AJNR

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AJNR Am J Neuroradiol 1988, 9 (5) 899-902
http://www.ajnr.org/content/9/5/899

Selective Embolization and Clot Dissolution with tPA in the Internal Carotid Artery Circulation of the Rabbit

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We describe a model of thromboembolic stroke in rabbits that utilizes the Seldinger technique and digital arteriography. The internal carotid arteries of 14 rabbits were catheterized selectively and embolized with autologous blood clots. After embolization, eight rabbits received IV tissue plasminogen activator (tPA); the remaining six were infused with saline and served as controls. After embolization, cerebral arteriograms were obtained at 30-min intervals for 180 min. Cerebral arteriograms obtained after tPA therapy revealed partial or complete thrombus dissolution in seven (88%) of the eight treated rabbits. In the control group, none of the arteriograms of the embolized internal carotid arteries showed thrombus dissolution. In the tPA-treated group, the median time for thrombus dissolution was 60 min.

This stroke model is economical, reproducible, and less traumatic to the brain than most of the previously described animal models. It also provides a means to compare the safety and efficacy of various thrombolytic agents in small animals.

The success of IV tissue plasminogen activator (tPA) in dissolving acute thrombi and restoring blood flow in the coronary arteries of humans has made clinicians optimistic that acute thromboembolic stroke may be treated in a similar fashion. However, before large clinical trials are begun, experiments with thrombolytic therapy in animals with acute cerebral arterial thromboemboli are needed. To determine the safety and efficacy of IV tPA in the treatment of thromboembolic cerebral ischemia and/or infarction, we developed a thromboembolic stroke model in rabbits. This article describes the model and recounts our experience with IV tPA in dissolving thrombi within the intracranial branches of the internal carotid arteries of rabbits.

Materials and Methods

Fourteen New Zealand white rabbits (2–3 kg each) were used in this study; eight were treated with tPA* and six served as controls. All animals were anesthetized with ketamine sulfate (15–20 mg/kg) and Rompun (5 mg/kg) administered intramuscularly. After the animals were effectively anesthetized, an incision was made in a hind leg, and the common femoral artery and vein were isolated. A 22-gauge angioset was positioned within the common femoral vein and a normal saline infusion was begun and maintained at a rate of 0.5 ml/min. The Seldinger technique was then employed to place a 3-French end-hole catheter into the common carotid artery (usually the right common carotid). The following technique was used: a 21-gauge minicath from which the delivery port was cut was inserted into the common femoral artery, a 0.018-in. straight guidewire was passed through the butterfly needle into the aorta, the needle was removed, and the 3-French end-hole catheter was passed over the guidewire into the aorta and positioned within its descending part. The 0.018-in. guidewire was removed and the catheter flushed with 3 ml of normal saline; a 0.014-in. steerable guidewire with a curved platinum tip was then used to guide the catheter into the common carotid artery. A 3-ml bolus of diatrizoate megalumine and diatrizoate sodium† was injected into the common carotid artery at a rate of 2 ml/sec, followed by rapid imaging (three frames/sec) of the rabbit's cerebral arteries in the lateral projection. Images of the cerebral vasculature

Received June 29, 1987; accepted after revision March 17, 1988.

This work was supported in part by grants from The Memorial Hospital Foundation and BRSG 632837.

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AJNR **9:899–902, September/October 1988**
0195–6108/88/0905–0899

* Genentech, Inc., South San Francisco, CA.
† Renografin-76. Squibb Diagnostics, New Brunswick, NJ.

were acquired with a Fisher-Adac digital subtraction unit. The rabbits were placed on a Bird respirator, which was set at 40–50 cycles/min, to maintain respiration at the time of embolization.

The internal carotid artery was identified and, using the steerable guidewire,[‡] the 3-French catheter was positioned within the proximal internal carotid artery. The aged (18 hr) thrombus (0.035 ml) was selectively embolized distally, and it usually completely occluded the ipsilateral distal internal carotid artery and its intracranial branches. The catheter was then pulled into the common carotid artery and another lateral cerebral arteriogram obtained. If the criterion for successful embolization was met—that is, complete occlusion of the anterior cerebral and/or the middle cerebral artery—thrombolytic therapy was begun. Selective internal carotid arteriograms after embolization were not done in order to minimize spasm caused by catheter manipulation. Severe internal carotid artery spasm may result in reduced cerebral blood flow, thereby reducing the amount of circulating tPA that reaches the site of thrombosis. We also concluded that a common carotid artery injection was less likely to cause alterations in blood flow to the internal carotid artery.

