
Labeling of Human Clots In Vitro with an Active-Site Mutant of t-PA

Edward T.A. Fry, David L. Mack, Juan C. Monge, Joseph J. Billadello,* and Burton E. Sobel

Cardiovascular Division, Washington University School of Medicine, St. Louis, Missouri

Prompt detection of acute thrombosis and its response to treatment with thrombolytic agents generally require angiography. Scintigraphic approaches with labeled antibodies to or components of the coagulation and fibrinolytic systems have been disappointing because of prolonged circulating half-lives of tracers and relatively slow or limited binding to thrombi. Accordingly, we developed and characterized a thrombolytically inactive, active-site mutant (Ser-478→Thr) of tissue-type plasminogen activator (t-PA) designed to detect thrombi in vivo. Binding of iodine-125 (¹²⁵I) labeled Ser→Thr t-PA to thrombi in vitro was time- and concentration-dependent, and specific judging from inhibition by pre-incubation with anti-t-PA IgG. Clearance of ¹²⁵I-labeled mutant t-PA in rabbits was rapid and biexponential (α $t_{1/2} = 1.9 \pm 0.4$ min, β $t_{1/2} = 39.8 \pm 11.2$ min). Thus, the amidolytically inactive mutant of t-PA designed binds rapidly and specifically to human thrombi in vitro and is cleared rapidly from the circulation in vivo—properties rendering it attractive as a potentially useful clot imaging agent.

J Nucl Med 1990; 30:187–191

Coronary thrombosis generally precipitates life-threatening phenomena including acute myocardial infarction (1). Fortunately, treatment with thrombolytic agents such as tissue-type plasminogen activator (t-PA) early in the course of acute myocardial infarction can reduce mortality and improve left ventricular function (2). However, prompt detection of intracoronary thrombi needed to provide definitive indications for treatment and prompt, objective detection of thrombolysis presently require coronary angiography. This procedure is invasive, labor- and personnel-intensive, associated with some risk, of considerable expense, and not universally available on demand. Despite their appeal, available noninvasive criteria of recanalization are generally nonspecific and often inadequate for accurate delineation of vessel patency judging from results of comparative studies with angiography (3).

Received July 13, 1989; revision accepted Sept. 5, 1989.
For reprints contact: Edward T.A. Fry, MD, Cardiovascular Division, Box 8086, Washington University School of Medicine, 660 S. Euclid Ave., St. Louis, MO 63110.

* Recipient of a Syntex Scholars Award.

Previous approaches to noninvasive detection of acute arterial thrombi have included scintigraphy with radiolabeled platelets (4), fibrinogen (5,6), anti-fibrin antibodies (7–9), and antibodies to specific membrane proteins of activated platelets (10,11,12). However, because of the relatively long half-lives and persistence of tracer in the circulation, low ratios of tissue (thrombus) to plasma concentrations of labeled material, and modest or markedly limited incorporation of label into thrombi in the absence of ongoing thrombosis, clinical applications for detection of coronary arterial thrombi have been somewhat disappointing.

Imaging of thrombi in experimental animals has been reported with indium-111- (¹¹¹In) labeled t-PA (13) and technetium-99m- (^{99m}Tc) labeled plasmin (14). However, administration of labeled protein with significant amidolytic and fibrinolytic activity (13) may predispose to bleeding or limit the sensitivity of scintigraphic detection of clots because of rapid lysis of those portions of the thrombus that become labeled and hence preclude visualization.

Recently, we utilized site-directed mutagenesis to substitute threonine for the active site serine (Ser 478) of t-PA, thereby, synthesizing a mutant (Ser 478→Thr) that retains only 0.35% of plasminogen activating activity compared with activity of wild-type t-PA (15). Because of the high affinity of t-PA for fibrin (16) and its rapid clearance from the circulation (17,18) we hypothesized that an appropriately labeled fibrinolytically inactive mutant that bound with high avidity to thrombi might be useful for their detection. The present study was designed to characterize the binding of the Ser-478→Thr mutant of t-PA to clots formed from human whole blood in vitro and to determine whether the labeled mutant exhibited properties in vitro and in vivo likely to render it useful as a clot-detecting tracer.

