The Effect of Intravenous Tissue-Type Plasminogen Activator in a Rat Model of Embolic Cerebral Ischemia

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Received December 12, 1986

Thrombolytic agents may be useful in the treatment of cerebral ischemia caused by arterial thrombosis or embolic occlusion. A trial of intravenous human tissue-type plasminogen activator (rt-PA) was carried out in seven male Sprague-Dawley rats subjected to embolic cerebral ischemia, with eight control animals. One-hour-old autogenous blood clot was injected into the internal carotid artery. A 30-minute infusion of 10 μg/kg/minute of rt-PA or saline followed. Areas of ischemia at two hours post-embolization were assessed by digital image processing of serial iodo-14C-antipyrine autoradiographic images. The volumes of "no-flow" (NF) and "low-flow" (LF) regions were calculated.

One animal in each group suffered no detectable ischemia; the remainder had well-defined regions of middle and posterior cerebral artery ischemia. No animal sustained a hemorrhagic lesion. Treatment produced no noticeable effect on the patency of cervical vessels. Total NF and LF volumes were less for the treated group but did not reach statistical significance by t-test. In middle cerebral distribution sections, however, LF volume was significantly less (p < 0.05) for treated animals (150 vs. 191 mm³), primarily due to a more significant decrease in LF volume in the anterior-middle cerebral overlap zone (47 vs. 90 mm³; p < 0.025). Fibrinogen levels were not altered by drug treatment (p > 0.30).

INTRODUCTION

Cerebrovascular ischemic events frequently involve a combination of embolic and thrombotic processes. Vascular thrombosis may occur as a result of abnormalities in the vessel intima (including the presence of a natural or foreign thrombogenic substance) or in the characteristics of the circulating blood. Treatment of a thrombotic cerebrovascular occlusion with a thrombolytic agent is a complex situation because of the risk of hemorrhage in an area of the brain in which there is dysautoregulation and ischemic damage to vascular structures. In theory, a thrombolytic agent might prevent expansion of an infarcted area by halting propagation of a thrombus or preventing occlusion of small collateral vessels.

Tissue plasminogen activator (t-PA), a peptide produced by vascular endothelium [1], converts plasminogen to plasmin, a key step in the fibrinolytic pathway. Tissue PA of human origin has recently become available in quantity by means of recombinant DNA technology [2]. Unlike other activators of plasminogen such as streptokinase and urokinase, human PA is effective when given intravenously, has not been shown to produce systemic anticoagulation with fibrinogen degradation, and carries a reduced

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risk of causing peripheral embolization [3,4,5]. In addition, PA is ten times more effective than urokinase in bringing about clot dissolution [6].

The effect of exogenously administered plasminogen activator in the setting of acute stroke due to cerebrovascular thrombosis is unknown. We have developed a model of ischemia involving embolization with homologous blood clot [7], a variant of that described by Kudo et al. [8] and Kaneko et al. [9]. Reproducible regions of hypoperfusion defined by iodo-[14C]-antipyrine autoradiography are produced in the middle and posterior cerebral artery territories secondary to occlusion of proximal vessels at the base of the brain. The purpose of this investigation is to assess the effect of intravenous PA infusion on the volume of ischemic tissue in this model of embolic cerebral ischemia in the rat.

METHODS

Experimental Procedure

A total of fifteen male Sprague-Dawley rats weighing from 314 to 459 grams were used. The animals were anesthetized lightly with ether and injected intraperitoneally with 40 milligrams per kilogram of sodium pentobarbital (Nembutal, 50 mg/cc, Abbott Laboratories). A tracheostomy was performed, followed by insertion of right femoral arterial and venous polyethylene catheters. Arterial pressure was continually recorded via transducer and chart recorder (Grass Instrument Co.). Temperature was monitored by rectal probe and maintained at approximately 37°C. Anesthesia was maintained with intermittent intravenous and intraperitoneal doses of pentobarbital. Arterial blood gases were measured by capillary tube sampling prior to embolization and just prior to sacrifice.

In accordance with the previously described procedure [7], the right pterygopalatine artery was dissected free, ligated with 4-0 silk suture, and coagulated distal to the ligature. The external carotid artery was ligated distally and cannulated with polyethylene catheter (PE-50, Clay Adams; inner diameter 0.58 mm, outer diameter 0.965 mm). With a temporary clip occluding the common carotid artery at the base of the neck, 0.1 cc of homologous clot (derived from approximately 0.7 cc of blood) was injected by hand into the external carotid catheter against resistance over two minutes. The common carotid clip was removed and the catheter withdrawn from the external carotid artery, which was then coagulated.

