Streptokinase Clot Lysis in Acute Occlusions of the Cranial Circulation: Study in Rabbits

Ricardo S. Centeno[1]
David B. Hackney[1,2]
John R. Rothrock[3]

An animal model of acute cerebrovascular thromboembolism was developed to evaluate the feasibility of selective fibrinolysis. In 20 Flemish Giant rabbits, autologous clot was deposited via selective catheterization of the distal end of the common carotid artery. The rates of clot lysis with streptokinase were compared in eight control rabbits and after three different dosage regimens in four rabbits each. Group A received selective infusion of 5000 U/hr; group B received 4000 U/min for 1 hr, 3000 U/min for 2 hr, and 5000 U/hr for 2 hr; and group C was treated similar to group B plus receiving an initial 20,000 U bolus of streptokinase. Lysis was evaluated by serial angiograms obtained hourly for up to 6 hr after the clot was deposited. Five proximal vessels arising from the common carotid artery were chosen for the angiographic grading system.The control group showed beginning lysis after 5–6 hr. Group A appeared to show a slight improvement about 4 hr after clotting. Group B demonstrated an early and greater improvement at 2 hr that appeared to be sustained throughout the experiment. Group C showed no difference compared with the control group. The Dunnet *t* procedure and the Kruskel-Wallis nonparametric analysis of variance were used for comparing the angiograms of the treatment groups with those of the control group at corresponding study times. The results of these animal experiments do not indicate a definite benefit of streptokinase treatment alone over no treatment. Further controlled studies are needed before the value of streptokinase in routine clinical practice can be determined.

Optimal treatment of acute cerebrovascular disease, including transient ischemic attack, reversible ischemic neurologic deficit, and acute stroke, remains a controversial issue. Medical management has been attempted with a variety of agents, most notably anticoagulants, but no clear-cut clinical benefit has been established [1–3]. Some physicians favor surgical management for acute stroke [4, 5]. While there are probably certain clinical situations where acute surgical intervention can prove beneficial, this is not yet an accepted therapy for most patients with acute stroke [2, 3]. Cases have been reported regarding attempts at transcatheter lysis of acute clots in the cranial circulation, but no controlled studies are available, nor have statistical analyses been performed [6, 7]. We tried to determine the efficacy of streptokinase in this setting, using an animal model that might further serve for evaluation of other similar agents.

Materials and Methods

Streptokinase as a fibrinolytic agent was used in 20 Flemish Giant rabbits (weight range, 2.9–5 kg). Eight of the rabbits were used for controls and four each were assigned to three different dose regimens (groups A, B, and C). A drop of Thrombin was placed on a milliliter of the rabbit's blood, which was allowed to stand for 1 hr in a glass beaker. Then, 0.75 ml of that clot was deposited at the distal end of the common carotid artery of each of the 20 rabbits through a 4-French catheter inserted via the femoral artery. A test injection of a small amount of contrast material verified the position of the clot; the carotid artery was then left undisturbed for 1 hr, after which a baseline angiogram was obtained.

Treatment was initiated after the baseline angiogram. The group A rabbits received 5000

Received April 17, 1984; accepted after revision November 23, 1984.

[1] Department of Radiology (H–755), University of California, San Diego, Medical Center, 225 Dickinson St., San Diego, CA 92103. Address reprint requests to R. S. Centeno.

[2] Present address: Department of Radiology, Harvard Medical School, Massachusetts General Hospital, Boston, MA 02114.

[3] Department of Neurology, University of California, San Diego, Medical Center, San Diego, CA 92103.

