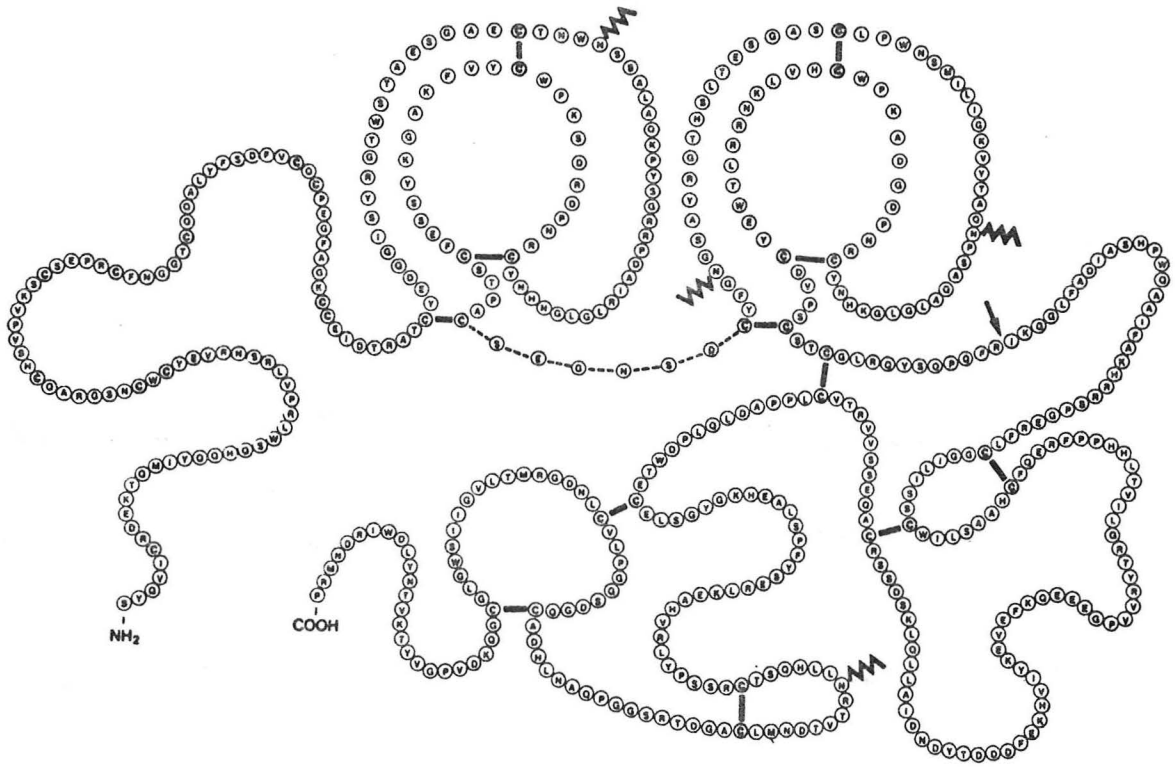
TISSUE PLASMINOGEN ACTIVATOR:
FROM MOLECULAR BIOLOGY TO MYOCARDIAL INFARCTION



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MEDICAL GRAND ROUNDS

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UNIVERSITY OF TEXAS HEALTH SCIENCE CENTER AT DALLAS

SOUTHWESTERN MEDICAL SCHOOL

INTRODUCTION

In 1628, Dr. William Harvey made the following observations:

"It has been shown by reason and experiment that blood, by the beat of the ventricles, flows through the lungs and heart and is pumped to the whole body. This is the only reason for the motion and beat of the heart".

In the 350 years since these initial observations of Dr. Harvey, it has become increasingly apparent that the cardiovascular system is much more than a simple mechanical pump which provides blood flow to various organs. Recent advances in catheterization techniques, radionuclide imaging, nuclear magnetic resonance, ultrasound technology and computer science have demonstrated the complexity of the cardiovascular system, and have allowed major improvements in the diagnosis and treatment of cardiovascular disorders in man. Molecular biology and recombinant DNA technology are now joining this list of standard tools used by cardiologists.

Certainly, one of the most lucid examples of the potential impact of recombinant DNA technology in clinical medicine is the recent development of recombinant tissue plasminogen activator as a therapeutic agent during acute myocardial infarction. This remarkable achievement would not have been possible without several key discoveries in molecular biology and clinical cardiology. In addition, this work exemplifies the synergistic relationship between basic and clinical research. The purpose of this Grand Rounds is to chronicle this journey from molecular biology to myocardial infarction.

CASE HISTORY

Perhaps the most appropriate starting point for our journey is to examine the effects of the acute intravenous administration of recombinant tPA on a patient recently admitted to Parkland Hospital with an acute anterior myocardial infarction.

TABLE I

Case History (PMH admission #1-09-28-70-6-099)

<u>April 9, 1986</u>

11:00am	RH is a 58 y/o WM who develops substernal chest pain, nausea, diaphoresis and shortness of breath.
11:40am	Admitted to PMH: EKG reveals 1½mm ST elevation in precordial leads V1 and V2, and patient continues to have chest pain and dyspnea

12:10pm	Intravenous tPA administered (9mg bolus; 25mg/hrx6 hr infusion)
12:30pm	EKG reveals normalization of anterior ST changes without loss of R waves; patient is pain free
7:00pm	Serum creatine kinase peaks at 413

April 11, 1986

9:00am	Cardiac cath reveals normal wall motion of all segments, including anterior wall, LVEF 68%, severe 3 vessel disease: 99% proximal RCA, 90% sequential proximal LAD, and 90% distal circumflex
6:00pm	CABG x 3 performed without complication