An intravenous infusion of human tissue-type plasminogen activator (t-PA; Genentech, Inc., South San Francisco, CA) was begun at a rate of 10 μg/kg/minute as the common carotid clip was removed. The drug was diluted in saline to provide a total volume of 1.0 cc. Seven animals received PA for 30 minutes post-embolization (approximately 12,000 IU). Eight animals received 1.0 cc of saline for the same period of time.

Prior to sacrifice at 120 minutes post-embolization, 0.9 cc of blood was withdrawn for fibrinogen determination. A dose of 0.24 milliCuries (mCi)/kg of 4-[N-Methyl-14C]-iodoantipyrine in saline (0.1 mCi/cc, American Radiolabeled Chemicals Inc., St. Louis, MO) was injected intravenously, followed ten seconds later by 2 cc of saturated potassium chloride solution.

Preparation of Tissue

Handling of tissue specimens proceeded as previously described [7]. Frozen sections of brain, 32 microns thick, were taken starting from the olfactory bulbs until occipital
pole tissue was no longer visible, which included the mesencephalon up to the level of the superior colliculus but below the interpeduncular nucleus. Two initial sections were saved from each series of 22 sections, resulting in approximately 50 saved sections per animal. Specimens were incubated with Kodak SB-5 film for six days.

**Volumetric Analysis**

Of two contiguous sections saved, the more intact specimen was chosen for photographic enlargement to 100 times original size. All autoradiographic images were processed by the Quandens image analysis system to define areas of "no perfusion" (of density equal to background) and "low perfusion" (20 percent of maximal density), which were then delineated on the enlargements [7]. Thus density and not actual blood flow was used to define regions of reduced flow. The areas of interest in the left and right halves of the hemispheres, brain stem, and diencephalon were measured for each image, using a microcomputer bit pad (Summagraphics Bit Pad One), as were the total areas of these specimens. The results of two measurements were averaged for each section. Correction for magnification in the process of enlargement was accomplished by measurement of an enlarged 10 mm by 10 mm standard. Volumes were then calculated, using the averages of the two analyzed sections to represent the entire segment of discarded slices which separated them.

**RESULTS**

One animal in each group failed to demonstrate significant regions of hypoperfusion (number 8 and number 15). Comparisons were made between the remaining 13 animals for purposes of assessing drug effect.

**Physiologic Measures, Anesthesia, and Fluids**

Mean animal weights for the two groups were not significantly different by one-tailed t-test (mean ± standard deviation, 413 ± 52 grams for the control group, \( n = 7 \); 382 ± 20 g for the treated group, \( n = 6 \)). Similarly, mean arterial pressures, arterial pO2, pCO2, and pH pre-embolization and pre-sacrifice were not significantly different (refer to Table 1). However, post-embolization pCO2 was significantly less than pre-embolization values for both groups (\( p < 0.05 \)). Mean arterial pressure fell in both groups, reaching statistical significance only in the treated group (\( p < 0.05 \)).

|                  | MAP (mm) | pO2 (mm) | pCO2 (mm) | pH       |
|------------------|----------|----------|-----------|----------|
| Control          |          |          |           |          |
| Pre-embolization | 134 ± 7  | 79 ± 9   | 40 ± 4    | 7.385 ± 0.08 |
| Pre-sacrifice    | 131 ± 19 | 88 ± 17  | 35 ± 4    | 7.412 ± 0.06 |
| p value          | NS       | NS       | NS        | NS       |
| Treated          |          |          |           |          |
| Pre-embolization | 129 ± 9  | 81 ± 19  | 39 ± 3    | 7.403 ± 0.05 |
| Pre-sacrifice    | 119 ± 8  | 88 ± 17  | 34 ± 4    | 7.428 ± 0.04 |
| p value          | <.05     | NS       | NS        | NS       |

(p < 0.05)