AJNR 6:589–594, July/August 1985
0195–6108/85/0604–0589

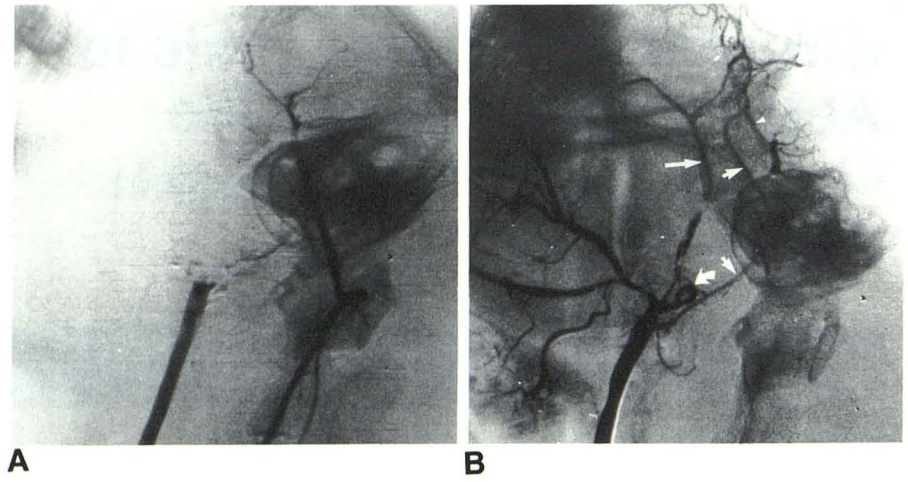


Fig. 1.—Control group, rabbit 3. **A**, Completely occluded common carotid artery 1 hr after embolization. Reflux of contrast material into aortic arch and demonstration of vertebral artery. **B**, 6 hr after embolization. Patency of most branches. Maxillary artery is partly patent, but superficial temporal artery (*curved arrow*) remains occluded. Internal carotid artery (*short arrows*), posterior communicating artery (*arrowhead*), maxillary artery (*long arrow*).

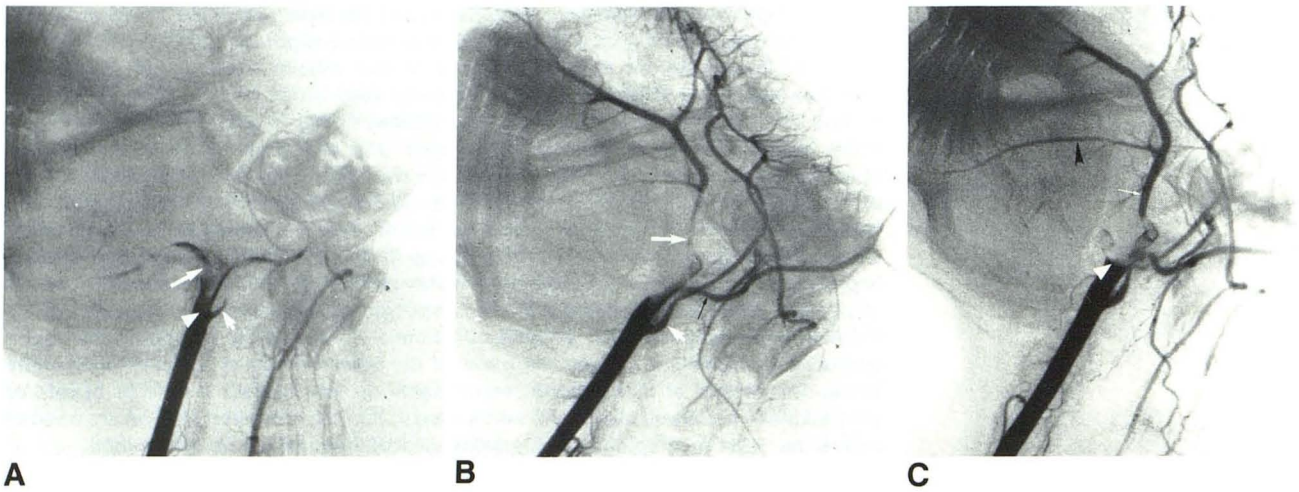


Fig. 2.—Group A (5000 U streptokinase/hr), Rabbit 6. **A**, Baseline (1 hr after embolization) angiogram. Complete occlusion of common carotid artery with clot (*arrowhead*) at level of origin of internal carotid artery, also occluded (*short arrow*). Lingual-facial artery (*long arrow*) also contains extensive clot. **B**, 2 hr after treatment. Patency of internal carotid (*short white arrow*) and superficial temporal (*black arrow*) arteries, partial reopening of maxillary artery (*long white arrow*). **C**, 4 hr posttreatment. Clot (*white arrowhead*) within distal common carotid has become consolidated, but lingual and facial arteries are still occluded. Origin and proximal parts of maxillary artery (*arrow*) contain large clot, but maxillary artery is bigger; small collateral (*black arrowhead*) to facial region caused by persistent occlusion of lingual and facial arteries.