All treated rabbits received 1 mg/kg of tPA beginning 15 min after embolization. Twenty percent of the total dose was administered as an IV bolus and the rest was dripped in over a 30-min period in an attempt to optimize the thrombolytic state within the rabbit. The control group received similar treatment with a saline infusion. At the end of the drip infusion, a lateral cerebral arteriogram was obtained and repeated at 30-min intervals until 180 min had elapsed. Each 30-min arteriogram was viewed by two of the principal investigators for presence or absence of thrombus dissolution. A concensus of opinion was required. A nonparametric Mann-Whitney U Test was used for data analysis because the scale of measurement times analyzed was not continuous but obtained at 30-min intervals. A median score for time to thrombus dissolution was obtained for each of the two groups.

The brains from all but one animal were removed postmortem, fixed, stained, sectioned, and examined for evidence of infarction and hemorrhage. The results of this neuropathologic investigation are presented in a separate publication [1].

Results

The cerebral arterial anatomy of the rabbit as depicted by digital subtraction angiography is shown in Figure 1. In our series, the internal carotid artery originated as the first branch from the dorsum of the common carotid artery 86% of the time. In 14% of the rabbits, the internal carotid artery and the occipital artery formed two separate branches from a common trunk that took its origin as the first branch off the dorsum of the common carotid artery.

Characteristically, after embolization, the column of contrast material abruptly ended at the site where the thrombus lodged in the internal carotid artery or its anterior or middle cerebral branches. The abrupt ending caused a squaring effect, as demonstrated in Figures 2B, 3B, and 4B. The loss of this squaring effect and antegrade contrast flow within the anterior and/or middle cerebral arteries was interpreted as evidence of thrombus dissolution (Figs. 2C and 3C). All images were analyzed as they were repetitively displayed on the digital subtraction monitor. After both observers committed themselves to an impression, films of selected images were obtained. In the tPA-treated group, the thrombus dissolved within 30 min in one rabbit, 60 min in four rabbits, and 90 min in two rabbits; the remaining rabbit in the tPA-treated group had no visible thrombus dissolution during the 180-min observation period in which cerebral arteriograms were obtained.

[‡] C. R. Bard, Inc. (USCI), Billerica, MA.

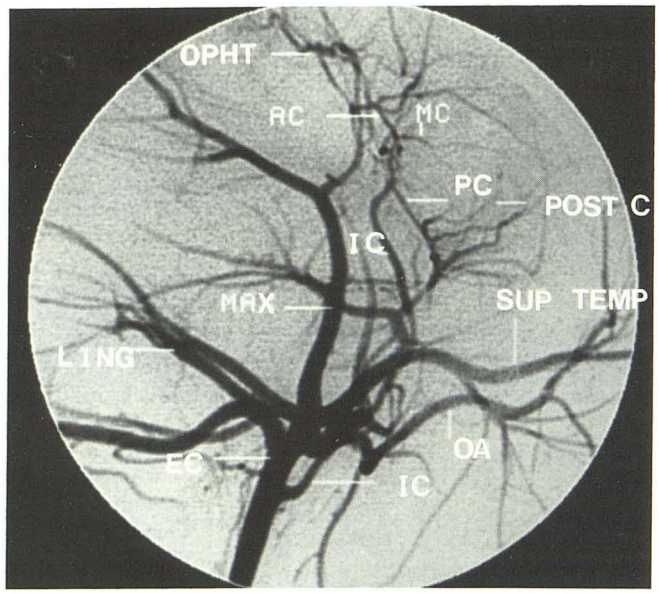

Fig. 1.—Lateral view of right common carotid arteriogram in a rabbit. Internal carotid artery originates as first branch off dorsum of common carotid artery and continues rostrally on a diagonal course away from common carotid until it exits from petrous bone and abruptly angles anteriorly at about 45° for a short course before reversing direction at almost a 90° angle and giving off its cerebral branches. IC = internal carotid, AC = anterior cerebral, MC = middle cerebral, PC = posterior communicating, POST C = posterior cerebral, EC = external carotid, LING = lingula, OA = occipital artery, SUP TEMP = superficial temporal, MAX = maxillary, OPHT = ophthalmic.