April 18, 1986

Patient discharged from hospital on aspirin, persantine and digoxin without further complication

RH is a 58 y/o WM who developed substernal chest pain, nausea, diaphoresis, and shortness of breath at 11:00am on April 9, 1986. At 11:40am, he was admitted to Parkland Hospital where he continued to have chest pain and an EKG revealed 1.5mm ST segment elevation in V1 and V2. The diagnosis of an acute anterior myocardial infarction was made and intravenous tPA was administered thirty minutes later. At 12:30pm the patient became pain free, and an EKG revealed normalization of the anterior ST segment changes without loss of R waves. At 7:00pm, the creatine kinase peaked at only 413. On April 11, a cardiac catheterization was performed which revealed normal wall motion of all segments, including the anterior wall, and a left ventricular ejection fraction of 68%. Severe 3 vessel disease was documented, consisting of 99% proximal RCA, 90% sequential proximal LAD, and 90% distal circumflex lesions. Due to the large portion of myocardium which was threatened by the coronary arterial thrombus and the severity of the underlying coronary artery disease, coronary artery bypass grafting was performed the same day. Nine days after his initial presentation, the patient was discharged from the hospital on aspirin, persantine, and digoxin without further complication.

This dramatic case history illustrates the impact that recombinant DNA technology is having on the treatment of cardiovascular disorders in man. Ten years ago, the therapeutic approach to this patient would have been bed rest and intravenous morphine prn chest pain. The availability of recombinant tPA has allowed a more aggressive approach to the treatment of acute myocardial infarction. Instead of merely treating the symptoms of myocardial ischemia, therapy has now been designed to directly interrupt the pathologic process causing acute coronary insufficiency by lysing the

coronary arterial thrombus. Although no general conclusions can be drawn about the efficacy of tPA therapy from this single case study, it is fairly certain that the administration of intravenous tPA was largely responsible for reversing the myocardial ischemia, maintaining normal left ventricular function, and improving the ultimate prognosis of this particular patient.

EARLY BIOCHEMICAL STUDIES ON FIBRINOLYTIC SYSTEM

This remarkable therapeutic achievement stems from earlier work identified serum factors which were capable of regulating intravascular coagulation (1). As recently reviewed by Dr. Michael Brown in his Grand Rounds (2), coagulation was regulated by a "waterfall" cascade of proteins, which ultimately resulted in the conversion of fibrinogen to fibrin, the main protein component of blood clots (Figure 1). As a result of work by several investigators, it became apparent that human serum also contained factors which regulated the breakdown of fibrin, later known as the "fibrinolytic system". The major enzyme which degrades fibrin to fibrin split products was plasmin, a protein which is synthesized in the liver (3,4). Studies between 1940-1950 identified factors which stimulated the conversion of plasminogen, the inactive serum precursor, to plasmin, the active protein, i.e. plasminogen activators (5-7) (Table II). Although work in the fibrinolytic system continued after this time, further research on the molecular basis of plasminogen activation was largely dormant during the ensuing 30-35 years.

FIGURE 1

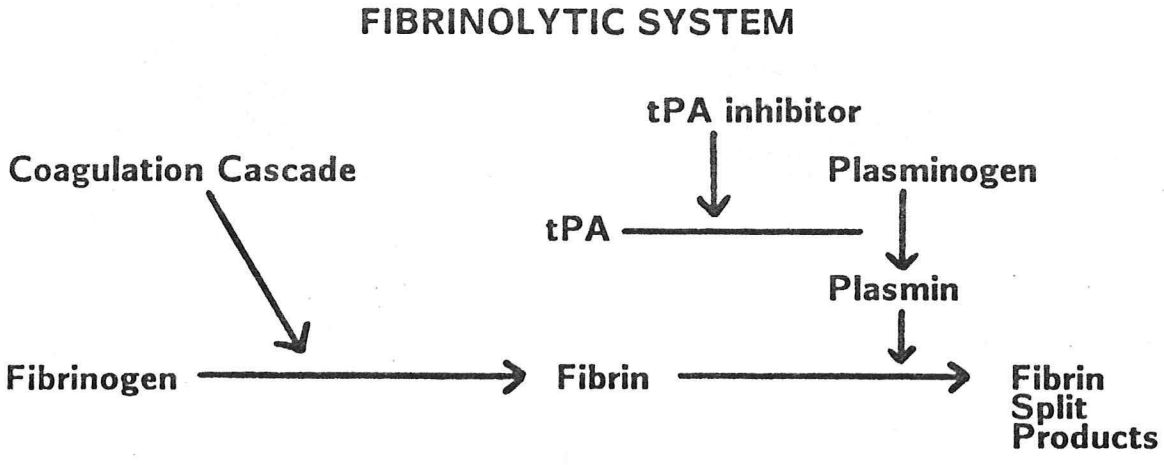


TABLE II

tPA DATELINE

Molecular Biology Studies

1940-50	Identification of plasminogen activators in human tissues and serum (5-7)
1979	Purification of tPA from a Bowes melanoma cell line [Rijken and Collen (15)]
1981-82	Molecular cloning and expression of tPA [Pennica et al (18); Sambrook, Hanahan, and Gething (19)]
1983	High level expression of tPA achieved by gene amplication during methotrexate selection in surrogate cell system [Kaufman et al (21)]
1983	Rapid-acting inhibitor of tPA found in human serum [Wiman et al (24)]
1984	Purification of tPA inhibitor from endothelial cells (28)
1986	Molecular cloning of tPA inhibitor [Ginsberg (29); Loskutoff (30); Pannekoek (31); Gerard, Sambrook, Gething, and Chien (32)]
1986	Production of tPA mutant proteins [Pannekoek (33); Bang, et al; Sambrook and Gething]

