IN AUGUST 1983, it was anticipated from biochemical observations and physiologic results in experimental animals that human tissue-type plasminogen activator (t-PA) held considerable promise as a clot-specific, coronary thrombolytic agent for patients with acute myocardial infarction.1 This anticipation has been fulfilled remarkably promptly by clinical research.2-5 Recently reported results from several randomized clinical trials3-5 punctuate a long series of antecedent laboratory, preclinical, and clinical investigations. Accordingly, research focused on t-PA serves as a particularly good example of productive, sequential developments and collaborations involving investigators from disparate disciplines in academy and industry. This report summarizes some of the seminal studies in the development and characterization of native t-PA and the recombinant DNA-produced t-PA used in multicenter trials reported recently.3-5

Although t-PA was identified in the 1940s, its isolation and characterization were hampered by inadequate sources and purification procedures. In 1980, an attractive procedure for the isolation of human t-PA was developed by Rijken et al.,4 yielding approximately 1 mg of t-PA from 5 kg of human uterine tissue. Subsequent development of t-PA and its application to coronary thrombolysis has progressed along four main lines: (1) isolation and characterization of t-PA from the Bowes melanoma cell line, (2) cloning and expression of the human t-PA gene, (3) delineation of the efficacy of purified t-PA in experimental animals with induced coronary thrombosis, and (4) evaluation of the coronary thrombolytic efficacy of t-PA in patients with acute myocardial infarction.

Isolation and characterization of t-PA from the Bowes melanoma cell line. The Bowes melanoma cell line was provided to us by Dr. D. B. Rifkin, New York University Medical School, toward the end of 1978. It had been obtained originally from pulmonary, metastatic melanoma cells from a patient named Bowes by Dr. G. Moore in 1974 and was maintained and exchanged among investigators because it secreted large amounts of plasminogen activator activity. However, the nature of the activity had not been elucidated. In collaboration with Dr. A. Billiau in the Laboratory of Virology at the University of Leuven, we introduced the Bowes cell line in our laboratory to study the spectrum of inhibition of plasminogen activators of malignant cells by synthetic inhibitors. In retrospect, selection of this cell line was fortuitous. Most malignant cell lines in culture produce urokinase-like plasminogen activators. Some produce mixtures of urokinase and an activator that does not react with urokinase antibodies.7 Cell lines that secrete t-PA primarily or exclusively are quite rare. The Bowes cell line is unusually, if not uniquely, efficient in this regard. This explains why, after our initial description of t-PA isolated from the Bowes line, this source became the standard one for most investigators in the field.

When we sought initially to purify the plasminogen activator from Bowes melanoma cell culture fluid in 1979, we observed that the activator, like uterine t-PA but unlike urokinase, had a specific affinity for fibrin. When mixtures of fibrinogen and plasminogen activator were clotted, the activator remained associated with the clot but could be eluted with potassium isothiocyanate.

Thus the melanoma plasminogen activator resembled t-PA. However, with the use of purification methods developed previously for t-PA, no homogeneous, final product was obtainable initially.

In October 1979, Dr. D. C. Rijken joined us from the Gaubius Institute in The Netherlands. He had developed a method for the purification of the plasminogen activator from human uterus in which adsorption of the activator to surfaces was prevented by the use of Tween 80 and in which zinc-chelate agarose was used in the first chromatographic step. With a modified version of this purification procedure we were soon...
able to purify melanoma cell culture fluid plasminogen activator material shown to be homogeneous by sodium dodecyl sulfate gel electrophoresis and to be immunologically identical to the uterine plasminogen activator (t-PA).

Subsequently, the purification procedure was scaled upward to produce a total amount of approximately 2 g of t-PA, facilitating systematic characterization of its biochemical, biological, and physiologic properties.

With Dr. Matsuo from Miyazaki University in Japan who joined us in September 1979, thrombolytic effects of t-PA in plasma in vitro and thrombolytic effects in rabbits with experimental pulmonary embolus in vivo were demonstrated. With Drs. Rijken, M. Hoylaerts, H. R. Lijnen, I. Juhan-Vague, and C. Korninger, the kinetics of plasminogen activator were clarified and immunoassays for t-PA in plasma were developed.

In collaboration with Dr. W. Weimar in 1981, we treated two patients with renal vein thrombosis after kidney transplantation with intravenous infusions of 5 and 7.5 mg of t-PA over 24 hr. Despite the fact that lysis occurred, the dose was probably too low for general use judging from the results of analogous patients (unpublished observations) and from extensive information acquired later in studies of coronary thrombolysis in animals and patients (see below).

Cloning and expression of the human t-PA gene. At the Fifth Congress on Fibrinolysis in Malmo, Sweden, where our first results with t-PA were presented, I met a scientist from the Department of Molecular Biology in the Genentech Corporation. Collaboration devoted to the cloning and expression of the t-PA gene ensued, with results reported at the Sixth Congress on Fibrinolysis in Lausanne, Switzerland, in 1982 and published in January 1983. Building on the successful cloning and expression of t-PA, Genentech made a profound commitment to developing recombinant human t-PA (rt-PA). With the approval of the FDA, rt-PA was first administered to a patient on February 11, 1984. The rapid progress was possible only because of the efforts of many scientists at Genentech and concomitant investigations elsewhere characterizing some of the biological and thrombolytic properties of the material. We were able to demonstrate that the turnover of the rt-PA in rabbits is similar to that of melanoma cell t-PA and that its thrombolytic properties in a rabbit jugular vein thrombosis preparation are indistinguishable from those of the plasminogen activator of melanoma origin.

