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THE UNIVERSITY OF ALBERTA

INTRATHECAL FIBRINOLYTIC THERAPY IN THE PREVENTION OF CEREBRAL VASOSPASM IN A PRIMATE MODEL OF SUBARACHNOID HEMORRHAGE

BY

Jay Max Findlay

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This work is dedicated to
the neurosurgeon
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ABSTRACT

The structure of primate cerebral arteries in vasospasm (VSP) resulting from subarachnoid hemorrhage (SAH) were compared to both normal arteries and normal arteries vasoconstricted *in vitro*, by means of electron microscopy. The findings suggest that luminal narrowing and vessel wall thickening after SAH is due to medial contraction and not a mural proliferative reaction.

The efficacy of intrathecal administration of the fibrinolytic agent human recombinant tissue plasminogen activator (rt-PA) in the elimination of subarachnoid clot and prevention of VSP, was evaluated in a blind, randomized, placebo-controlled trial. Two groups of 12 monkeys underwent cerebral angiography and coagulation analysis, followed by placement of a clot of autologous blood on the right side to simulate SAH. Twenty-four hours later the treatment group received 1.5 mg of rt-PA suspension over 24 hours via an Ommaya reservoir, while the placebo group received the same volume of saline. Diffuse right-sided VSP was observed 7 days after SAH in placebo animals ($p<0.01$), but not in those treated with rt-PA. The placebo group had large subarachnoid clots remaining at 7 days, but 11 of the 12 treatment animals were free of clot. Coagulation changes and brain inflammation did not occur with rt-PA treatment.

In a further study, 6 monkeys received 10 mg of intrathecal rt-PA. Systemic fibrinolysis did not occur, and pathologic examination of the brain was normal at 4 days. The efficacy of unilateral, intraoperative administration of rt-PA suspension (0.5

mg) plus sustained-release gel rt-PA (1.25 mg) in clearing a bilateral subarachnoid clot was evaluated in 16 monkeys. Significant vasospasm occurred in all major anterior cerebral vessels in the placebo group, but was not seen in treatment animals.

In a study involving 16 monkeys it was found that 0.75 mg of gel rt-PA was sufficient to lyse a 4.25 ml subarachnoid clot and prevent VSP.

The effect of gel rt-PA administered at various times from 0 to 72 hours after SAH was examined in 30 monkeys. In the control (no treatment) and 72-hour treatment groups significant VSP developed in most major clot-side arteries ($p<0.05$), but was not seen in the 0-, 24-, or 48-hour treatment groups. It was concluded that clearance of subarachnoid hematoma within 72 hours of SAH is effective in preventing VSP.

Key Words: Primate, Subarachnoid hemorrhage, Vasospasm, Fibrinolytic therapy, Plasminogen activator.

PREFACE

The first chapter of this work reviews some general aspects of cerebral vasospasm, including its definition, pathophysiology, clinical features, diagnosis, and treatment. The remainder of the thesis deals with studies performed using the primate model of subarachnoid hemorrhage and vasospasm developed in the Cerebrovascular Research Laboratory at the University of Alberta. This begins, in the second chapter, with an analysis of the morphological changes in the vasospastic cerebral arterial wall, leading to a more detailed discussion of the pathogenesis of cerebral vasospasm. The following four chapters, the main body of this work, deals with the use of intrathecal fibrinolytic therapy in the prevention of vasospasm in primates. The first study tested intrathecal fibrinolytic treatment after a unilateral subarachnoid hemorrhage. The second study included an additional test of the safety of this treatment, and then examined the efficacy of intrathecal fibrinolytic therapy after a more extensive, bilateral subarachnoid hemorrhage. Next was a dosage study, followed by a timing study which determined the length of time after subarachnoid hemorrhage during which this form of treatment remains effective. Chapter five contains the most thorough review of the basis and history of intrathecal fibrinolytic therapy. This ordering represents the sequence in which these fibrinolytic studies were completed. The final chapter reviews the progression of these studies and comments upon possible application of intrathecal fibrinolytic therapy in humans following aneurysmal subarachnoid hemorrhage.

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CHAPTER ONE

CEREBRAL VASOSPASM

The most common cause of primary subarachnoid bleeding, and the fourth most common cause of stroke, is ruptured saccular arterial aneurysms. Approximately 50% of patients are either killed or severely disabled by the initial rupture, and those who recover are then subject to a number of complications of aneurysmal subarachnoid hemorrhage (SAH). These include rebleeding from the aneurysm, obstructive hydrocephalus, and seizures, but the most important cause of morbidity and mortality in survivors of SAH is cerebral ischemia. Delayed ischemia occurs in 20 to 30% of patients and one-half of these will perish or be maimed as a result. The occurrence of cerebral ischemia after SAH is dependent on a number of factors, the most important of which is the development of narrowing of cerebral arteries known as vasospasm (VSP).