METHODS

Construction and Expression of Ser 478→Thr t-PA

Our construction and initial characterization of the active site mutant of t-PA has been described in detail recently (15). For use in the present study, the mutant protein was isolated and purified from media of transfected Chinese hamster ovary cells (Invitron Corporation, Redwood, CA).

Determination of Concentrations of t-PA Antigen

Wild-type t-PA (50 mg/ml, in 0.2 M arginine-phosphate buffer, pH 7.2) (Genentech Corp., South San Francisco, CA) was used. Concentrations of wild-type and mutant t-PA were determined by ELISA (American Diagnostica, Inc., Greenwich, CT) with specific, affinity-purified goat anti-human t-PA IgG as the capture antibody (19). Recognition by anti-t-PA IgG of mutant t-PA was confirmed on the basis of concordance of determinations of mass by ELISA and by a modification of the Lowry protein assay. SDS-PAGE of wild-type and mutant t-PA under nonreducing and reducing revealed that wild-type t-PA was predominantly single-chain while mutant t-PA was predominantly two-chain.

Determination of Serine Protease (Amidolytic) Activity

Proteolytic activity of t-PA was measured spectrophotometrically with a synthetic tripeptide chromogenic substrate, S-2288 (Kabi Vitrum, Stockholm, Sweden) specific for serine protease activity. Wild-type and mutant t-PA samples were diluted to selected final concentrations (0–4000 ng/ml) in 0.16 M NaCl, 0.1 M Tris-HCl, pH 8.4 and 0.01% Triton X-100 (assay buffer). Two hundred microliters of assay buffer and 200 μ l of t-PA solutions were incubated with 200 μ l of 5.0 mM S-2288 in a spectrophotometer cuvette at 37°. Absorbance at 405 nm (A_{405}) at 37° over 4 min was measured in a spectrophotometer with kinetic analysis capabilities (Gilson Corp., Oberlin, OH). Amidolytic activity of t-PA was expressed in units based on the change in A_{405} per minute. In control studies, wild-type t-PA was treated with 2 μ M D-Phe-Pro-Arg-chloromethyl ketone (PPACK, Calbiochem, San Diego, CA) to irreversibly inhibit its active site (20) prior to determination of amidolytic activity.

Radio-Labeling of t-PA

Wild-type, mutant, and PPACK-t-PA were labeled with iodine-125 (125 I) with a procedure incorporating a modification of the conventional chloramine-T method (21). Tissue-type plasminogen activator (~50 μ g protein) in phosphate buffered saline containing 0.01% Tween-80 (Sigma, St. Louis, MO, PBS-Tween) was labeled with 0.5 mCi Na 125 I (Amersham, Arlington Heights, IL) by exposure to 1% chloramine-T (Sigma) for 90 sec. The reaction was quenched with 2% sodium metabisulfite (Sigma) for >120 sec. Labeled t-PA was separated from unbound 125 I by gel filtration with Sephadex G-10 (Pharmacia, Piscataway, NJ) that had been equilibrated with PBS-Tween. Wild-type, mutant, and PPACK- 125 I-t-PA had specific radioactivities of $\sim 1.0 \times 10^7$ cpm/ μ g. Greater than 95% of the radioactivity recovered from the column was precipitable with 10% (v/v) trichloroacetic acid (TCA). Fibrinolytic activity of wild-type 125 I-t-PA measured by fibrin plate assay (22) was identical to that of unlabeled material (0.4–0.5 IU/ng). Structural integrity of wild-type and mutant 125 I-t-PA was verified by SDS-PAGE under reducing and nonreducing conditions.

Binding of t-PA to Clots

Clots were formed from whole human blood in vitro as previously described (23). Venous blood was collected without anticoagulants from normal volunteers and immediately transferred to Chandler tubes (27 cm length of Tygon tubing,