None of the control rabbits demonstrated thrombus dissolution as evidenced by reestablished flow of contrast material within the previously occluded distal internal carotid artery and/or its branches (Fig. 4). The tPA-treated group demonstrated an 80% success rate with a median thrombus dissolution time of 60 min ($p = .015$).

Cerebral infarcts within the distribution of the embolized ipsilateral internal carotid artery occurred in all rabbits; there were no qualitative differences between the control and treated animals in the size, distribution, and histologic characteristics of these infarcts [1]. Scattered microhemorrhages were identified within some infarcts in both control and tPA-treated animals, but neither group demonstrated gross hemorrhages.

Discussion

Our success with IV tPA therapy in rabbits for treating acute thrombosis within the distal internal carotid artery and/or its branches is similar to that reported in clinical trials of IV tPA therapy to relieve acute coronary thrombosis in humans [2–5]. We were successful 80% of the time in restoring blood flow within the rabbit's distal internal carotid artery and its branches, which had been completely occluded acutely with aged (18 hr) autologous clot. None of the controls showed

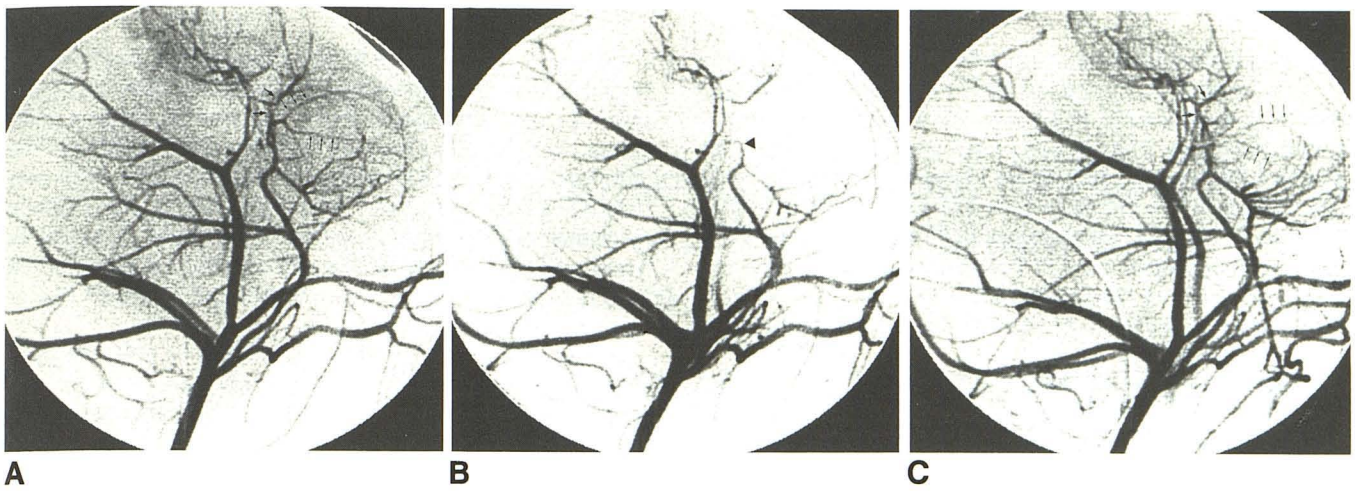

Fig. 2.—*A*, Cerebral arteriogram of a rabbit before IC embolization. *Large arrows* outline the course of AC artery. *Small arrows* outline MC artery.

B, Cerebral arteriogram of same rabbit after embolizing IC artery with 0.035 ml of autologous thrombus. IC artery (*arrowhead*) abruptly occludes about 1 cm beyond its PC branch. Note square appearance of the occlusion and absence of contrast within AC or MC arteries.

