tissue culture. The drug was supplied in vials containing 10 ml of a liquid formulation containing 5 mg rt-PA (active drug) or 10 ml of the formulation vehicle without active drug (placebo). The contents of the vials were administered intravenously or directly via a coronary arterial catheter.

Streptokinase (Streptase) was purchased from Hoechst-Roussel.

Results

Patient characteristics. Relevant characteristics of the patients entered into the randomized study are summarized in table 1. The five additional patients were not dissimilar as indicated in the table.

Coronary thrombolysis. The incidence of coronary thrombolysis elicited by the different treatment regimens is summarized in table 2. Overall, 14 patients were randomly assigned the placebo group; the study was run double blind with the primary angiographic end point at 45 min. In one patient there was complete recanalization at 45 min and in one partial recanalization at 15 min followed by complete reocclusion at 45 min. The 12 patients with persistent occlusion and the one with partial recanalization crossed over to intracoronary rt-PA and were given a total dose of 0.375 mg/

TABLE 1
Characteristics of patients studied

Randomized patients	
Age (yr; mean ± SD)	55.6 ± 8.7
Gender (M/F)	35/10
Locus of coronary occlusion	
Left anterior descending coronary artery	22
Right coronary artery	19
Left circumflex coronary artery	4
Interval from onset of pain to clot randomization (min; mean ± SD)	284 ± 99
Nonrandomized patients	
Age (yr; mean ± SD)	53.6 ± 7.6
Gender (M/F)	3/2
Locus of coronary occlusion	
Left anterior descending coronary artery	1
Right coronary artery	4
Left circumflex coronary artery	0
Interval from onset of pain to initiation of infusion (min; mean ± SD)	268 ± 53

TABLE 2
Efficacy of rt-PA for coronary thrombolysis

Dose (mg/kg)	Route	Duration (min)	No. of patients	Response to rt-PA: Number	Response to rt-PA: Type	Time to complete recanalization (min; mean ± SD)
Placebo	IV	30–45	14	1	R	
				1	PR	
				12	NR	
0.375	IC	15–30	13[A]	9	R	23 ± 8
				2	PR	
				2	NR	
0.25	IV	30	3	1	R	
				2	PR	
0.50	IV	30	3	1	R[B]	
				2	NR	
0.50	IV	60	15	11	R	46 ± 18
				4	NR	
0.75	IV	120	15	13	R	
				2	NR	

R = complete recanalization; PR = partial recanalization; NR = no response (for definition see Methods).

[A]As noted in the text, 13 of the 14 patients receiving placebo were crossed over to intracoronary rt-PA.

[B]This patient had high-grade but incomplete obstruction at the start of the rt-PA infusion.

kg over 15 or 30 min. At the end of a 45 min observation period nine patients had complete recanalization (69%), two had partial recanalization, and two had none. The interval before complete recanalization was 23 ± 8 min (mean ± SD).

Among the 18 patients given 0.5 mg/kg rt-PA intravenously (three given rt-PA over 30 min and 15 given rt-PA over 60 min), 12 had complete recanalization within 90 min (67%) and six had no response. In three of the patients in whom recanalization was complete there was reocclusion within the 30 min observation period after the end of the rt-PA infusion. Among the 15 patients given intravenous rt-PA in a total dose of 0.75 mg/kg over 2 hr, 13 had complete recanalization within 90 min (87%). Two of these patients manifested reocclusion during the subsequent 30 min observation period after discontinuation of rt-PA. Thus, of the 33 patients given 0.5 mg/kg rt-PA over 30 or 60 min, 25 had complete recanalization within 90 min (75%). The time to complete recanalization after the initiation of intravenous administration of rt-PA averaged 46 ± 18 (SD) min. Of those 25 patients with restoration of vascular patency, five (20%) exhibited reocclusion during the 30 min after discontinuation of the infusion. This early reocclusion rate is similar to that reported in studies of lysis induced with intracoronary streptokinase.[23]

Four patients with partial recanalization were given intracoronary streptokinase, which resulted in complete recanalization in each case. However, none of the six patients who were completely unresponsive to rt-PA were responsive to intracoronary streptokinase.