Clinical Studies

1979	Intracoronary streptokinase induces thrombolysis and reperfusion during acute myocardial infarction in man [Rentrop (8)]
1980	Clinical trials of streptokinase are initiated; demonstrate successful reperfusion (9-11)
1983	tPA induces coronary thrombolysis in animal models and patients with acute myocardial infarction (16,17)
1984-85	TIMI Trial reports intravenous tPA more efficacious than streptokinase (20)
1986	TIMI Trial reports high incidence of reocclusion following tPA therapy (23)
1986	Increased levels of tPA inhibitor observed in young patients following MI (35)

EARLY CLINICAL STUDIES ON THROMBOLYTIC THERAPY

During the late 1970's, renewed interest in the biochemistry of the fibrinolytic system was stimulated by the results of clinical studies in patients with acute myocardial infarction (8,9). In a set of pioneering studies, Dr. Peter Rentrop demonstrated that the intracoronary administration of streptokinase, an exogenous plasminogen activator, could induce thrombolysis and reperfusion during acute myocardial infarction in man (Table III).

TABLE III

EFFECT OF INTRACORONARY STREPTOKINASE ON CORONARY REPERFUSION DURING ACUTE MYOCARDIAL INFARCTION

Study	Reperfusion Rate
Rentrop et al (8)	85%
Rentrop et al (9)	74%
Serruys et al (10)	79%
Kennedy et al (11)	68%

Myocardial reperfusion rate is defined as: # of patients with angiographically documented reperfusion divided by # of patients receiving thrombolytic therapy. A summary of the most recent streptokinase trials has been compiled in the Appendix. The current protocol and contraindications for IV streptokinase therapy has also been included.

Subsequent to these studies, several randomized trials of streptokinase were initiated which demonstrated that the intracoronary administration of streptokinase could induce myocardial reperfusion in 68-75% of the patients with acute myocardial infarction (Table III). The results of these studies demonstrated the necessity for further isolation and characterization of the endogenous plasminogen activators which were present in human tissues and serum.

BIOCHEMICAL CHARACTERIZATION OF PLASMINOGEN ACTIVATORS

Although the studies with streptokinase were clearly encouraging, there were several inherent limitations in the use of streptokinase therapy. Table IV displays the properties of the three known plasminogen activators.

TABLE IV

PLASMINOGEN ACTIVATORS

	Streptokinase	Urokinase	tPA
Source	Bacterial	Human urine, Kidney cells	Human serum, Endothelial cells
Antigenicity	+	-	-
Plasminogen Activation	+	+	+
Fibrin Binding	-	-	+
Fibrinogen Depletion	+	+	-
Anticoagulation	+	+	-
Endogenous Inhibitor	-	+	+

Streptokinase is a bacterial product which activates plasminogen to plasmin, but displays no specific affinity for fibrin. The high affinity of streptokinase for circulating plasminogen results in the conversion of plasminogen to plasmin throughout the intravascular system, producing a profound depletion of plasminogen and fibrinogen (12). Furthermore, the marked accumulation of fibrin split products induces a systemic anticoagulated state, placing the patient at greater risk for bleeding complications (12). Urokinase is an endogenous protein activator of plasminogen, which is secreted into human urine by kidney cells. Since urokinase is an endogenous protein, antigenicity is less of a problem than with streptokinase. However, similar to streptokinase, urokinase has negligible affinity for fibrin and induces a systemic anticoagulated state (13). Tissue plasminogen activator is produced by endothelial cells and is present in human serum. In clear distinction from the other plasminogen activators, tissue plasminogen activator contains a high affinity for fibrin and requires fibrin to exhibit significant activity. This affinity for fibrin suggested the possibility that tPA will localize specifically to areas where fibrin has been deposited, i.e. at the site of the blood clot. Theoretically, this "targeting" effect of tPA might avoid the systemic anticoagulant effects observed with urokinase and streptokinase, since the activation of plasminogen would be limited to the site of the thrombus. Since fibrin degradation would occur only at the site of the clot, there would be minimal depletion of fibrinogen and plasminogen. In addition, this affinity for the site of the thrombus might allow the intravenous administration of tPA, thereby obviating the necessity for intracoronary administration. Obviously, a direct evaluation of the thrombolytic

efficacy of tPA would be necessary. Since the isolation of sufficient tPA from human tissue sources, such as placenta, would represent a formidable task, another technique would be necessary to purify large quantities of human tPA.

EARLY CLINICAL STUDIES OF tPA THROMBOLYSIS

One of the major advances in thrombolytic therapy occurred in 1979, when Dr. Daniel Rifkin demonstrated that a human melanoma cell line released large amounts of tissue plasminogen activator into the extracellular medium (14). Since these melanoma cells will grow continuously in culture, Dr. Desire Collen and other investigators were able to harvest larger quantities of tPA for in vivo studies (15). Using this purified tPA, coronary thrombolysis was achieved in animal models and in patients with acute myocardial infarction (16,17). Following the intracoronary administration of tPA, 6 of 7 patients with acute myocardial infarction developed myocardial reperfusion (17) (Table V). Furthermore, the depletion of fibrinogen and accumulation of fibrin split products was considerably less than previously reported with streptokinase therapy (17).