U streptokinase/hr for 6 hr; group B, 4000 U/min for 1 hr, 3000 U/min for 2 hr, and 5000 U/hr for 2 hr; and group C was treated similar to group B, except 20,000 U streptokinase was injected initially as a slow bolus. Serial angiograms were obtained hourly up to 6 hr. After the experiment each rabbit was observed for a period of 24–72 hr, at which point surviving rabbits were sacrificed and gross inspection of the abdominal and thoracic organs was performed. The brains were removed and inspected for gross morphologic abnormalities. The brain sections were stained with cresyl violet for microscopic analysis.

The proximal segments of five vessels arising from the common carotid artery were chosen for the angiographic grading system. Each artery was graded by two observers (R. S. C. and D. B. H.) according to four definable criteria: grade 1, normal; grade 2, patent with small thrombus; grade 3, patent with large thrombus; and grade 4, occluded. A maximum score of 20 could be obtained per angiogram if all the vessels were occluded, and a minimum score of 5 if all the proximal vessels were normal. These scores were adjusted for differences in baseline scores among the individual rabbits by subtracting the hourly posttreatment score for each rabbit from the baseline score of the same rabbit. These differences are plotted as the Y axis of the graphs and reflect improvement or worsening from the baseline score. A positive value indicates diminution of the clot; a negative value indicates a worsening.

As primary interest lay in comparing each of the three treatments to the control, we used the Dunnet *t* procedure for statistical analysis. Angiographic findings at the defined intervals were compared among the groups. The Kruskel-Wallis nonparametric analysis of variance was used for data analysis also.

Results

Angiography

Angiograms from each group are shown for comparison of the results (figs. 1–4). Spasm of partly occluded arteries was

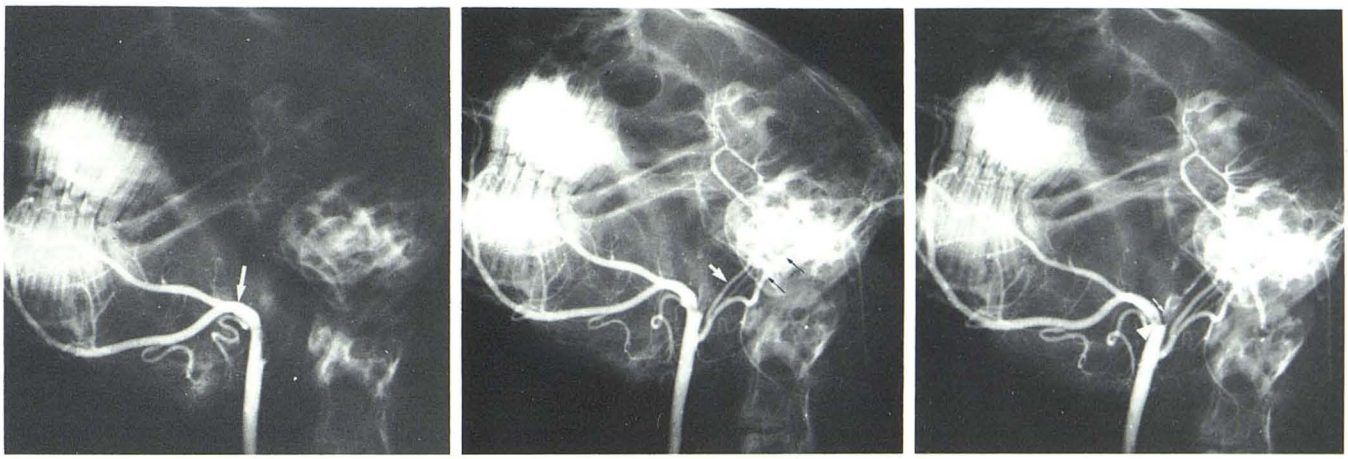
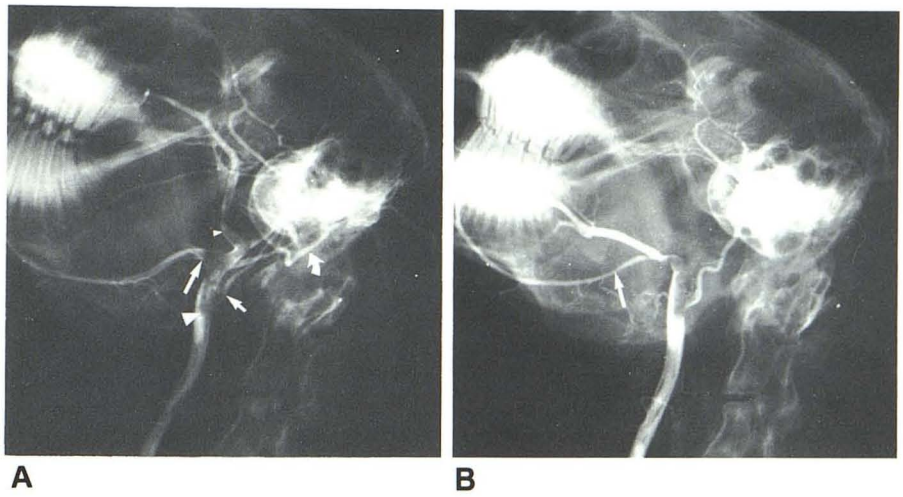