Representative coronary angiograms from a patient with complete recanalization and without reocclusion throughout the interval of the study period are shown in figure 1; those from a patient with recanalization followed by reocclusion during the 30 min observation period after discontinuation of the infusion of rt-PA are shown in figure 2.

Adverse effects. Clinical responses to infusion of rt-PA were generally favorable. No deaths occurred in association with the study. Two of the 50 patients (4%) died with left ventricular failure (on the third and fifth posttreatment days). One exhibited no recanalization. The other exhibited partial recanalization 15 min after the onset of the infusion followed by reocclusion 30 min later. No hemorrhagic complications requiring

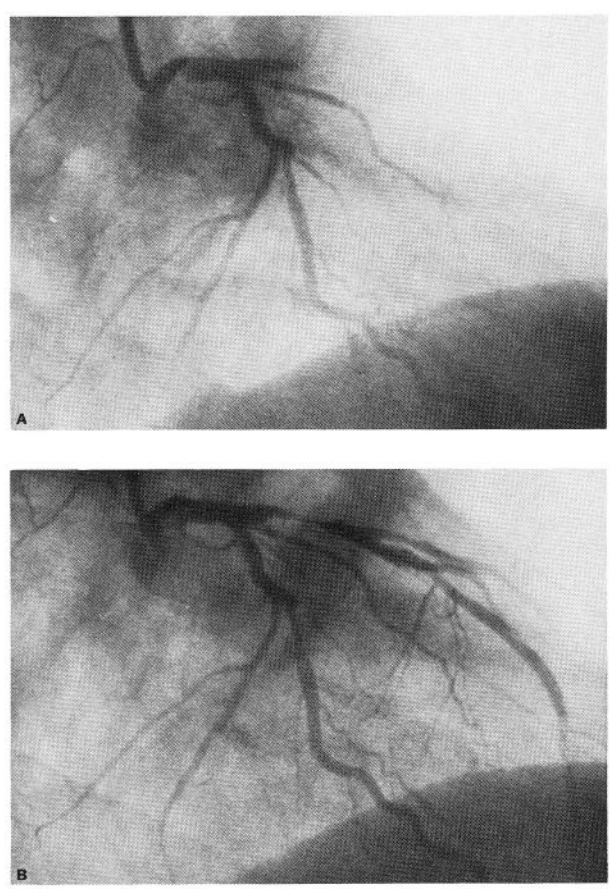

FIGURE 1. Right anterior oblique projections of left coronary arteriogram in a patient with persistent recanalization after infusion of rt-PA. *A*, Pretreatment arteriogram showing complete occlusion of the proximal left anterior descending (LAD) coronary artery. *B*, Arteriogram at 60 min after the start of intravenous rt-PA infusion showing recanalization of the LAD.