TABLE V

EFFECT OF INTRACORONARY tPA ON THROMBOLYSIS IN 7 PATIENTS WITH ACUTE MYOCARDIAL INFARCTION

Patient	MI (location)	Thrombolysis
1	Anterolateral	+, 50'
2	Anterior	+, 30'
3	Anterior	---
4	Inferior	+, 30'
5	Inferior	+, 37'
6	Anterior	+, 22'
7	Anterior	+, 19'

Thrombolysis was achieved in patients 1,2, and 4-7. Time required for reperfusion is included.

These studies by Collen demonstrated the necessity of directly evaluating the effect of tPA administration on the morbidity and mortality of patients with acute myocardial infarction. In particular, it would be critical to determine if the fibrin affinity of tPA would permit

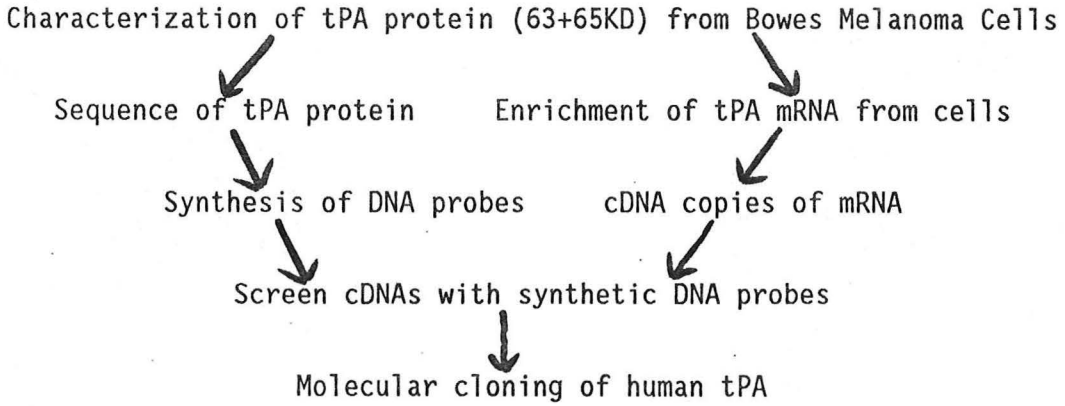
intravenous administration, thereby obviating the necessity of cardiac catheterization during acute myocardial infarction. A randomized study would require amounts of tissue plasminogen activator that could not be reasonably obtained by isolating tPA from melanoma cells. A new approach to the purification of human tPA would have to be adopted. Fortunately, the advent of recombinant DNA technology paralleled these clinical studies.

MOLECULAR CLONING OF HUMAN tPA

One of the major strengths of molecular biology techniques is the ability to clone the genes of proteins which are normally present in very trace amounts in human material. These cloned genes can be transferred into cells which are designed to make large quantities of the desired protein. Thus, to procure sufficient tPA to perform a large scale clinical trial, it became necessary to clone and express the tissue plasminogen activator gene. The molecular cloning of human tPA was accomplished by two independent groups: a Belgium group led by Dr. Collen, in collaboration with Genentech (18); and a Cold Spring Harbor group led by Dr. Joseph Sambrook (19). These investigators utilized similar strategies to clone the human tPA gene (Table VI). First, human tPA was isolated from the Bowes melanoma cell line, revealing a protein of about 63-65000 molecular weight. Subsequently, the amino acid sequence of a small stretch of the tPA protein was determined. Since the DNA code for each amino acid was known, a series of short DNA molecules (probes) were then designed to specifically bind to the tissue plasminogen activator gene. In parallel, the mRNA of the melanoma cells (enriched with tPA mRNA) was isolated, and copies of all of the melanoma protein genes, including tPA, were synthesized and inserted into bacteria. The synthetic DNA probes were then used as "bait", to "fish out" the tissue plasminogen activator gene from bacteria which have DNA copies (clones) of all of the proteins which are normally made from the melanoma cells. By screening hundreds of thousands of these clones, a DNA clone which codes for tissue plasminogen activator was obtained. The isolated tPA gene was then inserted into cells, which allowed the production of human tPA.

TABLE VI

CLONING STRATEGY OF HUMAN tPA



RESULTS OF PHASE I TIMI TRIAL

The availability of recombinant tPA allowed a direct comparison of the efficacy of intravenous tPA and streptokinase in patients with acute myocardial infarction. The Thrombolysis in Myocardial Infarction trial, known as TIMI, was initiated in 1983. The results of Phase I of the TIMI trial reported successful reperfusion in over 65% of the patients who received intravenous tPA, whereas intravenous streptokinase was successful in only 30% of the patients (20) (Table VII). Thus, these studies suggested that intravenous tPA was more efficacious than intravenous streptokinase. In fact, further evaluation of streptokinase therapy was eliminated in Phase II of the TIMI trial, which was designed to directly examine the effect of intravenous tPA on patient morbidity and mortality.