ETIOLOGY AND PATHOPHYSIOLOGY

Vasospasm and cerebral ischemia have a delayed onset following SAH, peaking in incidence approximately one week after aneurysm rupture. Arterial narrowing occurs mostly in larger, extraparenchymal cerebral arteries which course through the network of basal subarachnoid cisterns, the same vessels from which saccular aneurysms arise. When an aneurysm bursts it frequently encases the parent and other arteries in the

*A version of this chapter has been accepted for publication. Findlay JM, Weir BKA. Subarachnoid and Intracerebral Hemorrhage. In: Weinstein PR, Faden AI, eds. Protection of the Brain from Ischemia. Baltimore: Williams & Wilkins, 1989.

subarachnoid cisterns with blood clot. It has been shown that the location and volume of subarachnoid hematoma is directly related to the incidence, distribution and severity of ensuing VSP [21]. There is no longer any question that VSP is a delayed response of the vessel wall to thick periarterial blood clot, but the precise biochemical interaction leading to the condition is still not fully clarified. The major contemporary theory for the pathogenesis of VSP is that the periarterial blood clot, during its degradation, releases certain vasoactive substances that elicit sustained vascular smooth muscle spasm [36], as well as vasotoxic substances that injure the vessel wall, impairing its ability to synthesize endogenous vasodilatory substances [29], and inciting a destructive vessel wall reaction. Although histological examination of vasospastic human and animal cerebral arteries have shown structural changes such as intimal hypercellularity [40], corrugation and fragmentation of the internal elastic lamina [26], and medial myonecrosis and edema [10], it is unlikely that these changes are by themselves sufficient to cause any significant degree of luminal narrowing. The predominant morphologic change accounting for VSP on ultrastructural analysis is smooth muscle contraction [27,43]. This explains the frequent finding of angiographic resolution of VSP within a few weeks of SAH, yet persistence of the aforementioned structural abnormalities seen on histological examination [19]. However, the irreversibility of vasoconstriction during the VSP period [22], and the finding of medial myonecrosis on ultrastructural studies of vasospastic vessels, suggest that the condition is more than simple physiologic smooth

muscle contraction and that extreme vasoconstriction damages the structural integrity of the arterial wall.

Although a number of clot-derived spasmogens have been postulated, evidence is accumulating that products of erythrocyte breakdown, such as oxyhemoglobin (OxyHb), are especially important in the genesis of VSP [7,27]. Liberated during the process of hemolysis, the time course of OxyHb release into the periarterial clot from erythrocytes appears to follow the time course of angiographic and clinical VSP [33]. It has been speculated that during oxidative conversion of OxyHb to methemoglobin free radicals are produced, which in turn generate vasoconstrictive and vasotoxic lipid peroxides and prostaglandins [2,39]. Finally, it has been suggested that the periarterial clot may prevent cerebrospinal fluid (CSF) circulation from reaching the surface of the arteries and intraadventitial rete vasorum of the vessel wall (analogous to the vasa vasorum of non-cerebral vessels), thereby interfering with normal vessel wall nutrition and oxygenation [8,49]. Vessel wall hypoxia might also play a role in the pathogenesis of VSP [30,42].

While as many as two-thirds of patients suffering aneurysmal SAH will develop some degree of angiographic VSP, only about one-half of these will develop a delayed ischemic deficit. This is because a number of factors influence the development of ischemia in an individual patient. These include: the severity of VSP (in degree of vessel narrowing and the extent of its distribution); the prevailing blood pressure, blood volume, blood viscosity and intracranial pressure, all of which influence cerebral perfusion; the available collateral blood supply to the affected brain; the presence

of any coincident stenosing atherosclerotic cerebrovascular disease; any associated direct brain insult from the aneurysmal hemorrhage or surgical procedures; the hemoglobin content of the blood and its oxygenation; and brain compliance and shifts. These factors act in concert to determine the degree of impairment in local cerebral blood flow and oxygen delivery, as well as influencing the cerebral ischemia thresholds, below which ischemic symptoms and brain infarction will occur. While normal cerebral tissue will tolerate cerebral blood flow levels down to approximately 18ml/100gm/min without neurological symptoms, levels between 12 to 18ml/100gm/min will interrupt neuronal function but maintain the viability of these neurones for a period of time, and levels below 12ml/100gm/min will result in cerebral infarction [3]. An appreciation of factors affecting brain perfusion after SAH is important in the management of VSP.

Other etiologies of SAH are rarely associated with VSP and cerebral ischemia. Only aneurysm rupture is routinely capable of discharging a large enough volume of blood into the basal cisterns to evoke VSP in the arteries exposed there.

CLINICAL FEATURES

Vasospasm rarely occurs before 4 days after SAH, and peaks in incidence at the end of the first week and beginning of the second after aneurysm rupture. The incidence of VSP depends on the severity of the SAH, and since there is also a positive correlation between the amount of subarachnoid blood in the basal cisterns and clinical grade of the patient, it follows that those at greatest risk of developing VSP are patients in poor neurological condition. The onset of symptomatic VSP is frequently heralded by

increasing headache and drowsiness. This is followed by focal deficits referable to the distribution of the affected vessels [12]. In the anterior circulation the most commonly affected territory is that of the middle cerebral artery (MCA), resulting in hemiparesis, hemianopsia, dysphasia (dominant hemisphere), and neglect (nondominant hemisphere). Vasospasm of the anterior cerebral arteries can result in abulia, somnolence and lower extremity weakness. Vasospasm is commonly associated with mild pyrexia. Severe and multi-vessel VSP can lead to massive cerebral infarction, intracranial hypertension, cerebral herniation and death.

