Recombinant human tissue-type plasminogen activator therapy in acute thromboembolic stroke

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Systemic fibrinolytic therapy for acute stroke is no longer recommended because of resulting systemic fibrinolysis and the risk of intracerebral hemorrhage. Human tissue-type plasminogen activator (TPA) is a native enzyme that converts plasminogen to plasmin with subsequent clot lysis. The affinity for plasminogen is increased several-fold when the substrate is bound to fibrin. At appropriate dosage, "clot-specific" thrombolysis may be achieved at the surface of the thrombus without creating systemic fibrinolysis.

The authors designed a study to evaluate the effect of intravenous TPA administered 2 hours after acute thromboembolic stroke in rats. This time course was chosen to simulate an analogous clinical situation. Middle cerebral artery embolic stroke was caused by intracarotid injection of 0.025 cc of human blood clot in 16 rats. Regional cerebral blood flow, measured by the hydrogen clearance technique, and electroencephalographic (EEG) recordings were obtained every 30 minutes for 5 hours after thromboembolism. Eight rats received a 1-hour infusion of intravenous TPA (1.5 mg/kg) 2 hours after injection of emboli. Ipsilateral blood flow increased significantly within 30 minutes after intravenous TPA and reached preembolic levels within 60 minutes. Blood flow did not improve in the eight control rats throughout the experiment. Power spectral analysis of the EEG recordings showed improvement in the treated group compared to the control group. Postmortem angiography revealed proximal middle cerebral artery occlusion in control animals and patent middle cerebral arteries in TPA-treated animals. Serum fibrinogen and fibrin split products were unchanged in both groups, indicating the absence of systemic fibrinolysis. There were no intracerebral hemorrhages.

It is concluded that, in this rat model, TPA increases blood flow with subsequent improvement in the EEG recording after thromboembolic stroke without evidence of systemic fibrinolysis. Intravenous TPA may be useful in the treatment of acute stroke in man.

KEY WORDS cerebral ischemia plasminogen activator thrombolysis cerebral infarction rat

The efficacy of intravenous streptokinase or urokinase in thrombolytic therapy for acute stroke has not been proven, and the incidence of systemic fibrinolysis and associated intracerebral hemorrhage has discouraged further clinical investigation. Recently, limited clinical experience with intra-arterial injection of streptokinase or urokinase early after acute stroke has been promising, with a relatively decreased risk of intracerebral hemorrhage. Human tissue-type plasminogen activator (TPA) has a distinct advantage compared to previously available fibrinolytic agents in that it is "clot-selective." The affinity of TPA for circulating plasminogen is low, but there is marked affinity of TPA for fibrin-bound plasminogen. At an appropriate dosage, "clot-selective" fibrinolysis may thus be achieved without creating systemic fibrinolysis, thereby decreasing the theoretical risk of intracerebral hemorrhage. The intravenous route of administration eliminates the time and risk entailed in intra-arterial access, and the relatively short half-life of TPA in humans (approximately 5 minutes) affords excellent temporal control of clot lysis. Since TPA is a human enzyme, the problems with antigenicity experienced with other thrombolytic agents can be avoided.

Recently, TPA has been produced by recombinant deoxyribonucleic acid (DNA) techniques* in sufficient

* TPA produced by Genentech, Inc., South San Francisco, California.
TPA in acute thromboembolic stroke

quantities for laboratory and clinical investigation. Initial experience with TPA therapy for acute myocardial infarction has been encouraging. We have studied the effect of intravenous TPA therapy after acute thromboembolic stroke in the rat in order to determine whether this agent may have a role in the treatment of acute stroke in humans.

Materials and Methods

Sixteen male Sprague-Dawley rats, weighing 350 to 400 mg each, were used in this study. Prior to the experiment, the animals were allowed free access to food and water. The model for cerebral thromboembolism was adapted from that described by Kaneko, et al. The rats were anesthetized with thiameylal sodium, 50 mg/kg intraperitoneally, and atropine sulfate, 0.1 ml intramuscularly. A PE-240 polyethylene tracheostomy tube was inserted, and the animals were placed on a mechanical ventilator. Anesthesia was maintained with a 70% nitrous oxide/30% oxygen mixture. The femoral artery and vein were then cannulated with PE-50 tubing. The animals were paralyzed with Flaxedil (gallamine triethiodide), 10 mg/kg intravenously, and the dose was repeated every hour.

The right carotid artery bifurcation was exposed under an operating microscope. The pterygopalatine artery was identified at the base of the skull and coagulated with a bipolar electrocautery. A temporary clip was then placed at the origin of the external carotid artery. The external carotid artery was cannulated retrogradely with PE-50 tubing, the clip was removed, and the catheter tip was positioned near the origin of the internal carotid artery (Fig. 1). Material injected through the catheter entered the internal carotid artery circulation without altering the blood flow. The neck wound was then closed with surgical clips.