TABLE VII

RESULTS OF PHASE I TIMI TRIAL COMPARING INTRAVENOUS tPA WITH INTRAVENOUS STREPTOKINASE

Therapy	# Patients	Reperfusion Rate
Streptokinase	115	36%
tPA	99	66%

PRODUCTION OF RECOMBINANT tPA BY GENE AMPLIFICATION

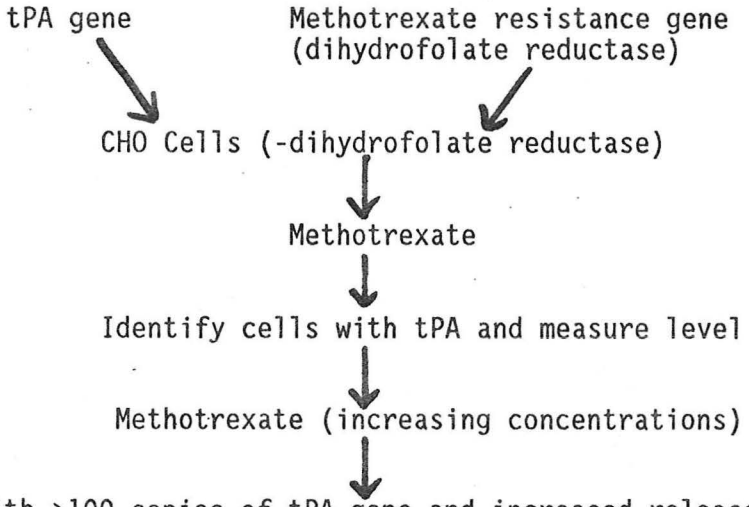
If Phase II of the TIMI trial continues to document a beneficial effect of thrombolytic therapy, the administration of intravenous tPA will become standard therapy in patients who present with an acute myocardial infarction within 4 hours of the onset of chest pain. Obviously, worldwide clinical use would require quantities of tPA which are several orders of magnitude greater than those needed for the TIMI trial. This point raises the important question: Can modern molecular biology meet the demands of cardiologists and their patients? Although the tPA protein can be produced efficiently by bacteria, this recombinant protein product has lost significant portions of its activity. Apparently, bacteria do not allow the correct processing of the tPA protein, resulting in the production of inactive tPA. Thus, to isolate recombinant tPA in the active form, it was necessary to insert the tPA gene into animal cells which will overexpress the protein.

In 1983, Kaufman and coworkers utilized an ingenious system to obtain maximal expression of tPA genes in animal cells (21). This system is based upon the clinical observation that cancer patients eventually become refractory to the effects of methotrexate therapy. The basis for this drug resistance is a marked increase in the protein which detoxifies methotrexate, dihydrofolate reductase. As the drug therapy is repeated, the dihydrofolate reductase (DHFR) gene replicates, eventually resulting in the presence of several hundred copies of the DHFR gene within each tumor

cell. This phenomenon of "gene amplification" is responsible for the increase in DHFR activity in tumor cells of patients undergoing serial treatment with methotrexate (22). The ability of the DHFR gene to stimulate gene amplification in cultured animal cells is the basis of the unique expression system utilized by Kaufman (Table VIII). First, the tPA and DHFR genes are inserted into cultured animal cells. Subsequently, the cells are treated with methotrexate, which allows only those cells which take up the DHFR gene to grow. The cells which do not contain the DHFR gene lose viability. A portion of the growing cells will contain both the DHFR and tPA gene and should release tPA into the medium. The cells containing the tPA and DHFR genes are identified, and then treated with increasing doses of methotrexate, resulting in the co-amplification of the DHFR and tPA gene. The net result is the production of animal cells which contain several hundred copies of the tPA and DHFR genes, which release tPA in 100-fold or greater amounts into the culture medium. Using this system, it is currently anticipated that sufficient tPA will be produced for worldwide clinical use.

TABLE VIII

STRATEGY FOR EXPRESSION OF tPA BY GENE AMPLIFICATION



CHARACTERIZATION OF AN ENDOGENOUS INHIBITOR OF tPA

The initial Phase I results of the TIMI trial have revealed a high incidence of coronary reocclusion following the intravenous administration of tPA [Table IX; (23)]. Over 30% of those patients with successful coronary thrombolysis subsequently developed reocclusion and further ischemic events. Unfortunately, many patients required further interventions, such as angioplasty or coronary artery bypass grafting. The basis for this high incidence of reocclusion was not completely clear. However, one possibility is that the high incidence of reocclusion was a result of the early inactivation of tPA by an endogenous inhibitor of tPA. The half-life of tPA was 4-8 minutes, supporting the concept of rapid inactivation of tPA (24). In 1983, several groups discovered a rapid acting inhibitor to tPA in human serum (24-27) (Figure 2). The inhibitor formed a complex with tPA, and completely inactivated the molecule (27). In fact, the serum level of inhibitor was ten fold higher than the endogenous level of tPA (24). Thus, the net fibrinolytic state might be determined by a balance between the level of tPA and the level of the tPA inhibitor.