DIAGNOSIS

While the diagnosis of delayed cerebral ischemia is suggested by the typical time of onset and nature of the neurological deterioration, other complications such as aneurysmal rebleeding, hydrocephalus, seizures, increasing edema around an intracerebral hematoma, postoperative intracranial clots, electrolyte disorders (most commonly hyponatremia), hypoxemia and sepsis must be considered. Computerized tomography, useful in the diagnosis of SAH and identification of patients with a large volume of subarachnoid clot and therefore at high risk of developing VSP, should be done to rule out alternative intracranial causes for deterioration and to look for evidence of cerebral swelling or hypodensity suggestive of impending infarction. Magnetic resonance imaging is more sensitive in detecting the presence and distribution of edema, and may become useful in detecting evolving cerebral ischemia before it is recognizable on computerized tomographic scans, providing the patients have not been treated with a

ferromagnetic aneurysm clip or are too seriously ill to undergo the imaging procedure.

It is becoming increasingly clear that transcranial Doppler sonography is very useful in the detection of VSP [14,17,37]. As the lumen of a vessel narrows the blood flow velocity increases (velocity is inversely related to the diameter squared), and this acceleration in blood flow in narrowed vessels at the base of the brain can be detected as an increase in Doppler frequency shifts with a hand-held ultrasound probe. The absolute velocity calculated from this Doppler signal is not always accurate, so that changes over time or side-to-side differences often give more important information than absolute values. The easiest vessel to record is the MCA, which is useful in the present context since it is usually involved in patients with clinically significant VSP. In one center with extensive experience with transcranial Doppler it was found that VSP could be diagnosed with a sensitivity of 75% and a specificity of 85% [14]. The simplicity, noninvasiveness and repeatability of this investigation make it a valuable tool in the evaluation of SAH patients and diagnosis of VSP. Ideally, the detection of an early, significant increase in cerebral blood flow (CBF) velocity will permit early institution of measures to prevent impending symptomatic cerebral ischemia and infarction.

The CBF response to SAH and VSP has been examined in a number of reports [15,47], providing insight into the pathophysiology of VSP and cerebral ischemia. The majority of these studies have shown a rough correlation between VSP, a reduction in CBF and delayed ischemic deficit. Positron emission tomography has also

been used to investigate SAH patients[25]. Inhalation xenon-enhanced computerized tomographic blood flow mapping[46] promises to overcome many of the difficulties encountered in clinical application of CBF studies.

At this time the definitive diagnosis of VSP still depends upon cerebral angiography and demonstration of arterial narrowing. Vasospasm may appear as a tapering and smooth reduction in arterial caliber or it may be segmental (between bifurcations). It may remain localized, affecting even a single vessel, or it may be diffuse in distribution, extending out to the parenchymal vessels. A recent review of 1,002 cerebral angiographic procedures for a number of different indications, 33% of which were aneurysm related, revealed a total ischemic event rate of 3.1%, approximately one-half of which were permanent[6]. The use of nonionic contrast media and arterial digital subtraction angiography may reduce this complication risk even further. In the authors' experience, the difficulty in making a clinical diagnosis of VSP in some instances necessitates cerebral angiography, particularly when surgery, or an experimental or potentially dangerous treatment for VSP is being contemplated.

TREATMENT

The delayed onset of VSP and cerebral ischemia after aneurysm rupture, combined with the clinician's ability to predict which patients are at greatest risk of developing these complications, would appear to create an ideal situation for effective intervention and prevention. It is discouraging to report, therefore, that despite an enormous effort to identify an agent or treatment

protocol capable of preventing or reversing VSP, none has met with unqualified success. These treatments can be placed in five categories: 1. vasodilator treatment intended to prevent or reverse arterial smooth muscle contraction; 2. hemodynamic therapy intended to augment CBF despite VSP; 3. cerebral protection with agents intended to prevent neuronal damage in the face of an ischemic insult; 4. anti-inflammatory, anti-thrombotic and miscellaneous other drugs intended to prevent vessel wall damage and inflammation; and 5. methods to remove the subarachnoid periarterial blood clot and thereby prevent the occurrence of VSP. Wilkins has provided comprehensive referenced lists of these agents and therapeutic protocols [45,46].

Vasodilator Treatment

Since its first recognition VSP has been considered largely a problem of vascular smooth muscle contraction, and consequently many vasodilator agents have been tested experimentally and clinically. Recently much interest has focused on the calcium antagonists, particularly the more cerebroselective agent nimodipine. Nimodipine is thought to relax smooth muscle by inhibiting influx of extracellular calcium and preventing the accumulation of free calcium ions in the myoplasma which are necessary for myofilament activation. There is evidence that cerebral vessels may be particularly susceptible to certain calcium entry blockers since there is a greater dependence on extracellular pools of calcium in initiating and maintaining contraction in cerebrovascular smooth muscle compared to vascular smooth muscle elsewhere in the body. Numerous *in vitro* investigations have demonstrated the

effectiveness of calcium antagonists in preventing and reversing cerebrovascular smooth muscle contraction in response to various vasoactive agents, including blood. Interest has focused on the effect of calcium antagonists on VSP induced by SAH.