The rat was placed in a stereotaxic head frame. Two craniectomies (3 mm in diameter) were made bilaterally, 1 mm posterior and 5 mm lateral to the bregma. Teflon-coated platinum wire electrodes with bare tips 0.5 mm long and 0.2 mm in diameter were inserted stereotaxically into the parietal cortex to a depth of 0.5 mm. An Ag/AgCl reference electrode was placed in the dorsal aspect of the neck. A platinum reference electrode for electroencephalographic (EEG) recording was inserted subcutaneously over the midfrontal region. The craniectomies were then sealed with acrylic cement.

Regional cerebral blood flow (rCBF) was determined by inhalation of 5% hydrogen gas for 3 to 5 minutes, followed by desaturation. The rCBF was calculated using the 2-minute initial-slope index determined by the least-squares method. Electroencephalographic recordings were obtained and were later analyzed by computerized power spectral analysis. Sample lengths of 40 seconds were digitized, and total power and mean frequency were determined. Mean systemic blood pressure was monitored continuously and remained between 80 and 120 mm Hg. Arterial blood gases were measured periodically: \( \text{pO}_2 \) remained greater than 100 mm Hg, \( \text{pCO}_2 \) was 30 to 40 mm Hg, and pH was between 7.35 and 7.45. Base deficit greater than 5 mEq/liter was corrected with 7% sodium bicarbonate. Body temperature was monitored with a rectal thermometer and was maintained at 37°C with a heating pad.

Human thrombus was prepared by modification of the method described by Kudo, et al. Briefly, 18 hours prior to the experiment, 25 \( \mu \)l of human blood was obtained and allowed to clot at room temperature. The clotted blood was then diluted with 0.3 ml of saline and fragmented once through a No. 27 needle. When measured with a micrometer the clot fragments were found to be 100 to 300 \( \mu \)m. Once baseline rCBF and EEG data were obtained, the thromboembolic material was injected into the internal carotid artery circulation without altering the blood flow. The neck wound was then closed with surgical clips.

Two hours after thromboembolism, eight animals received a 60-minute continuous intravenous infusion of TPA (1 mg/cc in sterile water) for a total dose of 1.5 mg/kg. The eight animals in the control group were treated with a similar volume of sterile water. The animals were sacrificed 5 hours postembolization; prior to sacrifice, 3 cc of blood was obtained for determination of serum fibrinogen and fibrin split products. Postmortem angiography was performed, for which a 75% solution of barium sulfate in gelatin was injected through the left ventricle. The brains were fixed in formalin, photographed, and x-ray films were made.

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† Ventilator manufactured by Harvard Apparatus, Inc., Millis, Massachusetts.
Results

There was no difference in blood pressure, arterial blood gases, temperature, or pulse rate between the control and the treated groups. In all experimental animals ipsilateral rCBF in the middle cerebral artery distribution dropped to less than 10 ml/100 gm/min immediately after thromboembolism. There was no significant improvement in the control group throughout the remaining 5 hours of the experiment. However, rCBF did increase significantly (p < 0.05) within 30 minutes after TPA infusion was begun and reached preembolic levels within 60 minutes (Fig. 2 left). The recovery in rCBF was maintained throughout the 5-hour duration of the experiment in the TPA-treated animals.

Power spectral analysis of the EEG recordings showed a sharp drop in total power immediately after thromboembolism. There was no significant recovery in the control group. On the other hand, the TPA-treated group showed a trend toward improvement in total power, although a significant difference between the control and treated groups (p < 0.05) was only apparent at the 150-minute time point (Fig. 2 right).

Successful postmortem angiography was performed...
angiograms revealed patent middle cerebral artery flow in the TPA-treated animals and nonfilling in the control animals (Fig. 3). All eight control animals had visible intraluminal thrombus in the proximal middle cerebral artery. In four rats that had been treated with TPA there was residual clot adherent to the arterial wall but no reduction in middle cerebral artery flow.

There was no significant change in serum fibrinogen or fibrin split products between the control and treated groups, indicating the absence of systemic fibrinolysis (Fig. 4). Neither group exhibited intracerebral hemorrhages.

Discussion

Stroke is currently the third most frequent cause of death, and occurs at a rate of over 400,000 cases per year in the United States. Presently no specific therapy has proved beneficial for acute stroke. Intravenous thrombolytic agents such as streptokinase and urokinase have not been shown to be beneficial in acute stroke, and the incidence of systemic fibrinolysis and associated intracerebral hemorrhage has discouraged further clinical trials. Streptokinase and urokinase activate fibrinolysis similarly by converting circulating plasminogen to plasmin, free circulating plasmin degrades both fibrin clot and circulating fibrinogen, in addition to inactivating prothrombin, Factor V, and Factor VII, thereby not only lysing clot but also inhibiting the coagulant phase of thrombus formation. The resulting systemic thrombolytic state is marked by a depletion in fibrinogen, plasminogen, Factor V, and Factor VII, with a reciprocal increase in fibrin split products. Additionally, fibrin split products interfere with fibrin multimerization, further limiting thrombus formation, and have an inhibitory effect on platelet aggregation. This sequence of events results in a transient anticoagulant state, and the risk of hemorrhage at the site of injury is increased.