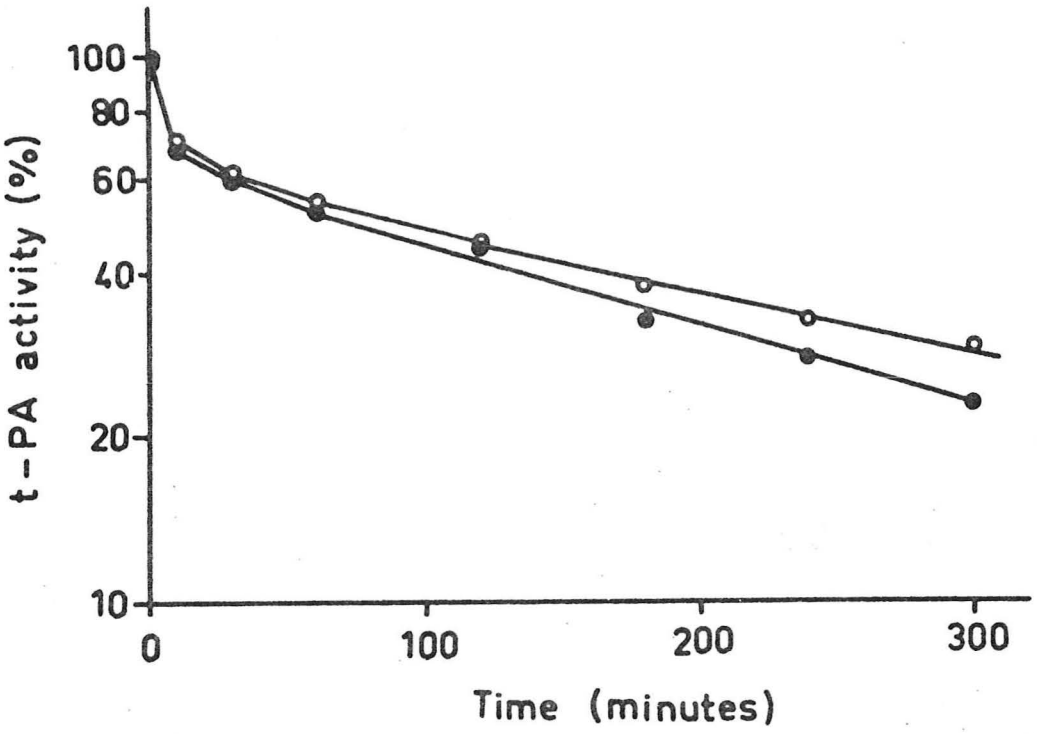
TABLE IX

EFFECT OF INTRAVENOUS tPA THERAPY DURING ACUTE MYOCARDIAL INFARCTION

# Patients	Reperfusion Rate	Time	Fibrinogen Level	Plasminogen Level
47	68%	40	-30%	-60%

FIGURE 2

TIME COURSE OF THE INACTIVATION OF tPA IN HUMAN SERUM



The high incidence of reocclusion following tPA therapy, coupled with evidence of an endogenous tPA inhibitor, have emphasized the importance of the isolation and characterization of the tPA inhibitor. In 1984, the tPA inhibitor was purified from endothelial cells, which presumably are the major source of the protein (28). Since the inhibitor was present in trace amounts in human serum, it became necessary to obtain sufficient quantities of the protein to characterize the nature of its interaction with tPA.

Molecular cloning of the tPA inhibitor has now been achieved by several independent groups (29-32), which will undoubtedly lead to a better understanding of the nature of the interaction between the inhibitor and tPA. If the tPA inhibitor can either be down-regulated or inhibited by synthetic compounds, it may not be necessary to administer exogenous tPA to induce thrombolysis. Study of the tPA inhibitor gene may eventually unravel the mechanisms which regulate the level of tPA inhibitor in human serum. The production of large amounts of tPA inhibitor will hopefully allow crystallization of the protein and the direct design of synthetic compounds to specifically inhibit the tPA inhibitor. These compounds may eventually allow the induction of a chronic state of thrombolysis in patients at high risk for acute coronary insufficiency, thrombotic, and embolic phenomenon. Since decreased tPA inhibitor activity would not result in systemic anticoagulation, this type of therapy might have several advantages over standard anticoagulation therapy with either coumadin or heparin.

DESIGN OF INHIBITOR-RESISTANT tPA PROTEINS BY SITE-DIRECTED MUTAGENESIS

Alteration of the tPA molecule might provide an alternative approach to prolonging the half life of tPA. Human tPA contains at least 3 sections (domains) which are responsible for various functions of the protein: 1) a fibrin binding domain; 2) a plasminogen binding domain; and 3) a domain which interacts with the tPA inhibitor (Figure 3). Recombinant DNA technology now permits the direct modification of any portion of the tPA protein. By creating mutations in specific portions of the tPA gene, "second generation" tPA proteins can be designed which are insensitive to the inhibitor, but maintain the ability to activate plasminogen and binding affinity for fibrin. Several groups have now produced a series of altered tPA molecules and are currently screening these proteins to determine which tPA proteins will maintain activity, but will lose the affinity for the endogenous inhibitor (33,34) (Table X).