(\( p < 0.05 \))
TABLE 2

| No. | Total Brain Volume (mm$^3$) | Right Brain Volume (mm$^3$) | NF Volume (mm$^3$) | LF Volume (mm$^3$) | NF % of Right Brain | LF % of Right Brain | NF/LF Fibrinogen mg% |
|-----|-----------------------------|-----------------------------|--------|-----------------|-----------------|-----------------|--------------------|
|     |                             |                             | NF     | LF              | NF/LF           |                 |                    |
|     |                             |                             | Volume | Volume          | Volume          |                 |                    |
|     |                             |                             | (mm$^3$)| (mm$^3$)        | volume%         |                 |                    |
|     |                             |                             |        |                 |                 |                 |                    |
| Control Animals | | | | | | | |
| 1   | 1,800                       | 912                         | 339    | 564             | 37.2            | 61.8            | 60.1               |
| 2   | 1,655                       | 913                         | 214    | 385             | 23.5            | 42.2            | 55.6               |
| 3   | 1,823                       | 889                         | 471    | 651             | 53.0            | 73.2            | 72.4               |
| 4   | 1,813                       | 897                         | 417    | 583             | 46.5            | 65.0            | 71.5               |
| 5   | 1,692                       | 886                         | 117    | 263             | 13.2            | 29.6            | 44.4               |
| 6   | 1,628                       | 899                         | 283    | 467             | 31.5            | 51.9            | 60.6               |
| 7   | 1,618                       | 895                         | 176    | 328             | 19.7            | 36.6            | 53.8               |
|     | 1,718                       | 899                         | 288    | 463             | 32.1            | 51.5            | 59.8               |
|     | SD                          | 91                          | 10     | 129             | 144             | 14.5            | 9.9                |
| Treated Animals | | | | | | | |
| 9   | 1,893                       | 945                         | 273    | 420             | 28.9            | 44.4            | 65.1               |
| 10  | 1,591                       | 885                         | 172    | 330             | 19.4            | 37.3            | 52.2               |
| 11  | 1,592                       | 896                         | 119    | 237             | 13.3            | 26.5            | 50.2               |
| 12  | 1,662                       | 908                         | 225    | 390             | 24.8            | 43.0            | 57.7               |
| 13  | 1,836                       | 909                         | 189    | 358             | 20.8            | 39.3            | 53.0               |
| 14  | 1,520                       | 925                         | 328    | 461             | 35.5            | 49.9            | 71.2               |
|     | 1,682                       | 911                         | 218    | 366             | 23.8            | 40.1            | 58.2               |
|     | SD                          | 149                         | 21     | 75              | 78              | 7.8             | 8.3                |

There is no clear explanation for this phenomenon. Although the drop was not great, it should serve to increase the volume of ischemic tissue, lessening the anticipated drug effect. Arterial blood gas values for the two animals without substantial perfusion defects were well within the ranges measured for the other animals. The mean arterial pressure for one of these (number 15), was higher than average (150 mm Hg pre-embolization and 144 mm Hg pre-sacrifice).

The control animals received an average of 0.06 ± 0.01 mg/kg of pentobarbital during the experiment, as compared with 0.07 ± 0.02 mg/kg for the treated group. The difference was not statistically significant. Animals 8 and 15 (without significant lesions), received doses which were well within the range given to the other animals. Controls and treated animals received 0.02 ± 0.01 cc of saline per gram during the experiment. The two animals without quantifiable lesions were given fluid doses well within the observed range.

**Observations**

Treatment with PA produced no noticeable effect on the patency of cervical vessels. All cervical internal carotid arteries appeared to contain significant blood clot except for those of animals 6, 7, and 12. The common carotid arteries of animals 2, 3, 9, 11, and 15 also appeared darkly discolored and barely pulsatile. But for number 8, the proximal middle and posterior cerebral arteries at the base of the brains in both drug-treated and control animals appeared to harbor clot. Animal number 8 demon-
FIG. 1. Rostral-caudal anatomic map of affected regions for animal number 11, with smallest LF (light hatching) and NF (dark hatching) volumes. Based on atlas of König and Klippel, used with permission.

A, cerebral aqueduct; AC, anterior commissure; AM, amygdala; CC, corpus callosum; CP, cerebral peduncle; D, dentate nucleus; ENT, entorhinal cortex; F, fornix; GP, globus pallidus; H, hippocampus; IC, internal capsule; IPN, interpeduncular nucleus; LGN, lateral geniculate nucleus; MGN, medial geniculate nucleus; NA, nucleus accumbens; NC, nucleus caudatus; OB, olfactory bulb; SC, superior colliculus; SN, substantia nigra; TH, thalamus.

Stratified a small proximal middle cerebral artery (MCA) clot. Animal number 15, the treated animal with no significant lesion, had an average amount of blood clot in middle cerebral and internal carotid arteries by visual inspection.

Except for number 15, hyperemia of the cortical surface of the right hemisphere was observed in all treated animals. The control animals with lower than average “no-flow” (NF) volume (numbers 2, 5, and 7) also demonstrated hemispheric hyperemia.

No hemorrhagic lesions were identified on inspection of brain sections of either control or drug-treated animals.