A number of controlled trials have now shown that nimodipine treatment results in a modest but significant reduction in severe neurologic deficits from VSP alone, although this may not be due to any reduction in large-vessel VSP as visualized on angiography, and it may not result in any significant reduction in mortality after SAH. Allen et al [1] showed in a randomized, prospective, double-blind, multi-institutional trial in good grade patients that oral nimodipine reduced the number of severe neurological deficits attributable to VSP alone. In this trial the treatment group received 0.35 mg/kg nimodipine every four hours for 21 days. Severe neurologic deficits occurred in 8 of 60 (13.3%) placebo treated patients but only 1 of 56 (1.8%) nimodipine treated patients ($p=0.03$, Fisher's exact test). Overall outcome was not different between the two groups and because angiography was not performed consistently it was not possible to determine the effect of nimodipine on the incidence or severity of VSP. Similarly, Philippon et al [35] showed a significant reduction in poor outcomes from VSP in a group of patients receiving 60 mg of nimodipine every four hours for 21 days. Severe neurologic outcomes including death from VSP occurred in 10 of 39 (25.6%) placebo treated patients compared to 2 of 31 (6.4%) nimodipine treated patients. Again overall outcome did not differ significantly between the two groups. Seiler [38] studied 70 consecutive patients admitted within 4 days of SAH, determining

their risk of developing VSP from the amount of subarachnoid blood on computerized tomographic scans, and then evaluating them daily with transcranial Doppler. The first 33 patients received no nimodipine, while the second 37 received intravenous nimodipine, 2 mg/hr intravenously for one to two weeks followed by oral nimodipine, 60 mg every 4 hours for an additional week. Although nimodipine did not prevent VSP as determined by Doppler studies, it did significantly reduce the incidence of delayed ischemic deficits and improved functional outcome in patients felt to be high risk for the development of VSP. Most recently a multicenter, randomized, placebo-controlled trial of oral nimodipine in poor grade aneurysm patients demonstrated a significantly better outcome at 3 months in nimodipine treated patients[34]. Delayed ischemic deficits from VSP alone were significantly less frequent in the nimodipine group, with permanent deficits occurring in 5 of 72 (6.9%) nimodipine treated patients and 22 of 82 (26.8%) placebo treated patients. No difference was seen in the incidence of moderate to severe diffuse VSP, which occurred in 64.3% of nimodipine treated patients and 66.2% of placebo treated patients. This apparent failure of nimodipine to affect angiographic VSP is in keeping with a clinical study showing nimodipine treatment resulted in a mild lowering in CBF[28], and with primate studies that failed to show any effect of oral nimodipine on the development of angiographic VSP[9,31].

Several uncontrolled studies have reported the use of intravenous nimodipine after SAH[4,24], and these also suggest it is efficacious in preventing delayed ischemia. Lewis et al[23] reported

15. Espinosa F, Weir B, Shnitka T, Overton T, Boisvert D: A randomized placebo-controlled double-blind trial of nimodipine after SAH in monkeys. Part 2: Pathological findings. J Neurosurg 60: 1176-1187, 1984.

16. Espinosa F, Weir B, Shnitka T: Electron microscopy of simian cerebral arteries after subarachnoid hemorrhage and after the injection of horseradish peroxidase. Neurosurgery 19: 935-945, 1986.

17. Fein JM, Flor WJ, Cohan SL, Parkhurst J: Sequential changes of vascular ultrastructure in experimental cerebral vasospasm. Myonecrosis of subarachnoid arteries. J Neurosurg 41: 49-58, 1974.

18. Findlay JM, Weir BKA, Steinke D, Tanabe T, Gordon P, Grace M: Effect of intrathecal thrombolytic therapy on subarachnoid clot and chronic vasospasm in a primate model of SAH. J Neurosurg 69: 723-735, 1988.

19. Fisher CM, Roberson GH, Ojemann RG: Cerebral vasospasm with ruptured saccular aneurysm - The clinical manifestations. Neurosurgery 1: 245-248, 1977.

20. Frazee JG, Bevan JA, Bevan RD, Jones KR, Bivens LV: Early treatment with diltiazem reduces delayed cerebral vascular narrowing after subarachnoid hemorrhage. Neurosurgery 23: 611-615, 1988.

21. Fujii S, Fujitsu K: Experimental vasospasm in cultured arterial smooth-muscle cells. Part 1: Contractile and ultrastructural changes caused by oxyhemoglobin. J Neurosurg 69: 92-97, 1988.

22. Grote E, Hassler W: The critical first minutes after subarachnoid hemorrhage. Neurosurgery 22: 654-661, 1988.

23. Hughes JT, Schianchi PM: Cerebral artery spasm. A histological study at necropsy of the blood vessels in cases of subarachnoid hemorrhage. J Neurosurg 48: 515-525, 1978.

24. Kapp JP, Clower BR, Azar FM, Yabuno N, Smith RR: Heparin reduces proliferative angiopathy following subarachnoid hemorrhage in cats. J Neurosurg 62: 570-5[illegible], 1985.

25. Kawasaki S: Sequential ultrastructural changes of experimental cerebral vasospasm in dogs. Neurol Med Chir (Tokyo) 20: 237-245, 1980.

26. Lehman RM, Kassell NF, Nazar GB, Weir BK, Johshita H, Nosko M, Duling BR, Owens GK: Morphometric methods in the study of vasospasm, in Wilkins RH (ed): *Cerebral Vasospasm*. New York, Raven Press, 1988, pp 113-117.