o.d. = 3/16 inch, i.d. = 1/8 inch, 1 ml blood/tube). The ends of each tube were brought together to form a loop, secured with a collar of tubing (o.d. = 5/16 inch, i.d. = 3/16 inch), and rotated on a tube rotator (Scientific Equipment Co., Baltimore, MD) for 60 min at 37° at a 60° angle. Clotting was complete within 1 hr. Clots were ~2 mm in diameter, 4–8 mm in length, 5–10 mg (dry weight), and similar morphologically to arterial thrombi formed in vivo, containing a platelet head and fibrin tail. After 60 min, the tubes were opened, and 125 I-labeled wild-type, mutant, or PPACK t-PA in PBS-tween was introduced in a volume of 25 μ l at selected final concentrations (0–500 ng/ml). Tubes were closed, rotated for 60 min, and then reopened. Their contents were poured onto pre-weighed polyethylene mesh filters (Spectrum Medical Ind., Inc., Los Angeles, CA) and washed thoroughly with 0.9% NaCl containing 0.01% Tween-80. Radioactivity of washed clots was measured with a gamma-counter (LBK-Wallace, Finland). The filter and clots were dried to a constant weight, and the mass of each clot was determined as the difference between the weight of the filter and the clot combined minus the weight of the filter alone. The amount of t-PA bound per milligram of clot was calculated by dividing the radioactivity of each clot by its dry weight and then by the specific activity (cpm/mg) of 125 I-t-PA. The time course of binding of 125 I-t-PA to clots was determined by incubating clots with 125 I-labeled wild-type or mutant t-PA at a final concentration of 62.5 ng/ml for selected intervals.

Specificity of binding of 125 I-mutant t-PA to clots was demonstrated by inhibition of binding of t-PA to clots in the presence of anti-t-PA antibodies. Iodine-125-labeled mutant t-PA (final concentration = 250 ng/ml) was pre-incubated with selected concentrations (0–100 μ g/ml) of affinity-purified goat anti-human t-PA IgG (American Diagnostica, Inc., Greenwich, CT) in PBS-Tween at 37° for 60 min. Binding of 125 I-mutant t-PA treated with anti-t-PA IgG to clots was measured in the same fashion as that used to measure binding without antibody present.

Pharmacokinetics of Mutant t-PA In Vivo

The half-life of mutant t-PA in the circulation in rabbits was determined by measuring clearance of 125 I-labeled mutant t-PA administered by bolus intravenous (i.v.) injection (18). New Zealand White rabbits (2.0–2.5 kg) were anesthetized with xylazine (18 mg/kg i.m.) and ketamine (60 mg/kg i.m.). Hemodynamics were stable throughout all experiments. Labeled mutant t-PA was administered as an i.v. bolus injection via a femoral venous catheter. Samples of whole blood were collected from a femoral arterial catheter into Vacutainer tubes containing sodium citrate (13 mM) with a two-syringe technique at zero time and at 1, 2, 4, 6, 8, 10, 15, 30, 45, and 60 min after injection. All samples were placed immediately on ice. Catheters were flushed with 2 ml of sterile saline after injection of t-PA and after each sample had been drawn to maintain blood volume near normal.

Samples were centrifuged at 1000 g at 4° for 10 min. Duplicate aliquots (500 μ l) of separated citrated plasma were treated with 1 ml of 10% TCA and kept on ice for 10 min. The samples were centrifuged at 1000 g for 10 min. Supernatant fractions were removed carefully and precipitates washed twice with PBS-Tween. Radioactivity in TCA precipitates reflecting the concentration of 125 I-mutant t-PA in plasma was measured with a gamma counter. Clearance curves were re-

solved into individual components from semilogarithmic plots (log[cpm] as a function of time) to obtain fast-phase (alpha) and slow-phase (beta) rate constants of elimination (k_α and k_β) with the use of a multiexponential curve-fitting computer program. Half-lives were calculated from rate constants for each phase: α -phase- $t_{1/2} = (\ln 2)/k_\alpha$, β -phase- $t_{1/2} = (\ln 2)/k_\beta$.

RESULTS

As anticipated, the amidolytic activity of the mutant t-PA was virtually zero. Activity of Ser \rightarrow Thr-t-PA was identical to that of wild-type t-PA treated with PPACK in concentrations that specifically and irreversibly inhibited the active site serine in the protease domain of t-PA (Fig. 1).

When clots formed in whole blood were exposed to ^{125}I -mutant t-PA in concentrations readily achieved in vivo with modest amounts of administered protein (17), specific binding occurred in a time-dependent manner (Fig. 2). Binding of mutant t-PA to clots occurred rapidly with maximal binding observed within 30 min. Increasing the concentration of wild-type or mutant t-PA increased the extent of binding of labeled t-PA to human clots (Fig. 3).