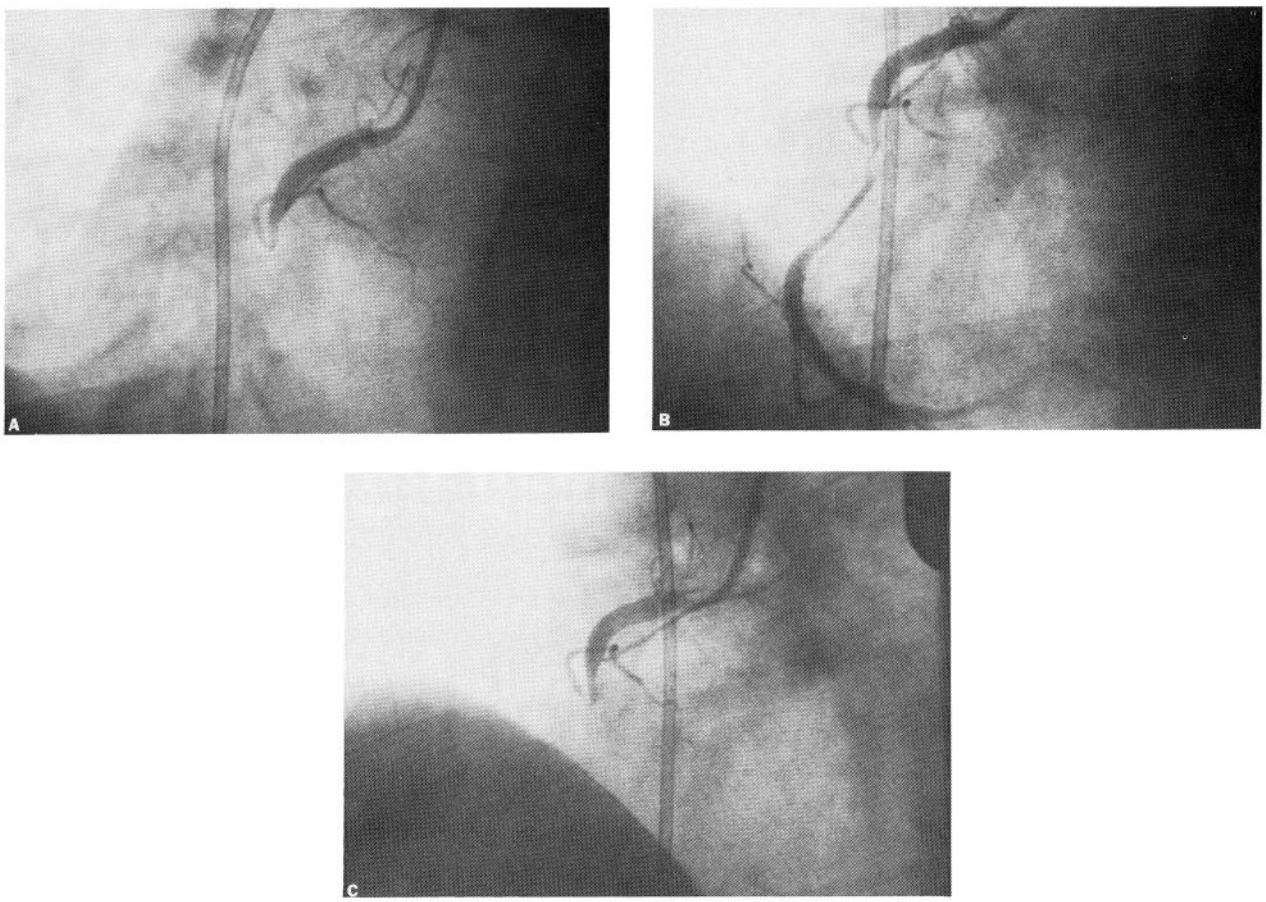

FIGURE 2. Left anterior oblique projections of right coronary arteriogram in a patient with recanalization during infusion of rt-PA followed by acute reocclusion. *A*, Pretreatment arteriogram showing complete occlusion of the right coronary artery (RCA). *B*, Arteriogram at 75 min after the start of the intravenous rt-PA infusion showing recanalization of the RCA. *C*, Arteriogram at 30 min after the conclusion of the rt-PA infusion showing reocclusion of the RCA.

transfusion occurred within the first 24 hr after infusion of rt-PA. One patient had an episode of gross hematuria within hours of rt-PA treatment, and two had gingival bleeding during the infusion. Their fibrinogen values at baseline were 202, 360, and 300 mg/dl. Values subsequently declined modestly (168, 145, and 200 mg/dl at 90 to 150 min and 193 and 130 mg/dl in the two studied at 4 hr). Corresponding FDP titers were 1:4, 1:2, and 1:2 at baseline; 1:16, 1:32, and 1:18 at 90 to 150 min; and 1:1 and 1:16 at 4 hr. Reperfusion arrhythmias that were benign and readily manageable, including accelerated idioventricular rhythm, bradycardia, and ventricular premature depolarization, were seen frequently.

Effects on the coagulation system. Results of assays of fibrinogen and FDPs are summarized in table 3. Blood samples from the first nine patients were not collected with aprotinin. Data from these nine patients plus the one placebo-assigned patient who did not receive rt-PA were excluded from coagulation system analysis. Fibrinogen levels in the samples from the remaining 40 patients decreased after 90 min, but only modestly (to an average of 92% of preinfusion values).