27. Liszczak TM, Varsos VG, Black PMcL, Kistler JP, Zervas NT: Cerebral arterial constriction after experimental subarachnoid hemorrhage is associated with blood components within the arterial wall. J Neurosurg 58: 18-26, 1983.

28. Mayberg MR, Houser OW, Sundt TM Jr: Ultrastructural changes in feline arterial endothelium following subarachnoid hemorrhage. J Neurosurg 48: 149-157, 1978.

29. Mizukami M, Kin H, Araki G, Mihara H, Yoshida Y: Is angiographic spasm real spasm? Acta Neurochirurgica 34: 247-259, 1976.

30. Mizukami M, Kawase T, Tazawa T, Nagata K, Yunoki K, Yoshida Y: Hypothesis and clinical evidence for the mechanism of chronic

cerebral vasospasm after subarachnoid hemorrhage, in Wilkins RH (ed): *Cerebral Arterial Spasm*. Baltimore, Williams & Wilkins, 1980, pp 97-112.

31. Nazar GB, Kassell NF, Povlishock JT, Lee J, Hudson S: Subarachnoid hemorrhage causes adherence of white blood cells to the cerebral arterial luminal surface, in Wilkins RH (ed): *Cerebral Vasospasm*. New York, Raven Press, 1988, pp 343-356.

32. Nosko M, Weir B, Krueger C, Cook D, Norris S, Overton T, Boisvert D: Nimodipine and chronic vasospasm in monkeys: Part 1. Clinical and radiological findings. Neurosurgery 16: 129-136, 1985.

33. Peerless SJ, Kassell NF, Komatsu K, Hunter IG: Cerebral vasospasm: Acute Proliferative Vasculopathy? II. Morphology, in Wilkins RH (ed): *Cerebral Arterial Spasm*. Baltimore, Williams & Wilkins, 1980, pp 88-96.

34. Peterson JW, Kwun B, Nishizawa S, Hackett JD, Zervas NT: Immunoreactivity, immunosuppression and cerebral vasospasm following SAH. Stroke 19: 130, 1988 (abstr).

35. Pickard JD, Graham DI, Matear E, MacPherson P, Tamura A, Fitch W: Ultrastructure of cerebral arteries following experimental subarachnoid haemorrhage. J Neurol Neurosurg Psych 48: 256-262, 1985.

36. Ross R: The pathogenesis of atherosclerosis - an update. N Engl J Med 314: 488-509, 1986.

37. Sacher M, Tenner M: Case reports. Structural changes of the intradural arteries following subarachnoid haemorrhage. Br J Radiol 51: 736-738, 1978.

38. Sakaki T, Tanigake T, Kyoi K, Utumi S, Murata Y, Hiasa Y, Kikuchi H: Pathological study of late arterial spasm. Neurol Med Chir (Tokyo) 19: 1085-1093, 1979.

39. Seifert V, Stolke D, Kunz U, Resch K: Influence of blood volume on cerebrospinal fluid levels of arachidonic acid metabolites after subarachnoid hemorrhage: Experimental study on the pathogenesis of cerebral vasospasm. Neurosurgery 23: 313-321, 1988.

40. Shaw MDM, Foy PM, Conway M, Pickard JD, Maloney P, Spillane JA, Chadwick DW: Dipyridamole and postoperative ischemic deficits in aneurysmal subarachnoid hemorrhage. J Neurosurg 63: 699-703, 1985.

41. Smith RR, Clower BR, Peeler DF Jr, Yoshioka J: The angiopathy of subarachnoid hemorrhage: Angiographic and morphologic correlates. Stroke 14: 240-245, 1983.

42. Smith RR, Clower BR, Grotendorst GM, Yabuno N, Cruse JM: Arterial wall changes in early human vasospasm. Neurosurgery 16: 171-176, 1985.

43. Takemae T, Branson PJ, Alksne JF: Intimal proliferation of cerebral arteries after subarachnoid blood injection in pigs. J Neurosurg 61: 494-500, 1984.

44. Tanabe Y, Sakata K, Yamada H, Ito T, Takada M: Cerebral vasospasm and ultrastructural changes in cerebral arterial wall. An experimental study. J Neurosurg 49: 229-238, 1978.

45. Tani E, Yamagatu S, Ito Y: Intercellular granules and vesicles in prolonged cerebral vasospasm. J Neurosurg 48: 179-189, 1978.

46. Van Citters RL, Wagner BM, Rushmer RF: Architecture of small arteries during vasoconstriction. Circulation Research 10: 668-675, 1962.

47. Weir B, Grace M, Hansen T, Rothberg C: Time course of vasospasm in man. J Neurosurg 48: 173-178, 1978.

48. Weir B: *Aneurysms Affecting the Nervous System*. Baltimore, Williams & Wilkins, 1987, pp 246-250.

49. Yamashima T, Yamamoto S: Cerebral arterial pathology in experimental subarachnoid hemorrhage. J Neurosurg 58: 1843-1850, 1983.

50. Yoshioka J, Clower BR, Smith RR, Tillman LJ: Pathogenesis of hemorrhage arteriopathy. Surg Forum 33: 488-490, 1982.

51. Yoshioka J, Yabuno N, Morooka H, Matsumoto Y, Ohmoto T, Nishimoto A: Morphological changes in cerebral vessels in experimental cerebral vasospasm. Neurol Med Chir (Tokyo) 21: 211-219, 1981.