Recently, clinical and laboratory investigations using local intra-arterial streptokinase and urokinase have been encouraging. Therapeutic dosage may be achieved at the site of the intraluminal thrombus by administering a lower total dose, which decreases systemic effects and the risk of intracerebral hemorrhage. Early therapy (less than 6 hours after the ischemic event) was given in a limited number of patients in uncontrolled prospective trials and appears to be efficacious and safe. In previous clinical trials in which systemic intravenous fibrinolytic agents were used, lack of response may have been because, with rare exception, treatment was not initiated within 6 hours of the ischemic symptoms. Neurological improvement has been demonstrated experimentally following restoration of middle cerebral artery flow after up to 6 hours of ischemia. Also, it is believed that reperfusion within 6 hours should decrease the risk of hemorrhage.

Tissue-type plasminogen activator is a recently available thrombolytic agent with many advantages compared to streptokinase and urokinase when used clinically. The affinity of TPA for circulating plasminogen is low relative to the affinity of TPA for fibrin-bound plasminogen. In addition, the reaction rate of conversion of plasminogen to plasmin is increased several-fold in the presence of fibrin. Once generated, fibrin-bound plasmin is relatively protected from degradation by α2 antiplasmin. The combination of these properties results in "clot-selective" thrombolysis. By administering TPA in appropriate doses, clot-specific thrombolysis may be achieved without significant systemic effects. Theoretically, in the setting of acute stroke this should decrease the risk of intracerebral hemorrhage. The intravenous route of administration eliminates the time and risk entailed in intra-arterial access, and the half-life in humans of approximately 5 minutes affords excellent temporal control of clot lysis.

Recent studies of TPA use in acute myocardial infarction have been very promising, demonstrating a reperfusion rate of greater than 70%, however, as demonstrated in the Thrombolysis in Myocardial Infarction Trial, intravenous infusion of large doses of TPA may produce depression of serum fibrinogen and plasminogen, although significant inactivation of Factors V and VII does not occur and a state of systemic anticoagulation is not achieved.

Data regarding thrombolytic therapy for acute stroke in experimental animals are limited. Del Zoppo and coworkers have shown a decrease in infarct size with intra-arterial urokinase given after middle cerebral artery thrombosis in the baboon. Zivin et al. have shown an improvement in gross neurological outcome and mortality following intracarotid thromboembolism treated immediately with intravenous recombinant TPA in the rabbit. The rat model was used in our experiment because, in comparison to other species such as the rabbit, cat, and dog, this animal has the cerebrovascular anatomy that most resembles that of man. Once the anastomotic pterygopalatine artery is ligated, the internal carotid artery vascular supply is isolated as in man. Thromboembolic occlusion of the middle cerebral artery or its branches will lead to focal cerebral ischemia in the appropriate distribution.

We chose to start the TPA infusion 2 hours after thromboembolism because of the practicality of initi-
ating therapy in an analogous clinical setting. Certainly, immediate treatment, as was administered in the previous experimental study using TPA, is not clinically feasible, and later therapy would diminish the degree of neurological recovery. We have demonstrated in this model that focal middle cerebral artery ischemia after intracarotid thromboembolism can be consistently reversed with intravenous TPA infusion, when begun 2 hours after the thromboembolic insult. Blood flow was reestablished within 30 to 60 minutes after beginning the TPA infusion. Improvement in the EEG recording indicated a trend toward recovery of neuronal dysfunction in the treated group. There was no change in levels of serum fibrinogen or fibrin split products, indicating the absence of systemic fibrinolysis and thus reducing the theoretical risk of intracerebral hemorrhage. There were no intracerebral hemorrhages in either animal group.

Although reestablishing blood flow acutely in thromboembolic stroke appears theoretically sound, there is no clear clinical evidence that it is beneficial. We have shown experimentally that intravenous TPA can effectively and reliably reestablish blood flow without significant risk. This is followed by at least a trend toward recovery of neuronal dysfunction as measured electroencephalographically. Whether this translates into improved clinical neurological outcome without significant risk needs further study. We believe TPA offers definite promise in the treatment of acute stroke and deserves further investigation.

Acknowledgment

We thank Mrs. Peggy Hoag for her help in typing this manuscript.

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Manuscript received October 24, 1986.

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