Fig. 3.—Group B (4000 U streptokinase/min for 1 hr; 3000 U/min for 2 hr; and 5000 U/hr for 2 hr), rabbit 17. **A**, Baseline angiogram. Complete occlusion of all major branches except lingual and facial arteries (*arrow*). **B**, Lysis of clot in internal carotid (*white arrow*) and superficial temporal (*black arrows*) arteries 2 hr after treatment. **C**, 4 hr after treatment. Edge of clot (*arrowhead*) is more defined at origin of maxillary artery (*arrow*), and there is still significant clot within it.

Fig. 4.—Group C (20,000 U streptokinase bolus followed by doses similar to group B), rabbit 14. **A**, 1 hr postembolus (baseline). Clot (*large arrowhead*) in distal common carotid, partly occluding internal carotid (*short straight arrow*), and extending into origin of lingual-facial (*long straight arrow*) arteries, large clot filling origin of maxillary artery (*small arrowhead*), and small clot at origin of superficial temporal artery (*curved arrow*). **B**, 6 hr postembolization. Although facial artery has reopened, lingual artery appears more stenotic; maxillary and superficial temporal arteries are now completely occluded, with overall appearance of worsening.



common among many rabbits. The grading method used was based on actual clot size and, therefore, spasm had no effect on the determinations. Within the control group ($n = 8$), there was a noticeable (30–40%) decrease in clot size in five subjects at 6 hr, with up to 90% in two. The other animal showed no change in clot size.

Two animals within group A did not show marked decrease in clot size, although the other two had decreases of about 75% after 4 hr. Animals in group B showed the most variable effects, ranging from almost no clot lysis to about 90% clot lysis after 4 hr. There was a tendency toward gradual clot reduction beginning at 2 hr. One rabbit in group C showed almost total occlusion of all the branches of the common carotid artery at baseline, which reverted to complete patency by 3–6 hr. All others belonging to this group, however, either showed worsening, no change, or mild improvement. There is obviously an overlap among the treatment groups; for example, similar improvement is noted in the "best result" subjects of group A, rabbit 6, and group B, rabbit 17.

Proximal parts of partly occluded branches of the common carotid artery were observed to convert to total occlusion in four cases, but no case was noted in which there were recognizable lucent emboli that may have detached from the main clot of the parent vessel. Smaller distal emboli were also not seen but could not be excluded (despite angiographic resolution comparable to currently available systems).

Statistical Results

Figures 5–8 demonstrate changes in occlusion scores for each rabbit from baseline. Despite the apparently better result obtained with group B, no significant differences exist between any of the streptokinase groups and the control group when tested by the Dunnet *t* procedure and the Kruskel-Wallis nonparametric analysis of variance.