52. Zervas NT, Liszczak TM, Mayberg MR, Black PMcL: Cerebrospinal fluid may nourish cerebral vessels through pathways in the adventitia that may be analogous to systemic vasa vasorum. J Neurosurg 56: 475-481, 1982.

TABLE II-I HAS BEEN REMOVED DUE TO POOR PRINT QUALITY

"PATHOLOGICAL ARTERIAL WALL CHANGES IN CHRONIC CEREBRAL VASOSPASM"

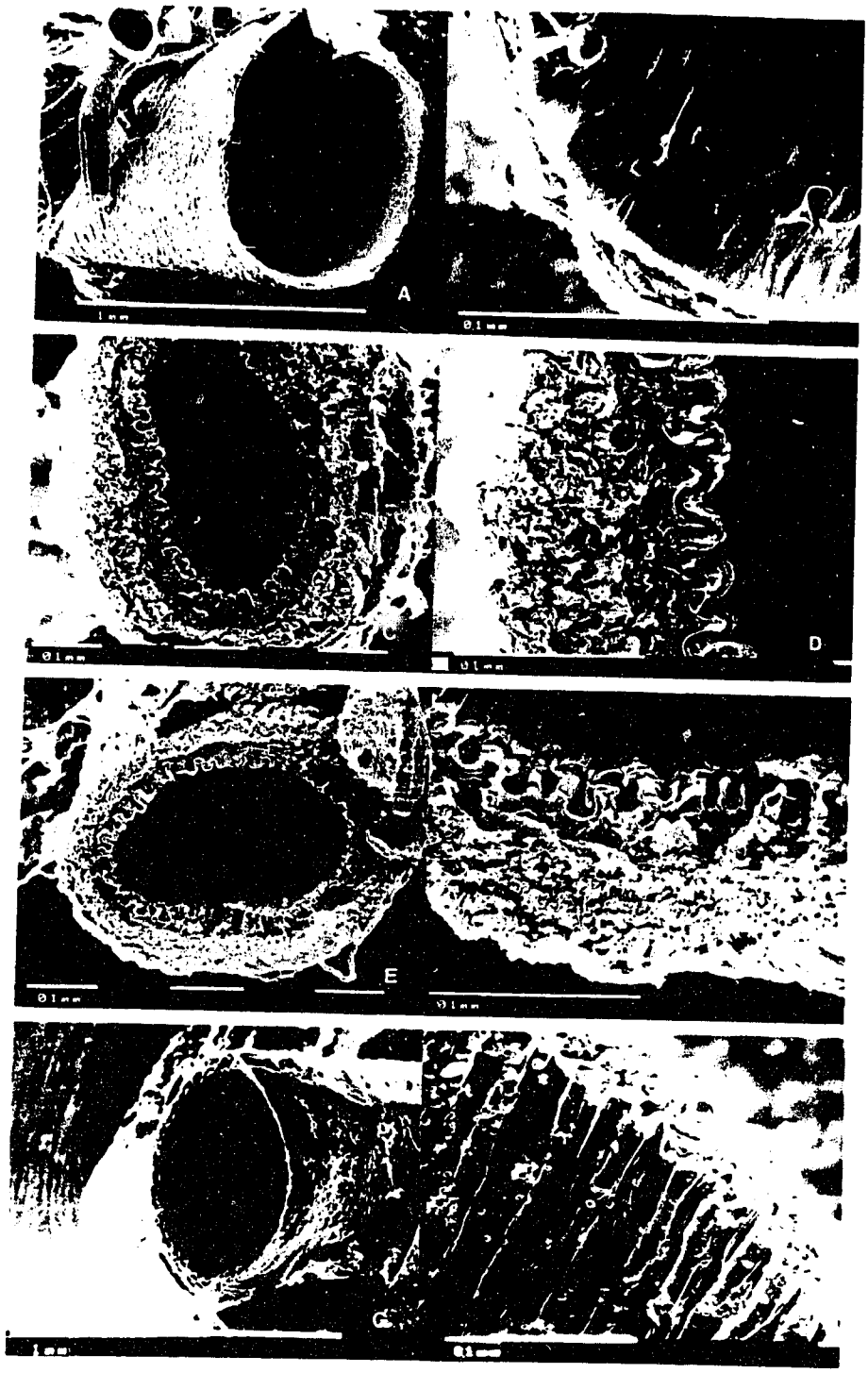

Figure II-1
Scanning electron micrographs from a normal left middle cerebral artery (MCA) (A & B), a normal left MCA vasoconstricted *in vitro* with prostaglandin $F_{2\alpha}$ (C & D), a vasospastic right MCA 7 days after subarachnoid hemorrhage (E & F), and a right MCA harvested 14 days after subarachnoid hemorrhage with resolving vasospasm (G & H). Note the similarities between the *in vitro* vasoconstricted and vasospastic vessels. Magnification bars are shown at the bottom of each micrograph.