Volumetric Data

Table 2 shows the volume values for both control and treatment groups, as corrected for a mean total brain volume of 1,702 mm³ for the two groups. The total volumes of hypoperfused tissue, both “no-flow” (NF) and “low-flow” (LF), was less for the treatment group but did not reach statistical significance by the one-tailed t-test at the 0.05 level of significance. There were no differences in the percentage of the right brain involved, percentage of total brain involved, the maximal NF area percentage for a given section, or NF/LF ratios for the two groups.

Figure 1 is a reconstruction of the anatomic location of areas affected in a treated animal (number 11) with the least NF and LF volumes based upon histological
identification of major anatomic structures [10]. Sequential histograms of right brain volume, NF volume, and LF volume demonstrate in standardized form the distribution of affected tissue for all animals (Fig. 2). The greatest region of ischemia is in the MCA distribution. As compared with the control animals, there is a significant reduction in lesion size in the lateral cortex, internal capsule, and lenticular nuclei. The hippocampus is nearly totally spared, as is the brain stem. There is almost no posterior cerebral distribution involvement. As can be seen from the histogram plots, the distribution of ischemic regions does not seem to differ substantially between the control and treatment groups except that the drug-treated animals seemed to show a greater amount of posterior cerebral sparing. If one considers the percentage of involvement in the MCA distribution alone, however, one finds that the volume of LF ischemic tissue from sections 10 through 16 inclusive (from just caudal to the caudate head to mid-thalamus) is less for the treated animals (LF volume mean of 191 ± 45 mm³ for the control group and 150 ± 22 mm³ for the treatment group; \( p < 0.05 \)). NF differences in this region do not reach statistical significance (124 ± 52 mm³ for control animals; 95 ± 23 mm³ for treated animals).

Further analysis of differences in average NF and LF volumes for control and drug-treated animals based on individual volume section size from rostral to caudal (Fig. 3) revealed a noticeable increase in average difference in sections 7 to 12 (between anterior cerebral artery and MCA primary distributions) and in sections 20 to 24 (posterior cerebral artery distribution).

LF volume for the anterior region (sections 7 to 12) was significantly less for treated animals (47 ± 26 mm³, 24.3 ± 11.9 percent of right brain) as compared with control animals (90 ± 40.9 mm³, 42.7 ± 17.7 percent; \( p < 0.025 \)). NF volumes were also reduced but did not reach statistical significance due to a greater amount of variability (23.6 ± 19.4 mm³ for the treatment group vs. 49.8 ± 39.3 mm³ for controls; \( p < 0.10 \)). Average NF/LF ratio was 0.55 for the control group and 0.50 for the treated group in this region.

LF and NF volumes for the posterior region (sections 20 to 24) were not significantly different for treated animals, concomitant with a greater amount of regional variability of involvement with this model. Average LF volume for the treatment group was 97.1 ± 41.7 mm³, as compared with 131.4 ± 65.4 mm³ for controls.

Finally, fibrinogen levels drawn just prior to sacrifice were not significantly different in the control and treatment groups (Table 2).

**DISCUSSION**

The use of fibrinolytic agents and anticoagulants in the setting of acute cerebral ischemia has been problematic. Attempts to use urokinase [11,12] and streptokinase [13] in the treatment of cerebral infarction have resulted in hemorrhagic complications without therapeutic benefit. The use of heparin is as yet controversial [14].

Plasminogen activator, produced by vascular endothelium, promotes fibrinolysis. PA is thought to play a role in the maintenance of vessel patency under normal conditions. In addition, as its activity is inhibited by circulating alpha₂-antiplasmin, PA primarily works within the protective fibrin matrix of a thrombus, sparing circulating fibrinogen, and effecting significant fibrinolysis in thrombosed vessels [15]. Patients with non-acute cerebrovascular disease have been found to have reduced plasminogen activator levels [16] and defective fibrinolysis [17]. A family group with
FIG. 2. A. Sequential rostral-caudal histograms for control group. Anatomic diagram from atlas of König and Klippel, used with permission. B. Sequential rostral-caudal histograms for treated group.
early and frequent onset of cerebrovascular disease associated with defective release of vascular plasminogen activator has been identified [18].

In this study using a rat model of cerebral ischemia, the intravenous administration of t-PA resulted in a significant difference in the volume of “low-flow” tissue in the control and treated animals in the middle cerebral artery vascular distribution, apparently owing to a greater relative increase in perfusion in the anterior-middle cerebral region of overlap in the treated animals. There was no consistent effect of the drug on the apparent patency of the cervical carotid system or on the presence of clot in the arteries of the circle of Willis, although this factor was not quantified; fibrinogen levels were unaffected by drug treatment, as expected.