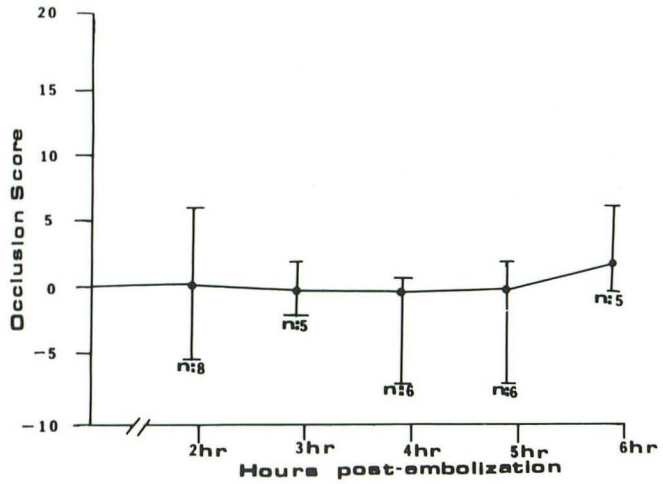


Fig. 5.—Control group. No significant change in clot size, although minimal improvement is noted 5–6 hr after embolization. *Circles* indicate median scores.

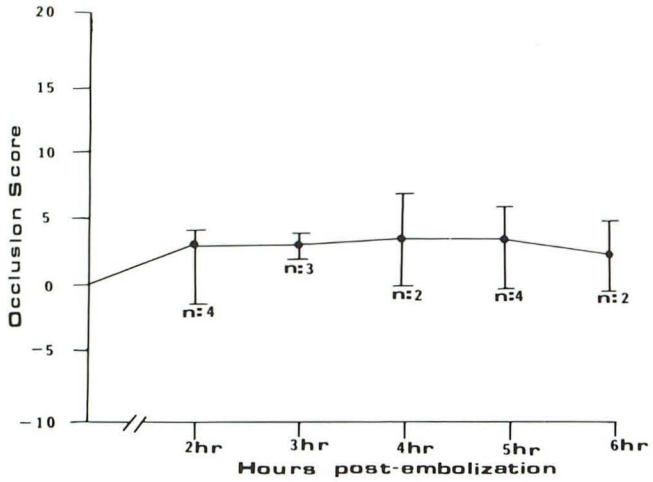


Fig. 7.—Group B. Early and greater improvement at 2 hr posttreatment is sustained throughout experiment. *Circles* indicate median scores.

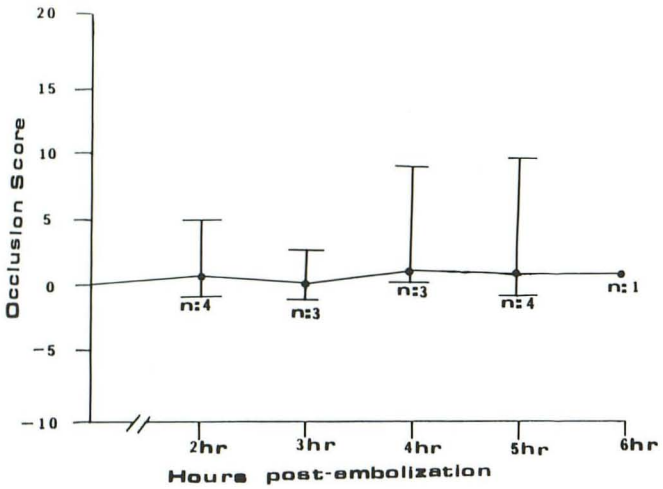


Fig. 6.—Group A. Slight improvement at 4 hr after embolization. *Circles* indicate median scores.

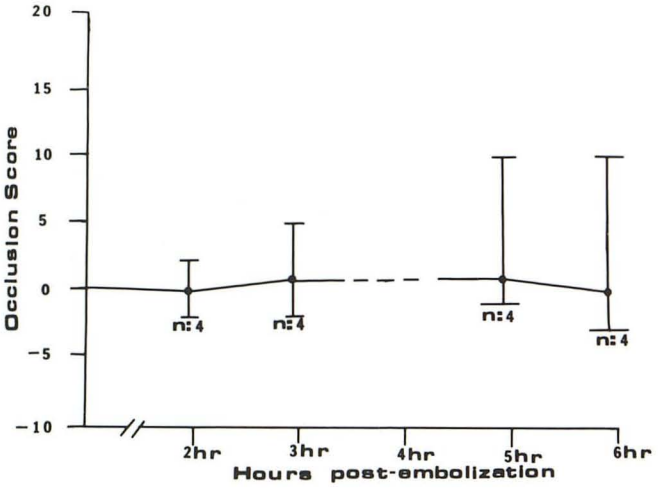


Fig. 8.—Group C. No essential difference in size of clot compared with controls and group A up to 5 hr, with worsening at 6 hr. *Circles* indicate median scores.