Delineation of coronary thrombolysis by natural t-PA and rt-PA in experimental animals. Late in 1981 at an NIH workshop on coronary thrombolysis, Dr. B. E. Sobel from Washington University initiated a collaboration in which our two groups explored the use of t-PA for thrombolysis in the treatment of acute myocardial infarction. The work focused also on the impact of the coronary thrombolytic effects of t-PA on myocardial metabolism and perfusion assessed with positron emission tomography. Within a short time the utility of this approach was demonstrated in closed-chest dogs with coronary thrombosis induced by advancing a copper coil into the left anterior descending coronary artery (LAD). Intravenous infusion of human t-PA purified from melanoma cell culture fluid resulted in prompt coronary recanalization without systemic activation of the fibrinolytic system. Furthermore, it restored myocardial blood flow and intermediary metabolism in the region at risk demonstrated tomographically with $^{11}C$-labeled palmitate and $^{13}C_2$O given intravenously. These results demonstrated that administration of native, human t-PA to animals with induced coronary thrombosis elicited prompt thrombolysis without predisposition to systemic bleeding attributable to a systemic lytic state. Subsequently, the observations were extended to rt-PA. In a collaborative study between the Cardiology Divisions of the University of Leuven, Belgium, and Washington University, the clot-specific coronary thrombolytic properties of rt-PA were demonstrated with the same experimental animal preparation. In a concurrent collaborative study with Dr. H. K. Gold, Massachusetts General Hospital, coronary thrombosis was produced between two ligatures of the LAD in open-chest dogs. Infusion of t-PA elicited clot lysis and myocardial salvage in this preparation as well. Subsequently, in a collaborative study with Dr. W. Flameng at the University of Leuven, the coronary thrombolytic properties, clot-specificity, and myocardial protection achievable with rt-PA were confirmed in baboons.

Administration of t-PA to patients with acute myocardial infarction. The first study in which t-PA was administered to patients with acute myocardial infarction was performed between February and September 1983 with purified t-PA obtained from the Bowes cell line. Participants included Dr. F. van de Werf and co-workers at the University of Leuven and Dr. Sobel and co-workers at Washington University. Intravenously administered t-PA in doses of 20,000 to 40,000 IU/min (200 to 400 μg) recanalized completely occluded coronary arteries within 30 to 60 min in six of seven patients without inducing a systemic, fibrinolytic state in any. These initial observations with native t-PA served as a template and stimulated initiation of a multicenter, blinded, randomized trial with rt-PA provided by Gen-
entech. Fifty patients were studied between February 11 and June 20, 1984, at Washington University with Dr. Sobel and co-workers, at the Massachusetts General Hospital with Dr. Gold and co-workers, and at Johns Hopkins University with Dr. M. Weisfeldt and co-workers. Intravenous infusion of 0.5 mg/kg body weight rt-PA over 60 min or of the same dose followed by 0.25 mg/kg over an additional 60 min resulted in recanalization of occluded coronary arteries in 75% of patients. Fibrinogenolysis was absent or modest in most but not all of the patients. Characterization of a subset of patients indicated that therapeutic plasma levels of t-PA were achievable in a dose-dependent fashion despite individual variation. These t-PA levels affected the fibrinolytic system in a fashion consistent with the kinetics of the participating biochemical reactions. The results obtained in this initial study of rt-PA in patients with acute myocardial infarction provided a foundation for the design of both the recently implemented NIH Thrombolysis in Acute Myocardial Infarction (TIMI) trial in the United States and the European Cooperative trial in Belgium, France, West Germany, and The Netherlands.

In the TIMI trial thus far, rt-PA has been infused over 3 hr (40 mg for 1 hr and 20 mg for each of the next 2 hr). After an initial open-label phase carried out in 87 patients in June and July 1984, a double-blind comparison with 1.5 million U of streptokinase infused over 1 hr was performed in 316 patients between August 1984 and February 1985. rt-PA was found to be approximately twice as effective as streptokinase for recanalization and to be associated with substantially less fibrinogenolysis.

In the European Cooperative trial, rt-PA was infused at a rate of 0.75 mg/kg over 90 min. Results were compared with those in patients given 1.5 million U of streptokinase over 1 hr. In 129 patients studied between July and December 1984, rt-PA was found to be more effective than streptokinase and to induce substantially less fibrinogenolysis.

The concordant conclusions of the three prospective, randomized clinical trials with rt-PA from which results are available to date, coupled with the extensive, antecedent biochemical, physiologic, and biological observations in experimental animals and patients, constitute a firm basis for the further evaluation of thrombolysis induced by t-PA in the management of patients with acute myocardial infarction. The relative safety and thrombolytic potential of t-PA appear to be well established. The ultimate benefit of coronary thrombolysis remains to be elucidated.

The author appreciates preparation of the typescript in the Departments of Biochemistry and Medicine at the College of Medicine, University of Vermont.

References

Human tissue-type plasminogen activator: from the laboratory to the bedside.

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Circulation. 1985;72:18-20
doi: 10.1161/01.CIR.72.1.18

Circulation is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0009-7322. Online ISSN: 1524-4539

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