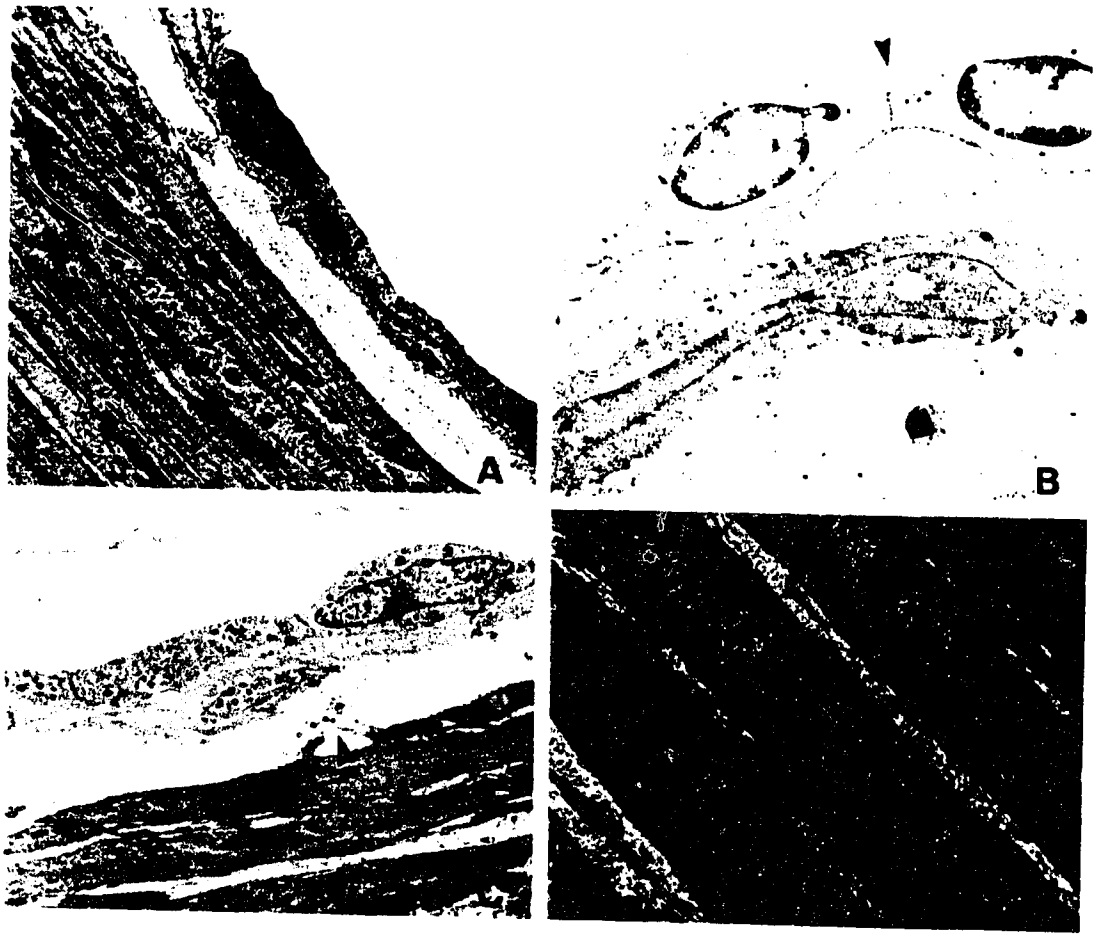

Figure II-2
Transmission electron micrographs of normal middle cerebral arteries. Flattened endothelial cells overlying a nonconvoluted internal elastic lamina (A). Tight-junction between endothelial cells (arrowhead), which overlay a myointimal cell in the subendothelium (B). Normal porosity in the internal elastic lamina (C). Nonconstricted myocytes in the tunica media (D).