FIGURE 3

SECONDARY STRUCTURE AND AMINO ACID SEQUENCE OF HUMAN tPA

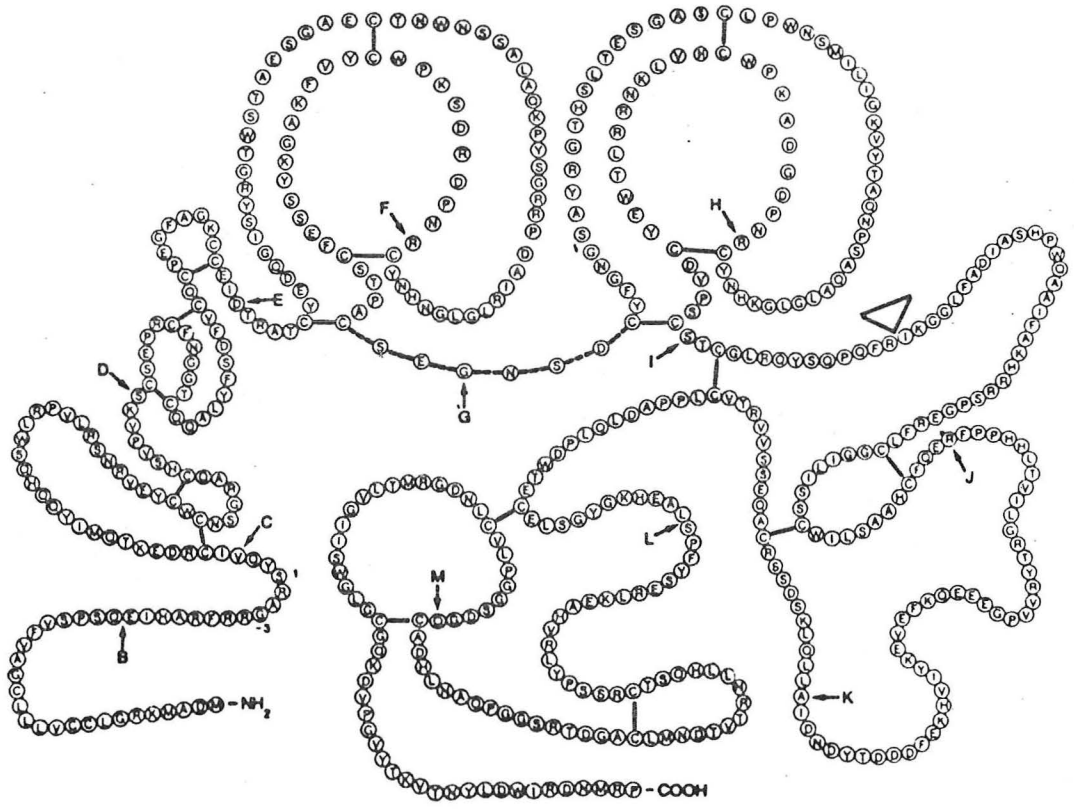


TABLE X

tPA MUTANTS

Deleted Exon	Deleted Domains
C-I	Heavy chain
C-D	Finger
C-E	Finger, EGF
E-G	Kringle 1
G-I	Kringle 2
E-I	Kringle 1+2

tPA mutant proteins were made by site directed mutagenesis of the wild-type tPA cDNA gene (Sambrook and Gething, 1986).

FUTURE DIRECTIONS

Since it is now clear that tPA will induce coronary thrombolysis during myocardial infarction, a logical question is whether a dysregulation of the fibrinolytic system is primarily involved in the pathogenesis of the coronary arterial thrombus. Data on this subject are not currently available. However, a recent report in the New England Journal of Medicine demonstrated that young patients with an acute myocardial infarction have higher levels of the endogenous tPA inhibitor (35) (Table XI). Thus, certain patients at high risk for myocardial infarction may have lower levels of endogenous plasminogen activator due to high levels of tPA inhibitor. Recombinant DNA technology will not only allow the production of large amounts of tPA and tPA inhibitor proteins, but also will permit a direct evaluation of the factors which regulate the expression of these genes. As noted in a recent review, it will certainly be important to study the regulation of the tPA inhibitor gene in animal models and patients with coronary artery disease (36).

TABLE XI

ACTIVITY AND CONCENTRATIONS OF tPA ANTIGEN AND INHIBITOR IN YOUNG PATIENTS WITH MYOCARDIAL INFARCTION

	Patients (N=71)	Controls (N=50)	P Value
	mean ±S.D.		
tPA activity (U/ml)	0.02±0.02	0.03±0.02	N.S.
tPA antigen (μg/liter)	5.8±1.6	4.9±1.6	N.S.
tPA inhibitor (AU/ml)	3.2±2.7	1.5±1.6	<0.001

AU denotes arbitrary units

CONCLUSIONS

Recombinant DNA technology has already had a profound impact on clinical cardiology. In addition to tPA, several important proteins, such as atriopeptin, have been cloned which have major beneficial effects on the cardiovascular system. Although recombinant DNA can be utilized to produce valuable proteins, the major contribution of molecular biology will be its ability to probe the molecular basis for cardiac function and cardiovascular disorders in man. Hypertrophic cardiomyopathy, congenital heart disease, Duchenne's muscular dystrophy, and other genetic disorders of the cardiovascular system are eminently suitable for analysis using recombinant DNA technology. Once the molecular basis for these and other disorders are known, there will undoubtedly be new advances in the treatment and diagnosis of cardiac disease. The journey from molecular biology to the treatment of myocardial infarction has been dazzling in its speed, spanning a time frame between 1981 (when the protein was originally cloned), to 1986, when thrombolysis was induced in our patient with an acute anterior myocardial infarction. It is a journey which would not have been possible without the combined insights of cardiologists and molecular biologists. Through the advent of molecular biology, a new renaissance in cardiac biochemistry and cardiovascular medicine is about to begin.

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APPENDIX

THROMBOLYSIS AND ACUTE MYOCARDIAL INFARCTION

(Compiled with the assistance of Michael Winniford, MD)

I. Assessment of Efficacy of Thrombolytic Therapy

A. Italian Intravenous Streptokinase (GISSI) Trial, Lancet 1986

Patients	5905 IV SK 5901 Control Randomized, unblinded
Entry criteria	Pain <12 hours ST elevation (96% of patients) or depression (4%)
Protocol	Streptokinase 1.5 million units IV over 1 hour No standard regimen for anticoagulation after treatment
Results	Hospital mortality rate reduced 18% (p=0.0002) in SK group: 13.0% control 10.7% SK

Influence of time to treatment on mortality:

Time from pain to enrollment	Reduction in mortality in SK group	
<1 hour	47%	p=0.0001
≤3 hours	23%	p=0.0005
3-6 hours	17%	p=0.03
>6 hours	4%	NS

Influence of infarct site:	Anterior	21%	p=0.0006 (18.4% vs 14.5%)
	Inferior	6%	NS (7.2% vs 6.8%)

Adverse effects attributed to SK:

Minor bleeding	3.7%
Major bleeding	0.3% (at least 2 unit transfusion)
Allergic reactions	2.4%
Anaphylaxis	0.1%
Hypotension	3.0%
Stroke	0.2% (not different from incidence of stroke in control patients)

Clinical reinfarction rate 2X higher in SK patients than in controls.

