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Clot-Selective Coronary Thrombolysis with Tissue-Type Plasminogen Activator

Abstract. *Coronary thrombolysis, an intervention that can abort the sequelae of acute myocardial infarction, was accomplished within 10 minutes in dogs by intravenous administration of clot-selective, tissue-type plasminogen activator. In addition to inducing clot lysis, this promising fibrinolytic agent restored intermediary metabolism and nutritional myocardial blood flow, detectable noninvasively with positron tomography, without inducing a systemic fibrinolytic state.*

Early coronary thrombolysis is being evaluated extensively as a means to abort the sequelae of acute myocardial infarction (*1*). However, its efficacy is difficult to assess and the procedure generally used requires cardiac catheterization (*2*). Intracoronary administration of activators of the fibrinolytic system such as streptokinase and urokinase induce proteolysis in the circulating blood and can induce a lytic state that may lead to systemic bleeding (*3*). Thus, this approach is not without risk and entails unavoidable delay that may limit the efficacy of the intervention. The interval after acute thrombosis during which reperfusion is salutary is clearly limited (*2*, *4*) and the necessity for cardiac catheterization contributes to additional delay.

Physiologically, fibrinolysis occurs only at the thrombus, where the binding of tissue-type plasminogen activator (tPA) to fibrin results in a complex with high affinity for circulating plasminogen (*5*, *6*). Plasmin, produced at the fibrin surface, is protected from interaction with α_2-antiplasmin because its lysine binding sites are occupied. Excess free plasmin forms complexes with circulating α_2-antiplasmin through unoccupied lysine binding sites and is thus inactivated (*5*).

We attempted to determine whether selective coronary thrombolysis could be achieved without induction of a systemic lytic state by intravenous administration of an agent with properties similar to those exhibited by the circulating physiological activator and whether favorable effects on metabolism of previously ischemic myocardium could be elicited. Accordingly, tPA was purified from the culture medium of a melanoma cell line and administered to dogs with induced coronary thrombi. Myocardial perfusion and metabolic integrity were evaluated noninvasively with positron emission tomography (PET) before and after administration of the tPA.

Coronary thrombolysis can be detected unambiguously by serial, coronary arteriography, but objective assessment of the metabolic and functional consequences of myocardium previously rendered ischemic is difficult. Relief of chest pain, rapid resolution of electrocardiographic ST-segment deviations, increases in left ventricular ejection fraction, and diminution of defects in thallium-201 scintigrams have been used as criteria of benefit in patients but are difficult to interpret (*2*). To characterize myocardial perfusion and the metabolic response to reperfusion, we used an approach applicable to patients as well as to experimental animals. Dogs were studied by PET after intravenous administration of ^{15}O-labeled water and ^{11}C-labeled palmitate (*2*, *4*, *7*, *8*).

Twenty-four dogs (20 to 26 kg) were injected subcutaneously with morphine sulfate (1.0 mg/kg) and anesthetized with sodium thiopental (12.5 mg/kg) and α-chloralose (60 mg/kg) administered intravenously. Coronary thrombus was induced by advancing a copper coil, under fluoroscopic control, into the left anterior descending coronary artery (*4*, *9*). Occlusive thrombus, heralded by typical electrocardiographic signs of ischemia, was confirmed angiographically. Dogs that developed ventricular fibrillation after coil placement and coronary thrombosis (N = 7) were excluded.

One to two hours after induction of coronary thrombus, the dogs were selected at random to receive intracoronary (i.c.) or intravenous (i.v.) streptokinase or tPA. Doses of streptokinase for both routes were 4000 IU/min (N = 8 i.c. and N = 6 i.v.) and were given for 90 minutes or longer if thrombolysis was not achieved. Although tPA has high specific fibrinolytic activity, its half-life ($t_{1/2}$) is short (2 to 3 minutes) (*10*). Accordingly, and because we wished to determine whether high dosage would be

Table 1. Effect of streptokinase (SK) and tissue-type plasminogen activator (tPA) in dogs with coronary thrombosis. The tPA was given by the i.c. route to five dogs and by the i.v. route to another five dogs. The SK (4000 U/min) was given to eight dogs by the i.c. route and to six dogs by i.v. administration. All values are expressed as means ± standard error. Differences between groups were assessed with the use of the Mann-Whitney U test; FDP, fibrinogen degradation product.