Binding of the thrombolytically inactive mutant t-PA to clots indicated that proteolytic activity of the molecule was not necessary for specific binding of the molecule to clots. As predicted from its lack of amidolytic and plasminogen activating activity, mutant t-PA did not lyse clots as measured by their persistent mass in the presence of mutant t-PA at concentrations up to 500 ng/ml in contrast to the case with wild-type t-PA.

To define the specificity of binding of mutant t-PA to human clots, mutant t-PA was treated with anti-t-PA IgG. As shown in Figure 4, pretreatment with increasing concentrations of anti-t-PA antibody inhibited

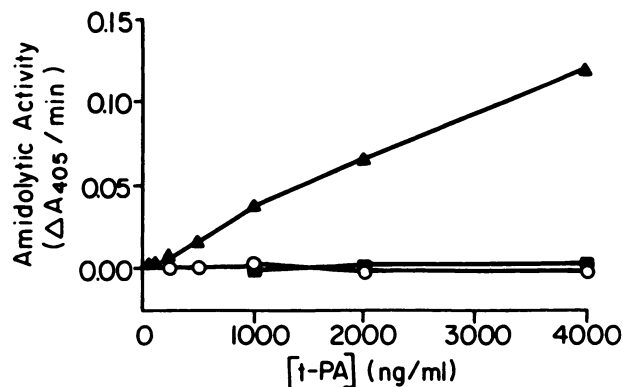


FIGURE 1

Amidolytic activity of t-PA was measured as the change in A_{405} per minute following incubation of selected concentrations of wild-type t-PA (closed triangles), Ser \rightarrow Thr-t-PA (closed squares), and wild-type PPACK-t-PA (open circles) with the chromogenic substrate S-2288 (5 mM) specific for proteolytic activity of t-PA. Points represent means of results of experiments performed in duplicate.

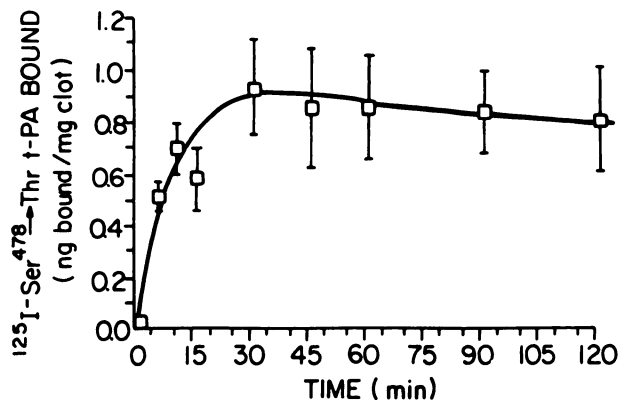


FIGURE 2

Binding of ^{125}I -Ser \rightarrow Thr-t-PA (125 ng/ml) to thrombi formed from human blood in Chandler tubes was measured at selected intervals at 37°C (mean \pm s.d., $n = 6$, for each time point).

ited binding of the labeled mutant t-PA to clots in a concentration-dependent manner.

Rapid clearance and consequent reduction of the blood pool-to-tissue ratio of label of an agent designed to label thrombi in vivo is needed to minimize the interval between administration of the label and acquisition of useful images. Therefore, the half-life in the circulation of rabbits of mutant t-PA was determined by measuring the rates of clearance of radiolabeled mutant t-PA. Clearance was rapid and biexponential with α -phase- $t_{1/2} = 1.8 \pm 0.3$ min and β -phase- $t_{1/2} = 38.1 \pm 8.6$ min ($n = 3$) (Fig. 5). Clearance of mutant t-PA was virtually identical to that of wild-type t-PA.

DISCUSSION

Acute thrombosis precipitates a number of potentially life-threatening processes, including acute myo-

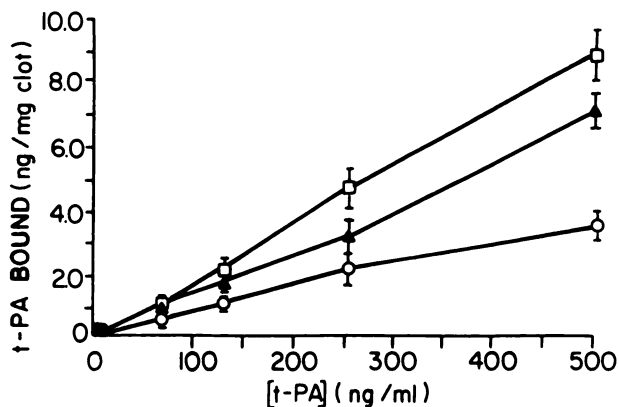


FIGURE 3

Binding of ^{125}I -labeled wild-type t-PA (open circles), Ser \rightarrow Thr-t-PA (closed triangles), and PPACK-t-PA (open squares) at selected concentrations to thrombi formed from human blood was measured after 1 hr at 37°C (mean \pm s.d., $n = 6$, for each t-PA at each concentration).