Results of assay of FDPs are summarized also in table 3. Data on FDPs from only two of the three participating centers are reported because results ob-

TABLE 3
Results of coagulation analyses

Fibrinogen[A]			FDPs[B]		
n	Before rt-PA (mg/dl)	After rt-PA (% of before values)	n	Before rt-PA (μg/ml)	After rt-PA (μg/ml)
40[C]	280 ± 70	92 ± 4	23[C,D]	5.7 (4.6–6.8)	23 (17–30)

[A]Mean ± SEM.

[B]Geometric means and ranges from mean − SEM to mean + SEM.

[C]Includes results from all patients except the first nine, whose samples were not collected with aprotinin, and one placebo patient who was not treated with rt-PA.

[D]Results include those from all patients from two centers (see text).

tained from samples at the third center were distorted as a result of incomplete clotting of samples from anticoagulated patients. In the 23 patients from whom adequate samples were available FDPs rose only modestly from a geometric mean of 5.7 μg/ml before to 23 μg/ml 90 min after the start of the infusion of rt-PA. Of interest, pharmacologic levels of heparin may result in false-positive elevations of FDPs because of interference attributable to inadequate clotting of fibrinogen in the assay system in vitro.

Four of the 23 patients exhibited a decrease in fibrinogen level of more than 15%, which was associated with an increase in FDPs of more than fourfold. Among the 17 patients from whom FDP data could not be analyzed, two exhibited a decrease in fibrogen level of more than 15%.

The level of fibrinogen did not fall below 100 mg/dl in any of the patients studied. Thus it remained in a range rarely implicated in clinical bleeding diatheses. Nevertheless, six patients exhibited changes in fibrinogen and FDP levels that were consistent with some degree of systemic activation of the fibrinolytic system. Partial activation in vivo in patients with high plasma concentrations of rt-PA is not unexpected.[24] Assuming a half-life of 6 min,[25] infusion of 0.5 mg/kg of rt-PA over 60 min will result, hypothetically, in a steady-state plasma concentration of approximately 2.5 μg/ml (0.03 μM). The activation of plasminogen by rt-PA in the absence of fibrin follows Michaelis-Menten kinetics with K_m = 65 μM and k_{cat} = 0.1 sec^{-1}.[13] The rate of plasmin formation (v) is represented by $v = k_{cat}/k_m [A] [P]$, where [A] = concentration of rt-PA and [P] = concentration of plasminogen (2 μM). Consequently, it can be calculated that with [A] = 0.03 μM, $v = 10^{-4}$ $\mu M \cdot sec^{-1} = 0.36$ $\mu M \cdot hr^{-1}$.

Thus, conversion of as much as 18% of the circulating plasminogen (baseline concentration of approximately 2 μM) and consumption of 36% of the α_2-antiplasmin (concentration 1 μM) may be anticipated. Due to biological variability in clearance rate and potential interactions of other plasma proteins (e.g., fibrinogen) affecting the kinetics of plasminogen activation, more significant activation of systemic fibrinolysis may occur in some patients subjected to infusion of 0.5 mg/kg of rt-PA over 60 min.

Because of the limited experience to date with t-PA for coronary thrombolysis,[9] it was not possible to establish a particular dose or duration of infusion a priori. Six of the first nine patients treated had no response or only partial recanalization followed by prompt reocclusion after discontinuation of the infusion of rt-PA. Four of these patients responded to intracoronary streptokinase, suggesting that the dose or duration of infusion of rt-PA selected initially was inadequate. Extension of the intravenous infusion to 1 hr resulted in lysis in 80% (24 of 30) of patients treated. It is possible that above a certain threshold of plasma rt-PA concentration, the duration of infusion is a critical determinant of efficacy. Accordingly, doses below 0.5 mg/kg as infusions maintained for 60 to 90 min may provide desirable efficacy with a minimal risk of systemic fibrinogenolysis.