Agent	Route of administration	Time of onset of lysis (min)	Percentage change in tomographically estimated jeopardized myocardium*	Percentage change after thrombolysis compared to prethrombolytic values†			
				Fibrinogen‡	FDP	Plasminogen	α_2-Antiplasmin
tPA	i.c.	8 ± 1§	−57 ± 18§	−10 ± 7	6 ± 5§	8 ± 17§	20 ± 10§
tPA	i.v.	8 ± 1§					
SK	i.c.	31 ± 2§	−25 ± 12§	1 ± 7	33 ± 18§	−12 ± 14§	−10 ± 10§
SK	i.v.	85 ± 19§					

*The percent salvage of myocardial metabolism assessed after [^{11}C]palmitate administration was calculated as previously described (*4*, *15*). †Values were expressed as percentages of the levels before thrombolytic therapy in order to normalize for variation from animal to animal. ‡Fibrinogen depletion is not anticipated with the dose of SK selected because of two factors: (i) SK binds less avidly to canine than human plasminogen; and (ii) the SK-plasmin complex does not activate plasminogen as efficiently in dogs as in humans (*13*). §P < .05.

limited by induction of a systemic lytic state, 10,000 IU/min for a total of 60 minutes was the dose used for both i.c. ($N = 5$) and i.v. ($N = 5$) administration. Thrombolysis, reflected by reperfusion arrhythmia, was confirmed angiographically. Heparin (10,000 U, i.v.) was administered to prevent reoccurrence of thrombosis after the administration of both streptokinase and tPA. Serial blood samples were obtained to monitor fibrinolysis as well as to determine whether the intervention induced a systemic lytic state.

Myocardial perfusion and metabolic integrity was assessed before and after thrombolytic therapy with PET. The dogs were placed supine in a plexiglass shell and evaluated with the fast-scanning PET VI. To estimate perfusion we used a procedure that quantified the regional distribution of labeled water in the heart after correction for blood pool activity (*8*). We injected 25 to 35 mCi of $H_2{}^{15}O$ ($t_{1/2} = 2.1$ minutes) intravenously as a bolus. Since water becomes distributed in the vascular compartment as well as in the tissue water pool, the $H_2{}^{15}O$ distribution measured tomographically during a 40-second interval beginning 20 seconds after injection of tracer was corrected for vascular radioactivity. Correction was achieved by subtraction of the radioactivity of the vascular pool that was assessed independently after administration of 10 mCi of $C^{15}O$ by inhalation (selectively labeling red blood cells) and repeat tomography. The $H_2{}^{15}O$ myocardial activity assessed in this fashion was closely correlated with the distribution of ^{68}Ga-labeled macroaggregated albumin microspheres (*8*).