Pathologic Results

Gross autopsy examination of the control and treated rabbits revealed no evidence of gross hemorrhage. Microscopic examination of the brain revealed infarcts of varying size in both the control and treated groups. It is worth noting that in only one rabbit was mild bleeding demonstrated by microscopic examination, and this hemorrhage occurred in the subarachnoid space. The same rabbit, belonging to group A, also demonstrated a large area of infarction.

With the staining method used and under varying powers of light microscopy, no toxic effects were demonstrated after sacrifice in any of the animals following the 24–72 hr observation period.

Discussion

Varying pathologic responses have been observed in the brain tissue of cats subjected to 1–3 hr of arterial occlusion versus 6 hr or longer [8]. Hemorrhagic infarction was noted most often in those with a longer duration of arterial occlusion. Changes in vascular permeability occurred even with transient occlusion of about 30 min duration. An increase in permeability through pinocytotic transport mechanism, indicative of heightened metabolic activity, was seen first, followed by actual leakage through the necrotic walls and vessels, signifying blood-brain barrier breakdown [9]. Initial brain edema in arterial occlusion is mainly intracellular. Vasogenic edema appears to have its onset at no earlier than 4–6 hr [10] and

may indicate irreversible ischemic damage. Theoretically, then, fibrinolytic therapy would be most effective if used rapidly after arterial occlusion, prior to the appearance of vasogenic edema.

Streptokinase and urokinase are the most commonly used fibrinolytic agents. Streptokinase functions by acting upon plasminogen to produce plasmin, accomplished by the formation of a streptokinase-plasminogen complex, converting plasmin into its active form to act on the fibrin clot [11]. Urokinase exerts its action directly, transforming plasminogen into plasmin by cleavage of certain peptide bonds [12].

To our knowledge no animal study is currently available comparing the results of streptokinase for treatment of acute stroke with controls. We elected to use an animal model to observe the efficacy of streptokinase on the actual clot itself, and we believe this has validity so long as certain limitations are recognized. Our study's failure to demonstrate a significant difference between the treatment and control groups using accepted methods of statistical analysis (at $\alpha = 0.05$) may reflect these limitations.

This apparent lack of effect is inconsistent with the reported benefit from intraarterial streptokinase infusion in other organ systems [13, 14]. These recent clinical reports, however, deal with arterial occlusion treated with low-dose streptokinase (5000 U/hr) for much longer periods of time, that is, hours to days (J. Bookstein, personal communication). There are also reports demonstrating the value of short-duration streptokinase infusion (1000–2000 U/min for 15–95 min) into completely obstructed coronary arteries [15]. The positive effect of streptokinase infusion in these reports, however, was based on "reopening" of the obstructed coronary artery. Actual change in clot size or degree of lysis was not specifically considered. Furthermore, the same patients were given direct infusions of vasodilators into the coronary artery before streptokinase infusion, and in some, the vasodilator effect was enough to establish antegrade flow past the occlusion.

Of the angiograms obtained using our stroke model, most demonstrated reopening of a totally occluded common carotid artery or one or more of its major branches. In an actual human clinical situation this effect could potentially be enough to prevent a large infarction. We believe that, using clot size reduction as the parameter, streptokinase may demonstrate a mild to moderate degree of potency as a fibrinolytic agent for major thrombotic stroke. If our assumption is correct, streptokinase may be capable of reestablishing some flow into a severely compromised cerebral circulation, optimally within the "golden period" of 3–4 hr; a more definitive form of treatment could then be initiated to correct the underlying cause of occlusion. A larger series of animals or actual controlled trials with humans will be necessary to establish definite benefit.

It was not possible to obtain internal controls (opposite carotid arteries), since initial trials verified poor toleration of bilateral occlusion. Different doses and their varying effects, however, can be compared, as all factors are similar except for individual variations within the subject species. The model is also useful for angiographic comparison with other drugs with potentially greater effect than streptokinase. Finally, the model can be used to refine current catheter materials and techniques, thus enhancing the effectiveness of this method of treatment once its clinical value becomes well established. Optimal vascular conditions for treatment of human patients have recently been proposed, and similar conditions can be duplicated experimentally in the animal model [16].