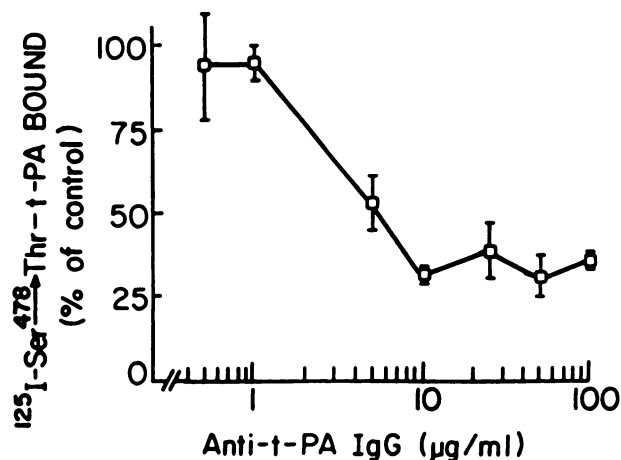


FIGURE 4
Specificity of binding of ^{125}I -Ser \rightarrow Thr-t-PA to human thrombi was demonstrated by inhibition of binding by pre-incubation of ^{125}I -Ser \rightarrow Thr-t-PA with selected concentrations of affinity purified anti-t-PA IgG (mean \pm s.d., $n = 3$, for each concentration).

cardial infarction, unstable angina, pulmonary embolism, and stroke. Timely intervention with thrombolytic agents, anticoagulation, or both can reduce morbidity and mortality otherwise attributable to some of these syndromes. Early detection and delineation of acute thrombosis is needed for prompt identification of patients most likely to benefit from immediate treatment with potentially hazardous agents and to identify those unlikely to respond or particularly prone to complications of contemplated treatment. For patients with incipient or suspected acute myocardial infarction, rapid and accurate detection of acute coronary thrombosis at the time of initial evaluation and early after acute

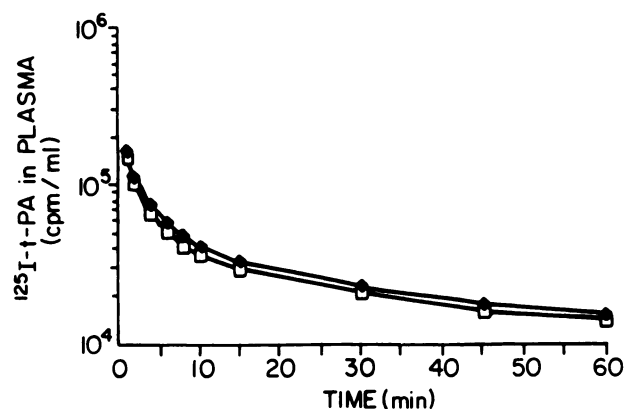


FIGURE 5
Half-lives of ^{125}I -labeled wild-type and Ser \rightarrow Thr-t-PA were determined by measuring concentrations of t-PA as TCA precipitable radioactivity in plasma as a function of time following i.v. bolus injections of labeled t-PA into rabbits. Representative clearance curves are shown for wild-type t-PA (open squares) and Ser \rightarrow Thr-t-PA (closed diamonds).

interventions such as pharmacologic thrombolysis or angioplasty should result in more effective patient selection for treatment and concomitantly contribute to reduction of overall risk.

Currently employed methods for definitive detection of coronary thrombi require invasive procedures such as selective angiography. Limitations of alternative methods include delay in scintigraphic imaging after the time of administration of the thrombus-detecting agent, low ratios of the concentration of label associated with the thrombus to the concentration of label in the circulation, and prolonged persistence of label in the circulation (24). Ideally, a clinically useful agent for rapid and specific radiodetection of acute thrombi in vivo would bind to thrombi with high affinity and specificity, be cleared rapidly from the circulation, persist in thrombi, exhibit no immunogenic properties, be readily administered intravenously, and be devoid of inhibitory effects on the response of the thrombus to subsequent treatment with lytic agents.