Discussion

Because of its potential for preserving jeopardized, ischemic myocardium, coronary thrombolysis is being investigated vigorously as a potential initial therapeutic step for patients with evolving transmural infarction,[2-6] associated often with occlusive coronary thrombi.[1] Unfortunately, however, conventional activators of the fibrinolytic system such as streptokinase and urokinase elicit fibrinogenolysis in doses required for successful lysis. Their use therefore entails the risk of bleeding. Recently, t-PA harvested from a human melanoma cell line has been shown to induce coronary thrombolysis in animals[10] and in patients[9] under conditions in which fibrinogenolysis can be avoided. Subsequently, t-PA produced by recombinant DNA technology (rt-PA) was shown to be effective in experimental animals with biological effects similar to those of t-PA harvested from the melanoma cell line.[11, 12]

This study evaluated rt-PA in patients for the first time. It was not possible, a priori, to be certain that rt-PA would exhibit thrombolytic properties and relative clot-selectivity[24] comparable to that seen in an earlier study of seven patients with t-PA harvested from melanoma cells.[9] The present placebo-controlled trial of rt-PA given to a substantially larger number of subjects confirmed the hypothesis that the recombinant DNA product exhibits pharmacologic properties required for relatively clot-selective coronary thrombolysis in patients. Thus, it extends the previous observations obtained with t-PA derived from melanoma cells to recombinant DNA t-PA that can be produced in large quantities. In view of the thrombolytic properties of the rt-PA delineated in this initial clinical study of its use, rt-PA appears likely to provide a promising approach to coronary thrombolysis.

Because the present study was the first in which rt-PA was used in patients, it was in part a dose-finding study. The results obtained indicate that either intravenous or intracoronary infusion of rt-PA in doses of 0.5 to 0.75 mg/kg elicits coronary thrombolysis generally within 90 min without compromising hemostasis.

Thus, rt-PA administered by intravenous or intracoronary infusion induced coronary thrombolysis without systemic fibrinogenolysis in a majority of treated patients. Early reocclusion occurred in approximately 20% of successfully treated patients but only after discontinuation of the infusion. The results obtained in this initial clinical, placebo-controlled trial of rt-PA suggest that intravenous administration of this agent offers considerable promise for coronary thrombolysis.