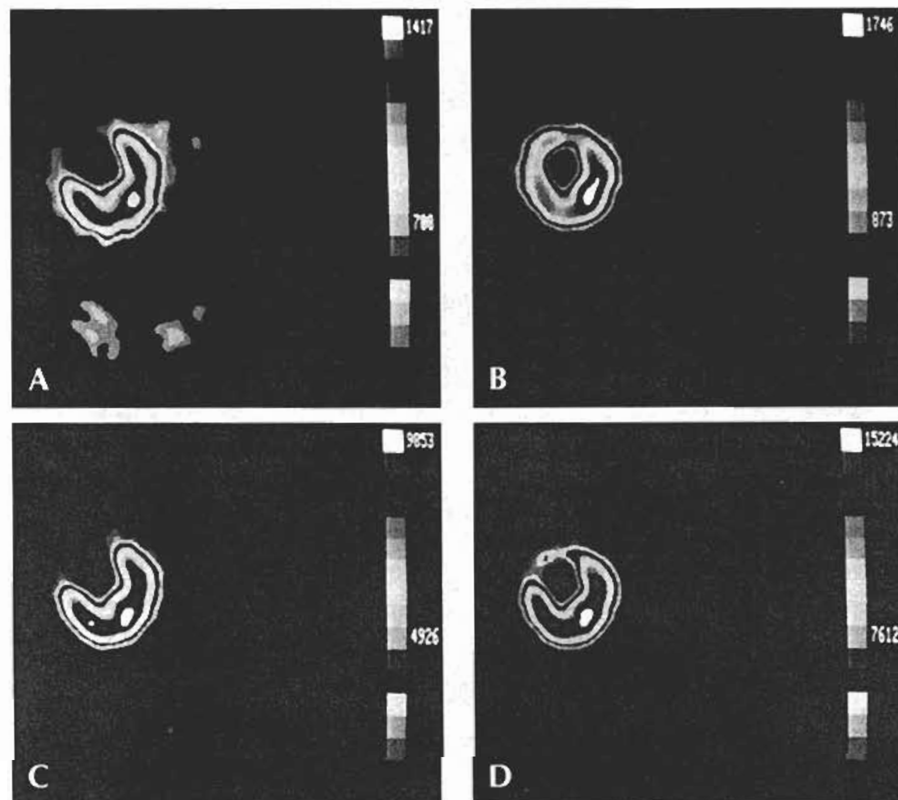

Fig. 1. These reconstructions are from a single mid-ventricular transverse slice obtained with PET before (A and C) and after (B and D) thrombolysis in one dog treated with intravenous tPA. In these tomograms, anterior is to the top, posterior is to the bottom, and the free lateral and septal walls of the left ventricle are to the reader's left and right, respectively. The right ventricle is not visualized because of its thin size. (A and B) Perfusion scans obtained after a bolus injection of $H_2{}^{15}O$ with correction for vascular pool activity with $C^{15}O$-labeled red blood cells (A) before and (B) after thrombolysis. (C and D) Metabolism after the administration of [^{11}C]palmitate (C) before and (D) after thrombolysis. The [^{11}C]palmitate tomograms were corrected analogously for radioactivity in the blood pool by using $C^{15}O$-labeled red blood cells to identify the vascular space. The initial tomograms (A and C) were obtained 1½ hours after induced coronary thrombosis and show a large anterior defect in both (A) perfusion and (C) metabolism scans. The post-thrombolysis scans (B and D) were obtained 1½ hours after the first scan and approximately 80 minutes after thrombolysis following a second administration of $H_2{}^{15}O$, $C^{15}O$, and [^{11}C]palmitate. Restoration of perfusion is virtually complete in (B). Significant restoration of [^{11}C]palmitate accumulation occurred, but a residual metabolic defect persisted in the center of the initially ischemic zone (D), consistent with death of some cells after ischemia of this duration (*4*). Total counts collected were greater than 800,000 in each scan, and the scale factor represents counts per pixel.

Myocardial metabolism was assessed by injecting 15 to 20 mCi of [^{11}C]palmitate ($t_{1/2} = 20.4$ minutes) intravenously and obtaining tomograms beginning 5 minutes after injection of the tracer to allow sufficient time for clearance from the blood pool. Under these conditions, diminished accumulation of radiolabeled palmitate characterizes the locus and extent of impairment of myocardial metabolism induced by ischemia (*4, 7*).

Immediately after completion of initial tomography, streptokinase or tPA was administered by the i.c. or i.v. route. Repeat perfusion and metabolism tomograms were obtained 90 to 120 minutes after initial tomography, by which time residual radioactivity in the heart due to ^{11}C was no longer appreciable. After completion of the tomographic studies, coronary arteriography was repeated. The tPA was isolated from purified culture fluid of a human melanoma cell line as described previously (*11*) and appeared to be identical to circulating activator obtained from human blood (*11*). The streptokinase was obtained commercially from Hoechst-Roussel Pharmaceuticals.

Blood samples, treated with citrate to prevent coagulation, were obtained prior to, during, and after infusion of either streptokinase or tPA, cooled promptly to 0° to 4°C, and centrifuged. One milliliter of plasma was mixed with 250 U of aprotinin (Trasylol; Bayer, Leverkusan, West Germany) to preclude proteolysis in vitro. Plasma samples were frozen and stored at −20°C until analysis of fibrinogen, fibrin (or fibrinogen) degradation products, plasminogen, and α_2-antiplasmin activity as described (*12*).

Both streptokinase and tPA induced coronary thrombolysis. Intracoronary streptokinase (4000 IU/min) elicited thrombolysis in an average of 31 minutes, heralded by electrocardiographically documented typical reperfusion arrhythmia (ventricular ectopic activity and idioventricular rhythm) and confirmed by repeat angiography. When administered by the i.v. route, it induced thrombolysis only after a considerably longer interval, averaging 85 minutes. As shown in Table 1, both i.v. and i.c. tPA elicited thrombolysis rapidly, within 10 minutes ($P < .01$ compared with the interval required for streptokinase). Coronary thrombolysis with tPA was accompanied by restoration of myocardial perfusion and metabolism reflected tomographically as shown in Fig. 1. Initial tomograms obtained 1.5 hours after induction of thrombosis generally showed large defects in the anterior region of the left ventricle in both perfusion and me-

tabolism tomograms. The tomographic reconstructions obtained 90 minutes later demonstrated restitution of perfusion and partial restoration of accumulation of palmitate. The myocardial salvage seen after reperfusion induced with tPA was greater than that seen in dogs in which thrombolysis was induced with streptokinase, perhaps because the earlier lysis resulted in diminished ischemic time. Despite the overall improvement in myocardial metabolism in the previously ischemic zone, a residual metabolic defect in the center of the initially ischemic zone generally persisted, consistent with death of some cells after ischemia of this duration (Table 1) (*4*).