Substitution of threonine for serine at amino acid position 478 within the active site of t-PA essentially abolished the proteolytic activity of the molecule without diminishing the binding of the genetically engineered mutant to clots formed from whole blood. This modest change is unlikely to induce substantial immunogenicity. Binding of the mutant t-PA to clots was concentration-dependent, time-dependent, and specific. The clearance of mutant t-PA was rapid. Thus its concentration in blood will undoubtedly decrease quickly after i.v. administration in diagnostic studies.

We have previously demonstrated that substitution of the active site serine with threonine appears to prevent formation of complexes between t-PA and its physiologic inhibitor, plasminogen activator inhibitor-1 (PAI-1) (15). Lack of formation of complexes of t-PA and PAI-1, which is substantial with infusion of pharmacologic doses of wild-type t-PA (25), may be partially responsible for the enhanced binding of mutant t-PA to clots compared with binding of wild-type t-PA. Mechanisms responsible for enhanced binding of PPACK-t-PA to thrombi as compared with mutant t-PA (Fig. 3) are unknown. Possible mechanisms include conformational changes in t-PA favoring binding when treated with PPACK as well as potentially enhanced fibrin binding of one-chain as compared with two-chain t-PA (26).

Persistence of t-PA within clots for prolonged intervals despite rapid clearance from the circulation is evidenced by continued release of cross-linked products indicative of continued dissolution of fibrin in vivo (27). Accordingly, persistence of mutant t-PA in thrombi can be anticipated despite its prompt disappearance from the circulation. Thus, the ratio of label bound to thrombi to label in the blood pool is likely to be high, enhancing detection of thrombi in vivo.

Results of the present study demonstrate that a fibrinolytically inactive mutant of t-PA, genetically engineered to retain properties accounting for specific binding of t-PA to thrombi and for rapid clearance from the circulation, exhibits characteristics rendering it attractive as a potentially useful clinical imaging agent for detection of coronary arterial and other thrombi in vivo. Further studies will be undertaken in several species when amounts of material harvested are sufficient to permit labeling with tracers with attractive energetics for imaging under conditions that do not alter binding to clots, clearance, or exhibit immunogenicity with procedures analogous to those we have used with other residualizing labeling procedures (28).

ACKNOWLEDGMENTS

The authors thank Mrs. Kelly Hall for assistance in preparing the manuscript.

Supported in part by NIH grants HL-17646, SCOR in Ischemic Heart Disease, and NIH grant HL-38868.