C, 60 min after the beginning of tPA therapy, AC (*large arrows*) and MC (*small arrows*) arteries demonstrate antegrade blood flow. MC artery branches do not fill as far distally as before (Fig. 2A), which suggests there is increased resistance to blood flow as a result of residual thrombi and/or vasospasm.

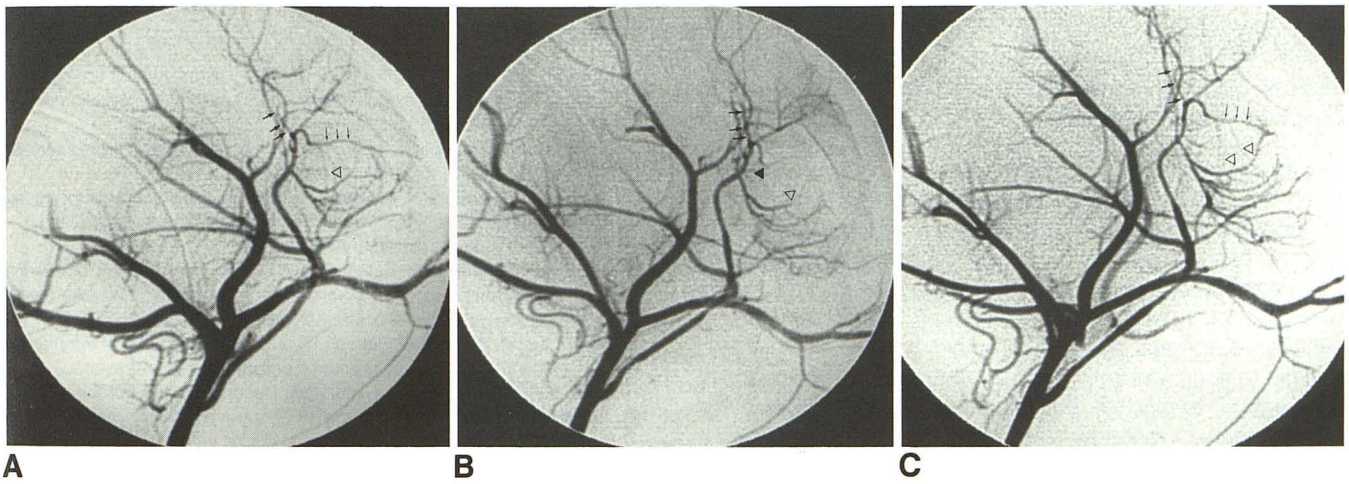

Fig. 3.—*A*, Cerebral arteriogram of a rabbit before IC embolization. *Large arrows* outline the course of AC artery. *Small arrows* outline the course of MC artery. POST C artery branch course is outlined by *open arrowhead*.

B, Cerebral arteriogram of same rabbit after embolizing IC artery with 0.035 ml of autologous thrombus. There is squaring of the contrast column within MC artery (*closed arrowhead*). There is similar square appearance to occlusion within POST C artery (*open arrowhead*). AC artery size has increased, presumably because of additional blood flow (*arrows*).

C, Cerebral arteriogram of rabbit shown in Fig. 3B 60 min later, after IV tPA therapy. There is reappearance of contrast flow within MC (*large arrows*) and POST C (*arrowheads*) arteries. Size of AC artery (*small arrows*) is back to normal.