The small reduction in total ischemic volumes in the treated animals as compared with controls was overshadowed by the wide range of values obtained, and the relative contribution of middle cerebral artery flow to overall brain perfusion. In this model, the anterior cerebral distribution escapes involvement. The greatest variability occurs in the posterior cerebral distribution. The failure to demonstrate a difference in ischemic volumes in this same vascular distribution was probably due to this inherent variability. In addition, NF volumes demonstrate greater variation than LF volumes, making NF volume a less sensitive measure of therapeutic effect.

The only observable factor which seemed to correlate with a reduction of size of the affected region was the presence of hyperemia. All of the treated animals had a hyperemic hemisphere, in spite of the generally lower pCO2. The three control animals with smaller than average NF and LF volumes also had hemispheric hyperemia. The significance of hyperemia in terms of effective cerebral flow is not well established. Post-embolic hyperemia may be associated with a change in the position of the embolus with partial or complete reperfusion [19].

Factors which are likely to affect the results of this or other experiments with
exogenously administered plasminogen activator include the activity of human tissue-type PA in animal species, the age of the occluding thrombus, and the degree of vessel occlusion.

The rat was chosen for this study as an inexpensive and readily available animal in this reliable model of embolic cerebral ischemia. Small amounts of plasminogen activator would be necessary because of the small animal size. However, rat tissue plasminogen activator, although immunologically similar to human plasminogen activator [20], is functionally different. In vitro studies of human tissue-type PA [21] have shown that, as compared with 95 percent dissolution of human clots, only 10 percent dissolution occurs with rat clots in the same period of time (80 percent for primate clots, 60 percent for cat and rabbit, and 30 percent for canine clots). A 30-minute infusion time and a dose of 12,000 IU was used in our study in order to compare favorably with the dose and time course of drug administration used in previous studies: human tissue PA will effectively lyse intravascular fibrin deposits in rat lung when given in doses of 300 IU or more over one hour [22]. In a rabbit model, a 1 mg/kg infusion of t-PA over 30 minutes following cerebral embolization with autogenous clot is effective in preserving neurological function [23]. Plasminogen activator has been shown to produce thrombolysis in canine femoral vein [3] and coronary arteries [4,5]; clot dissolution was observed in 10 to 14 minutes with doses of 10 μg (1,000 IU)/kg/minute intravenously; however, heparin was also administered in these studies to prevent recurrent thrombosis. Heparin was avoided in this study in order to focus on the effects of PA infusion.

Non–cross-linked clots lyse more extensively than totally cross-linked clots. In the rabbit model of jugular vein thrombosis induced by foreign body, 100,000 IU of PA infused over four hours produced 75 percent lysis of fresh clots and 35 percent lysis of one-day-old clots [24]. As cross-linking takes approximately one hour, there is no significant difference in the amount of clot lysed if the sample is between one hour and 24 hours old [21]. Thus in our study the exact age of the clot should not be relevant if it is more than one hour old. One must bear in mind, however, that the effect of immediate intervention in this model of stroke may poorly translate into a useful clinical effect in humans where the opportunity to intervene within the first hour of ischemia is infrequent. The passage of time may convert a partial occlusion to a total occlusion, and PA may be most effective with partially occluding thrombi. Mattson et al. [25] showed that, in the rabbit, a two-hour infusion of 0.5 mg (approximately 50,000 IU) produced a 60 percent decrease in the weight of a partially occluding thrombus but no significant thrombolysis in totally occluded vessels.

The relative resistance of rat blood clot to the effects of human PA is a limiting factor which may be overcome by the use of greater doses or duration of therapy. An assessment of dose-responsiveness would be of interest. Smaller volumes of clot could be used, but this generally results in less reproducible (and bilateral) lesions. The variability encountered with an embolic model of cerebral ischemia is a significant factor in assessment of a treatment regimen meant to reduce the ischemic effects of cerebral emboli.

Plasminogen activator as used in this study may improve cerebral blood flow only in partially occluded vessels, that is to say, those on the periphery of the region subjected to total vascular occlusion. Partial occlusion may occur not only because of a relatively small embolus, but also because of a regional low-flow state with secondary platelet aggregation, with or without ischemic endothelial injury. As vascular plasminogen
activator is thought to play an important role in the normal regulation of vascular patency, one may hypothesize that exogenously administered PA may be effective in situations of non-embolic cerebrovascular occlusion.

ACKNOWLEDGEMENTS

The authors would like to acknowledge Dr. Dennis Spencer for his helpful suggestions and careful reading of the manuscript, and Dr. Carole LaMotte for supplying the Quandens imaging system.

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