Neither tPA nor streptokinase led to significant depletion of fibrinogen. The lack of a decrease of fibrinogen induced by streptokinase is typical of results in dogs at the dose used and is in contrast to the marked fibrinogen depletion seen in patients given corresponding doses (*3*, *13*).

Analysis of fibrin (or fibrinogen) degradation products, plasminogen, and α_2-antiplasmin blood levels (Table 1) indicated that tPA did not induce a systemic lytic state. In contrast, streptokinase induced a mild systemic lytic state reflected by elevation of fibrin (or fibrinogen) degradation products.

Thrombolysis induced pharmacologically is an attractive therapeutic approach whether clot initiates or simply perpetuates acute myocardial infarction. However, prompt restoration of blood flow is essential (*2*, *4*). When given by the i.v. route, tPA elicits angiographically documented coronary thrombolysis promptly with tomographically demonstrable salvage of myocardial metabolism and perfusion and without induction of a systemic lytic state. The i.v. route of administration is as effective as the i.c. route because of the activator's avidity and selectivity for binding to fibrin. Since it may be possible to produce tPA by recombinant DNA technology (*14*), this agent offers particular promise for widely applicable, prompt, safe dissolution of coronary thrombi accompanied by restitution of metabolism in jeopardized myocardium in patients.

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15. Numerical values (PET numbers) throughout each tomographic slice were printed out with a Varian matrix printer. With the use of the transmission and $C^{15}O$ scan data as a guide to overall ventricular wall dimensions, regions compromised metabolically by ischemia were identified as those with less than 50 percent of peak intramural ventricular counts corrected for background. Jeopardized zones were defined as the number of pixels identified in the area of risk divided by the total number of pixels per slice.
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Genetically Obese Mice: Resistance to Metastasis of B16 Melanoma and Enhanced T-Lymphocyte Mitogenic Responses

Abstract. *The metastasis of B16 melanoma cells differed significantly in obese* (ob/ob) *and lean (+/?) female mice of strain C57BL/6J. When the mice were inoculated subcutaneously with melanoma cells at 10 to 11 months of age, the primary tumor grew more slowly in obese than in lean littermates and the frequency of lung metastasis was greatly reduced. When the mice were injected with the cells at 4 to 7 months, the primary tumor grew at the same rate in obese and lean mice, but the obese mice again showed a significantly reduced frequency of lung metastasis. That this effect was related to an enhanced immunocompetence in obese mice was supported by the finding that splenic lymphocytes of* ob/ob *mice showed three times the proliferative response to the T-cell mitogen concanavalin A compared with the proliferative response of lean control mice. The* ob/ob *mouse may provide a model for the study of enhanced immunocompetence in obese individuals.*

Obese humans often appear to be highly resistant to cancer. Malignancies occur less often in morbidly obese men than in the general population (*1*), and data from the Framingham study indicate that death rates due to cancer decrease steadily with increases in body build for men aged 40 to 69 years (*2*). Obese women show an increased incidence of breast cancer after menopause (*3*) but in younger women the reverse is true, with a negative association between body build and breast cancer risk (*4*). In both males and females, follow-ups of individuals who are initially disease-free indicate that those destined to develop malignancy weigh less at original screening than controls matched for other factors (*5*).

An animal model would be useful for investigating mechanisms that might link obesity to an increased resistance to cancer. One potential model is the genetically obese (C57BL/6J *ob/ob*) mouse, which develops a syndrome of obesity caused by a single recessive gene (*6*). In the experiments reported here we infected obese (*ob/ob*) and lean (+/?) mice with B16 melanoma cells and found that the former were more resistant to the growth and metastasis of this cancer than their lean littermate controls.

We used 58 pairs of genetically obese (*ob/ob*) mice and their lean (+/?) littermates. All animals were female. Sixteen pairs were injected with melanoma cells at 10 to 11 months of age (experiment 1), and 42 pairs were inoculated at 4 to 7 months of age (experiment 2). Ten days before the injection the mice were