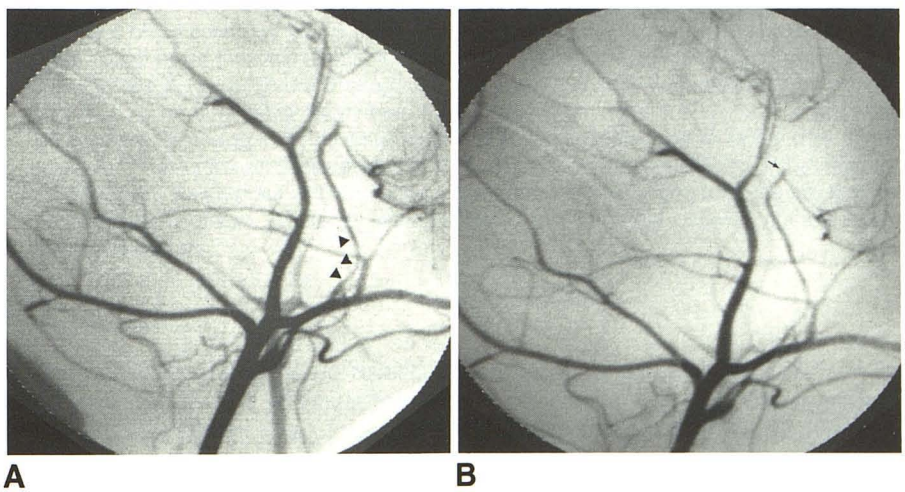

Fig. 4.—*A*, Cerebral arteriogram of a control rabbit immediately after embolization of 0.035 ml of autologous clot to distal IC artery. Note areas of narrowing (*arrowheads*) in IC artery, suggesting spasm.

B, Cerebral arteriogram of same control rabbit 180 min after embolization. *Arrow* shows square appearance of remains of occluded IC artery. Note that IC artery spasm has increased.

angiographic evidence of restoration of blood flow. Kamijyo et al. [6] suggested that reperfusing an ischemic brain region prior to 6 hr may result in significant protection of the corresponding tissues. The relatively rapid time to clot dissolution (median, 60 min) and lack of significant hemorrhage with IV tPA therapy raises optimism that this agent could be safely used to treat acute stroke resulting from thromboembolic disease. Enthusiasm for the possible success in restoring blood flow within a reasonable time before irreparable brain tissue damage occurs is in order. However, this assumption remains to be proved.

Centeno et al. [7] selectively embolized 0.75 ml of 1-hr-old thrombus (pretreated with thrombin) into the common carotid artery of rabbits and 1 hr later treated them with intraarterial streptokinase on the ipsilateral side. Common carotid arteriograms were obtained hourly up to 6 hr to assess thrombus dissolution. The resulting data did not indicate a definite shortening of time of thrombus dissolution in the streptokinase group versus the control group. Our more positive results may be explained in the following manner. First, we embolized a smaller amount of thrombus (0.035 ml versus 0.75 ml), which may have increased the likelihood of accelerated dissolution by tPA. Second, as has been suggested by other studies [2, 5], tPA has a thrombolytic action superior to that of streptokinase. Streptokinase forms a complex with circulating plasminogen, which converts circulating uncomplexed plasminogen to plasmin. Freely circulating plasmin degrades fibrin and fibrinogen and inactivates prothrombin, factor V, and factor VIII, with the result that streptokinase therapy has a tendency to cause significant bleeding as a side effect. Decreased circulating plasminogen may also serve to reduce the number of activated streptokinase plasminogen complexes, thereby reducing thrombolytic activity. tPA binds directly to fibrin and activates only fibrin-bound plasminogen, converting it to plasmin. Plasmin bound to fibrin is protected from rapid inactivation by alpha^2-antiplasmin, which allows it to effectively digest fibrin thrombi. This fibrin-specific property of tPA is thought to make it a superior thrombolytic agent and less likely to produce severe hemorrhage when used in moderation, as in the thrombolysis in myocardial infarction (TIMI) trials [2].

Our model offers several advantages over many earlier stroke models. It is economical, easily reproducible, and more physiological than most in that it requires no surgical intervention or tying off of the carotid artery or its branches. The latter advantage avoids excessive trauma to the brain and thus allows for better pathologic analysis of the cerebral hemispheres. The stroke created in the rabbit is similar to human thromboembolic stroke and enables the study of the angiographic efficacy and neuropathologic safety of tPA or other thrombolytic agents.

In conclusion, the immediate initiation of IV tPA therapy in eight rabbits after acute occlusion of their distal internal carotid artery with autologous clot resulted in rapid dissolution of the clot in seven animals without causing major hemorrhagic side effects.

ACKNOWLEDGMENTS

We thank Genentech, Inc., for kindly donating the tPA, and Janet Lambert for help in preparing the manuscript. We also thank Vanessa Brown and James Staruk for their technical assistance.

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