There was a *suggestion* of benefit in our subject group treated with 4000 U/min for 1 hr, 3000 U/min for 2 hr, and 5000 U/hr for 2 hr over the other groups. There was an overlap of results between the lower- and higher-dose groups, possibly reflecting intergroup variability in the state of the fibrinolytic system of each animal. The response of group C animals to the largest dose regimen may indicate that excessively large doses of streptokinase deplete plasminogen. Lack of available plasminogen curtails the conversion of plasminogen to plasmin and favors rethrombosis [17]. Although in a few cases there was conversion of high-grade stenosis into total occlusion within the proximal branches of the common carotid artery, showers of emboli were not observed after diminution of the main clot.

Gross and microscopic analysis of multiple brain slices did not reveal a specific toxic effect from streptokinase. Reports dealing with streptokinase treatment of thromboembolism in human vertebral artery thrombosis likewise did not seem to indicate a deleterious effect [7]. Hemorrhage appeared in only one of our 12 treated rabbits, and this was microscopically confined to the subarachnoid space. The precise mechanism for such bleeding is not known, although ischemia itself may be the primary factor.

The results of our animal experiments on the use of streptokinase alone for the treatment of acute cerebral thromboembolism do not indicate a definite beneficial effect over untreated animals. Further controlled studies are obviously required before its routine use in clinical practice can be recommended.

REFERENCES

1. Whisnant JP, Cartlidge NEF, Elveback LR. Carotid and vertebral-basilar transient ischemic attacks: effect of anti-coagulants, hypertension, and cardiac disorders on survival and stroke occurrence—a popular study. *Ann Neurol* **1978**;3:107–115
2. Millikan CH, McDowell FH. Treatment of progressing stroke. *Stroke* **1981**;12:397–409
3. Whisnant JP. The role of the neurologist in the decline of stroke. *Ann Neurol* **1983**;14:1–7
4. Fisher MC. Discussion. In: McDowell FH, Brennan RW, eds. *Cerebral vascular diseases. Eighth Princeton conference*. New York: Grune & Stratton, **1973**:216
5. Goldstone J, Moore WS. Emergency carotid artery surgery in neurologically unstable patients. *Arch Surg* **1976**;111:1284–1291
6. Zeumer H, Ringelstein EB, Hacke W. Local fibrinolytic therapy in basilar artery thrombosis and distal subtotal vertebral artery stenosis. In: Trübestein G, Etzel F. eds. *International symposium on fibrinolytic therapy*. Bonn: **1982**:49
7. Zeumer H, Hacke W, Ringelstein EB. Local intraarterial thrombolysis in vertebral basilar thromboembolic disease. *AJNR* **1983**;4:401–404

8. Kamijyo Y, Garcia JH, Cooper J. Temporary regional cerebral ischemia in the cat. *J Neuropathol Exp Neurol* **1977**;36:338–350
9. Petito CK. Early and late mechanisms of increased vascular permeability following the experimental cerebral infarction. *J Neuropathol Exp Neurol* **1979**;38:222–234
10. Hossmann KA, Schuier FJ. Experimental brain infarcts in cats: I. Pathophysiological observations. *Stroke* **1980**;11:583–592
11. Sherry S, Lindemeyer RI, Fletcher AP, Alkjaersig N. Studies on enhanced fibrinolytic activity in man. *J Clin Invest* **1959**;38:810–822
12. Barlow GH. Pharmacology of fibrinolytic agents. *Prog Cardiovasc Dis* **1979**;21:315–326
13. Dotter CT, Rosch J, Seaman HA. Selective clot lysis with low-dose streptokinase. *Radiology* **1974**;111:31–37
14. Risius B, Zelch MG, Graor RA, Geisinger MA, Smith JAM, Piraino DW. Catheter-directed low dose streptokinase infusion: a preliminary experience. *Radiology* **1984**;150:349–355
15. Rentrop P, Blanke H, Karsch KR, Kaiser H, Kostering H, Leitz K. Selected intracoronary thrombolysis in acute myocardial infarction and unstable angina pectoris. *Circulation* **1981**;63:307–316
16. Becker GJ, Rabe FE, Richmond BD, et al. Low-dose fibrinolytic therapy. *Radiology* **1983**;148:663–670
17. Brogden RN, Speight TM, Avery GS. Streptokinase: a review of its clinical, pharmacology, mechanism of action and therapeutic uses. *Drugs* **1973**;5:357–445