Conclusions In this study, intravenous streptokinase reduces 14-21 day mortality in patients with acute myocardial infarction, especially those treated within 3 hours and those with anterior MIs.

B. ISAM Study Group, Berlin (NEJM 1986)

Patients 1741 randomized to IV SK vs placebo

Protocol 1.5 million units SK IV
Time limit - 6 hours

Endpoint 21 day mortality

Findings Insignificant 11% reduction in mortality in SK pts with unexpectedly low mortality rate in control group (7.1%)

Higher global and regional LVEF 3-4 weeks after MI in SK patients:

56.9 SK
53.8 Control ($p<0.005$)

Smaller area under CK-MB time-activity curve in SK patients

Bleeding complications: 5.9% SK
1.5% control

Intracranial bleeding in 4 SK patients

C. Interuniversity Cardiology Institute in the Netherlands Randomized Trial of Thrombolysis (Simoons et al, Lancet 1985 and JACC 1986)

Patients 269 assigned to thrombolytic therapy (234 treated)
152 - IC SK alone (136 treated)
117 - IV SK in ER followed by IC SK (98 treated)
264 conventional treated controls

Randomized, unblinded

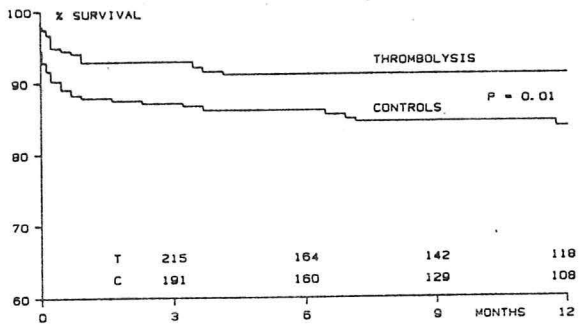
Protocol Time limit 4 hr to CCU admit
Streptokinase 4000 u/min IC
PTCA of high-grade residual stenosis in 46 patients

Results IC alone: patent vessel before SK in 18%
reperfusion of total occl. in 79%
At end of treatment, patent vessel in 83%

IC + IV: patent vessel before IC SK in 41%
reperfusion of total occl. in 67%
At end of treatment, patent vessel in 81%

Results — Hospital mortality rate reduced 47% in SK patients ($p<0.05$)
- 9.8% control
- 5.2% SK

1 year mortality rate reduced 44% ($p=0.01$)
- 16% control
- 9% SK



Life-table analysis of survival in patients allocated to thrombolysis (T) or conventional therapy (C).

LVEF improved 4% in SK patients vs 1% in controls ($p<0.0001$)

Greatest improvement in global and regional LV function in those with SK + PTCA

Complications — Nonfatal reinfarction in 36 (21%) SK
3 (7%) SK + PTCA
16 (6%) control

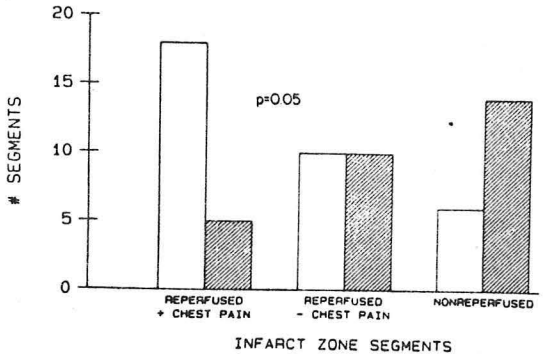
Angiographic reocclusion at late cath in 21% of successfully reperfused patients.

Transfusions in 18% SK
8% controls

II. Patient Selection

A. *Inclusion criteria:* *Pain duration less than 3-5 hours*
ST-segment elevation
No contraindications

Patients most likely to benefit: Early treatment (within 3 hours)
Continued pain at time of Rx
High risk patients
- Anterior MI
- Previous MI
- Rales
- Hypotension
- Age >60
- Shock



Change in regional function between treatment and 10 days in those with and without chest pain. Those with pain at start of Tx have greatest improvement in function (from Topol, JACC 1985)

B. Exclusions

Absolute contraindications

Active internal bleeding
Recent (within 2 months) cerebrovascular accident or neurosurgical prodedure
Recent (<10 days) major surgery, organ biopsy, previous puncture of noncompressible vessels
Recent serious gastrointestinal bleeding
Recent serious trauma, including prolonged cardiopulmonary resuscitation

Relative contraindications

Severe arterial hypertension (>200 mmHg systolic or >110 mmHg diastolic)
Recent minor trauma, including brief cardiopulmonary resuscitation
Hemostatic defects, severe hepatic or renal disease
Advanced age
Diabetic hemorrhagic retinopathy

IV. Protocol for IV Streptokinase Administration

Prophylactic lidocaine infusion
Heparin lock for blood sampling
Percutaneous femoral venous sheath if invasive procedures anticipated (eg. inferior MI with bradyarrhythmias)
Premedication with hydrocortisone or benadryl not routinely used
Streptokinase 750,000-1,500,000 units over 30-60 minutes. Use urokinase (1-2 million units) if SK therapy within previous 6 months. Optimal rate of infusion = 500 units/kg/min
After completion of SK infusion, start heparin infusion (1000 units/hr) to maintain PTT at 1.5-2 times upper limit of normal. Continue heparin for 2-5 days.
Measure fibrinogen, FDP, or thrombin time to insure fibrinolysis.
CPK levels every 6 hr to detect early peak
Substitute aspirin 325 mg/day (dipyridamole probably of no additional value) or warfarin for 3-12 months