REFERENCES

- DeWood MA, Spores J, Notske RN, et al. Prevalence of total coronary occlusion during the early hours of transmural myocardial infarction. *N Engl J Med* 1980; 303:897-903.
- Tiefenbrunn AJ, Sobel BE. The impact of coronary thrombolysis on myocardial infarction. *Fibrinolysis* 1989; 3:1-15.
- Califf RM, O'Neil W, Stack RS. Failure of simple clinical measurements to predict perfusion status after intravenous thrombolysis. *Ann Intern Med* 1988; 108:658-662.
- Fox KAA, Bergmann SR, Mathias CJ, et al. Scintigraphic detection of coronary artery thrombi in patients with acute myocardial infarction. *J Am Coll Cardiol* 1984; 4:975-986.
- Jeghers O, Abramovici J, Jonckheer M, Ermans AM. A chemical method for the labeling of fibrinogen with ^{99m}Tc. *Eur J Nucl Med* 1978; 3:95-100.
- Harwig JF, Welch MJ, Coleman RE. Preparation and use of ¹²³I-labeled highly iodinated fibrinogen for imaging deep-vein thrombi. *J Nucl Med* 1976; 17:397-400.
- Knight LC, Maurer AH, Ammar IA, Shealy DJ, Mattis JA. Evaluation of indium-111-labeled anti-fibrin antibody for imaging vascular thrombi. *J Nucl Med* 1988; 29:494-502.
- Liau CS, Haber E, Matsueda GR. Evaluation of monoclonal antifibrin antibodies by their binding to human blood clots. *Thromb Haemost* 1987; 57:49-54.
- Rosebrough SF, Grossman ZD, McAfee JG, et al. Thrombus imaging with indium-111 and iodine-131-labeled fibrin-specific monoclonal antibody and its F(ab')₂ and Fab fragments. *J Nucl Med* 1988; 29:1212-1222.
- Peters AM, Lavender JP, Needham SG, et al. Imaging thrombus with radiolabeled monoclonal antibody to platelets. *Br Med J* 1986; 293:1525-1527.
- Som P, Oster ZH, Zamora PO, et al. Radioimmunoimaging of experimental thrombi in dogs using technetium-99m-labeled monoclonal antibody fragments reactive with human platelets. *J Nucl Med* 1986; 27:1315-1320.
- Palabrica TM, Furie BC, Konstam MA, et al. Thrombus imaging in a primate model with antibodies specific for an external membrane protein of activated platelets. *Proc Natl Acad Sci USA* 1989; 86:1036-1040.
- Hnатовich DJ, Virzi F, Dohert PW, Wilson J, Rosa J, Ansell JE. Characterization of indium-111 labeled recombinant tissue plasminogen activator for the imaging of thrombi. *Eur J Nucl Med* 1987; 13:467-473.
- Tengborn L, Hedner U, Rohlin M, Stahlberg F. Demonstration of ^{99m}Tc-labelled plasmin on the surface of ex vivo thrombi. *Thromb Res* 1982; 28:783-791.
- Monge JC, Lucore CL, Fry ETA, Sobel BE, Billadello JJ. Characterization of interaction of active-site serine mutants of tissue-type plasminogen activator with plasminogen activator inhibitor-1. *J Biol Chem* 1989; 264:10922-10925.
- 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* 1982; 257:2912-2919.
- Tiefenbrunn AJ, Robison AK, Kurnik PB, Ludbrook PA, Sobel BE. Clinical pharmacology in patients with evolving myocardial infarction of tissue-type plasminogen activator produced by recombinant DNA technology. *Circulation* 1985; 71:110-115.
- Lucore CL, Fry ETA, Nachowiak DA, Sobel BE. Biochemical determinants of clearance of tissue-type plasminogen activator from the circulation. *Circulation* 1988; 77:906-914.
- Bergsdorf N, Nilsson T, Wallen P. An enzyme-linked immunosorbent assay for determination of tissue plasminogen activator applied to patients with thromboembolic disease. *Thromb Haemost* 1983; 50:740-744.
- Lijnen HR, Uytterhoeven M, Collen D. Inhibition of trypsin-like serine proteinases by tripeptide arginyl and lysyl chloromethylketones. *Thromb Res* 1984; 34:431-437.
- Hunter W, Greenwood FC. Preparation of ¹³¹I-labeled human growth hormone of high specific activity. *Nature* 1962; 194:495-496.
- Jespersen J, Astrup T. A study of fibrin plate assay of fibrinolytic agents. *Haemostasis* 1983; 13:301-315.
- Fry ETA, Sobel BE. Lack of interference by heparin with thrombolysis or binding of tissue-type plasminogen activator to thrombi. *Blood* 1988; 71:1347-1352.
- Krohn KA, Knight LC. Radiopharmaceuticals for thrombus detection: selection, preparation, and critical evaluation. *Semin Nucl Med* 1977; VII:219-228.
- Lucore CL, Sobel BE. Interactions of t-PA with plasma inhibitors and their pharmacologic implications. *Circulation* 1988; 77:660-669.
- Rijken DC, Hoylaerts M, Collen D. Fibrinolytic properties of one-chain and two-chain human extrinsic (tissue-type) plasminogen activator. *J Biol Chem* 1982; 257:2920-2925.
- Eisenberg PR, Sherman LA, Tiefenbrunn AJ, Ludbrook PA, Sobel BE, Jaffe AS. Sustained fibrinolysis after administration of t-PA despite its short half-life in the circulation. *Thromb Haemost* 1987; 57:35-40.
- Daugherty A, Thorpe SR, Sobel BE, Schonfeld G. Effects of diet-induced hypercholesterolemia on total catabolic rates and tissue accumulation of cholesterol ester-rich very low density lipoproteins and low density lipoproteins in rabbits in vivo. *J Lipid Res*, in press.