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References

1. DeWood MA, Spores J, Notske R, Mouser LT, Burroughs R, Golden MS, Lang HT: Prevalence of total coronary occlusion during the early hours of transmural myocardial infarction. N Engl J Med **303:** 897, 1980
2. Rentrop P, Blanke H, Karsch KR, Kaiser H, Kosterling H, Leitz K: Selective intracoronary thrombolysis in acute myocardial infarction and unstable angina pectoris. Circulation **63:** 307, 1981
3. Schroder R, Biamino G, von Leitner ER, Linderer T, Bruggeman T, Heitz J, Vohringer HF, Wegscheider K: Intravenous short-term infusion of streptokinase in acute myocardial infarction. Circulation **67:** 536, 1983
4. Anderson JL, Marshall HW, Bray BE, Lutz JR, Frederick PR, Yanowitz FG, Dats FL, Klausner SC, Hagan AD: A randomized trial of intracoronary streptokinase in the treatment of acute myocardial infarction. N Engl J Med **308:** 1312, 1983
5. Khaja F, Walton JA, Brymer JF, Lo E, Osterberger L, O'Neil WW, Colfer HT, Weiss R, Lee T, Kurien T, Goldberg AD, Pitt B, Goldstein S: Intracoronary fibrinolytic therapy in acute myocardial infarction. Report of a prospective randomized trial. N Engl J Med **308:** 1305, 1983
6. Kennedy JW, Ritchie JL, David KB, Fritz JK: Western Washington randomization trial of intracoronary streptokinase in acute myocardial infarction. N Engl J Med **309:** 1477, 1983
7. Tennant SN, Dixon J, Venable TC, Page HL Jr, Roach A, Kaiser AB, Fredericksen R, Tacoque L, Kaplan P, Babu NS, Anderson EE, Wooten E, Jennings HS III, Breinig J, Campbell WB: Intracoronary thrombolysis in patients with acute myocardial infarction: comparison of the efficacy of urokinase with streptokinase. Circulation **69:** 756, 1984
8. Walker ID, Davidson JF, Rae AP, Hutton I, Lawrie TDV: Acylated streptokinase–plasminogen complex in patients with acute myocardial infarction. Thromb Haemost **51:** 204, 1984
9. Van de Werf F, Ludbrook PA, Bergmann SR, Tiefenbrunn AJ, Fox KAA, deGeest H, Verstraete M, Collen D, Sobel BE: Coronary thrombolysis with tissue-type plasminogen activator in patients with evolving myocardial infarction. N Engl J Med **310:** 609, 1984
10. Bergmann SR, Fox KAA, Ter-Pogossian MM, Sobel BE, Collen D: Clot-selective coronary thrombolysis with tissue-type plasminogen activator. Science **220:** 1181, 1983
11. Van de Werf F, Bergmann SR, Fox KAA, deGeest H, Hoyng CF, Sobel BE, Collen D: Coronary thrombolysis with intravenously administered human tissue-type plasminogen activator produced by recombinant DNA technology. Circulation **69:** 605, 1984
12. Gold HK, Fallon JT, Yasuda T, Leinbach RC, Khaw BA, Newell JB, Guerrero JL, Vislovsky FM, Hoyng CF, Grossbard E, Collen D: Coronary thrombolysis with recombinant human tissue-type plasminogen activator. Circulation **70:** 700, 1984
13. Hoylaerts M, Rijken DC, Lijnen HR, Collen D: Kinetics of the activation of plasminogen by human tissue plasminogen activator. Role of fibrin. J Biol Chem **257:** 2912, 1982
14. Matsuo O, Rijken DC, Collen D: Thrombolysis by human tissue plasminogen activator and urokinase in rabbits with experimental pulmonary embolus. Nature **291:** 590, 1981
15. Korninger C, Matsuo O, Suy R, Stassen JM, Collen D: Thrombolysis with human extrinsic (tissue-type) plasminogen activator in dogs with femoral vein thrombosis. J Clin Invest **69:** 573, 1982
16. Collen D, Stassen JM, Verstraete M: Thrombolysis with human extrinsic (tissue-type) plasminogen activator in rabbits with experimental jugular vein thrombosis. Effect of molecular form and dose of activator, age of the thrombus, and route of administration. J Clin Invest **71:** 368, 1983
17. Weimar W, Stibbe J, Ven Seyen AJ, Billiau A, De Somer P, Collen D: Specific lysis of an iliofemoral thrombus by administration of extrinsic (tissuse-type) plasminogen activator. Lancet **2:** 1018, 1981
18. Collen D, Rijken DC, Van Damme J, Billiau A: Purification of human tissue-type plasminogen activator in centrigram quantities from human melanoma cell culture fluid and its conditioning for use in vivo. Thromb Haemost **48:** 294, 1982
19. Pennica D, Holmes WE, Kohr WJ, Harkins RN, Vehar GA, Ward CA, Bennett WF, Yelverton E, Seeburg PH, Heyneker HL, Goeddel DV, Collen D: Cloning and expression of human tissue-type plasminogen activator cDNA in *E. coli*. Nature **301:** 214, 1983
20. Clauss VA: Gerinnungsphysiologische Schnellmethode zur Bestimmung des Fibrinogens. Acta Haematol **17:** 237, 1957
21. Rampling MW, Gaffney PJ: The sulphite precipitation method for fibrinogen measurement; its use on small samples in the presence of fibrinogen degradation products. Clin Chim Acta **67:** 43, 1976
22. Merskey C, Lalezari P, Johnson AJ: A rapid simple sensitive method for measuring fibrinolytic split products in human serum. Proc Soc Exp Biol Med **131:** 871, 1969
23. Gold HK, Leinbach RC, Palacios IF, Yasuda T, Block PC, Buckley MJ, Akins CW, Daggett WM, Austen WG: Coronary reocclusion after selective administration of streptokinase. Circulation **68** (suppl I): I-50, 1983
24. Sobel BE, Gross RW, Robison AK: Thrombolysis, clot selectivity, and kinetics. Circulation **70:** 160, 1984
25. Korninger C, Stassen JM, Collen D: Turnover of human extrinsic (tissue-type) plasminogen activator in rabbits. Thromb Haemost **464:** 658, 1981