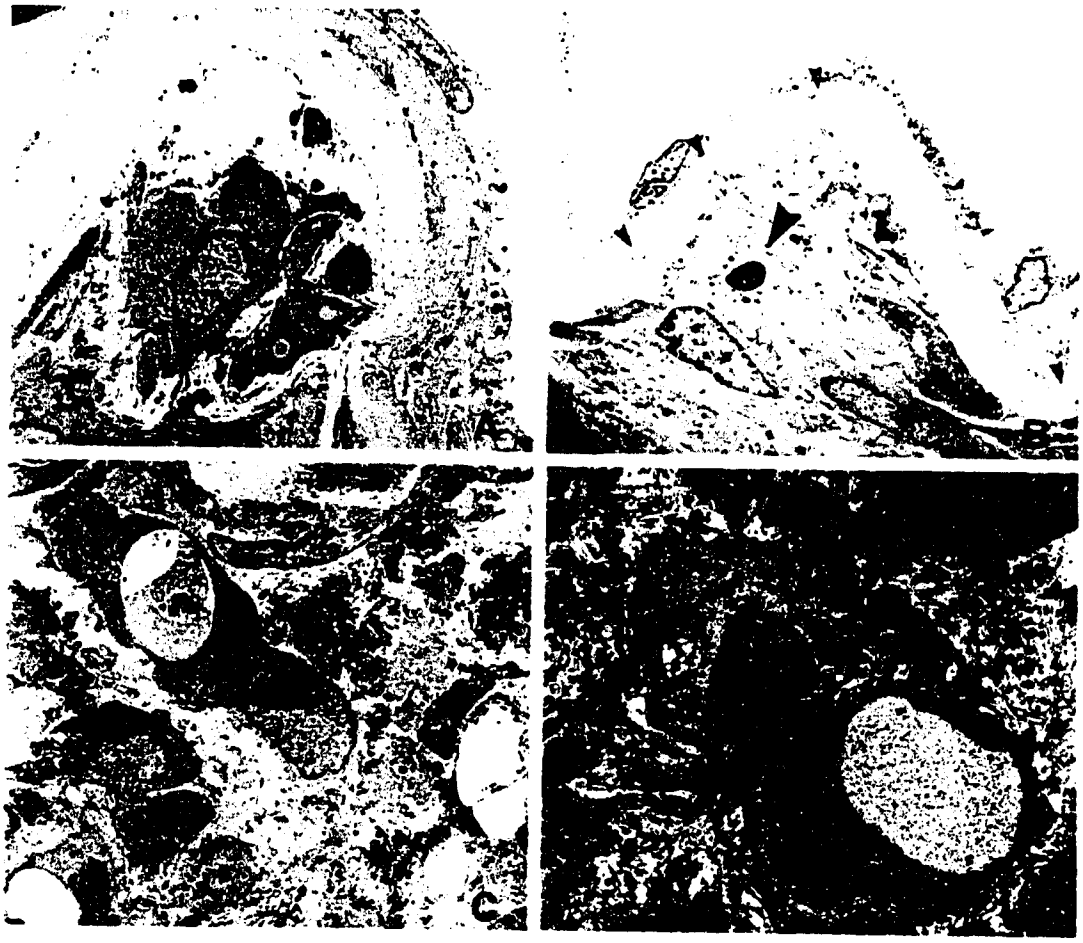

Figure II-3
Transmission electron micrographs of vasospastic middle cerebral arteries. Vacuolated endothelial cells with loss of tight-junctions 7 days post subarachnoid hemorrhage (A). Apparent fragmentation of the internal elastic lamina (small arrowheads) and a pyknotic myocyte in the tunica media (large arrowhead) at day 7 (B). Vacuoles in contracted smooth muscle cells of tunica media at 7 (C) and 14 (D) days after subarachnoid hemorrhage.

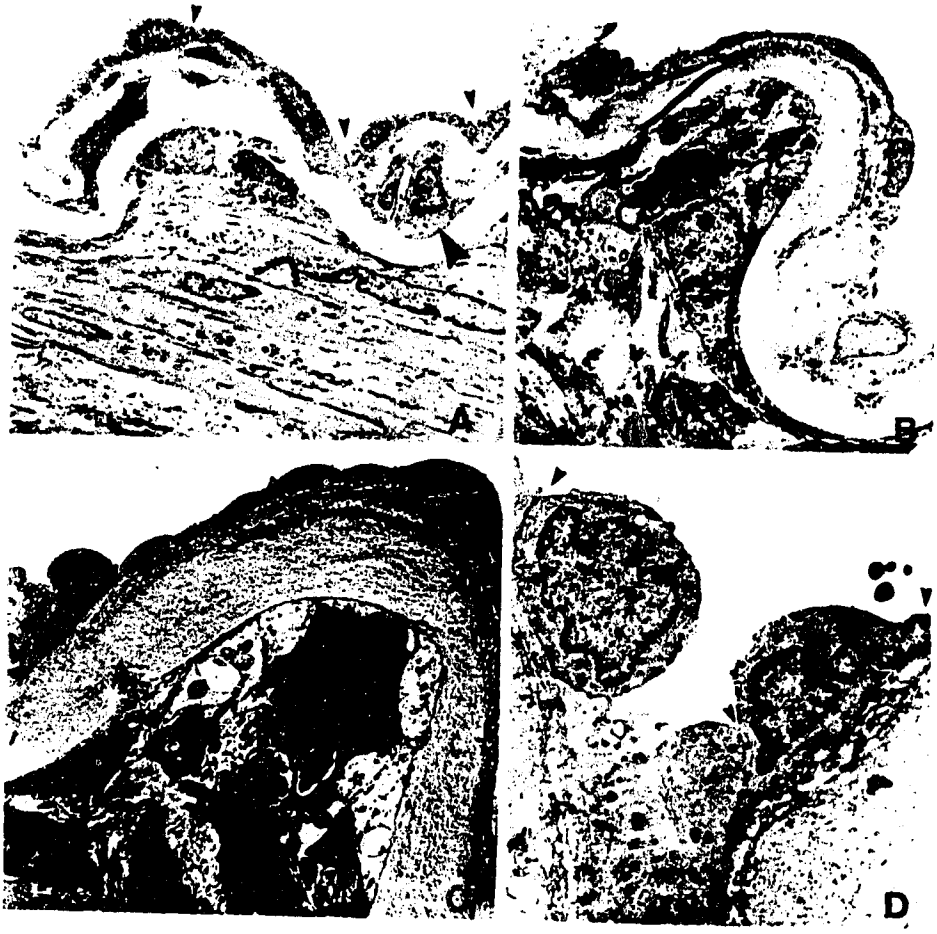

Figure II-4
Transmission electron micrographs of middle cerebral arteries vasoconstricted *in vitro* with prostaglandin $F_{2\alpha}$. Endothelial cells maintain their tight-junctions (small arrowheads), despite convolution of the intima that has crowded together several myointimal cells (large arrowhead) (A). Rounded appearance of otherwise normal endothelial cells overlying an intact internal elastic lamina (B). Contracted but non-vacuolated smooth muscle cells in the tunica media (C). Intact tight-junctions (D).

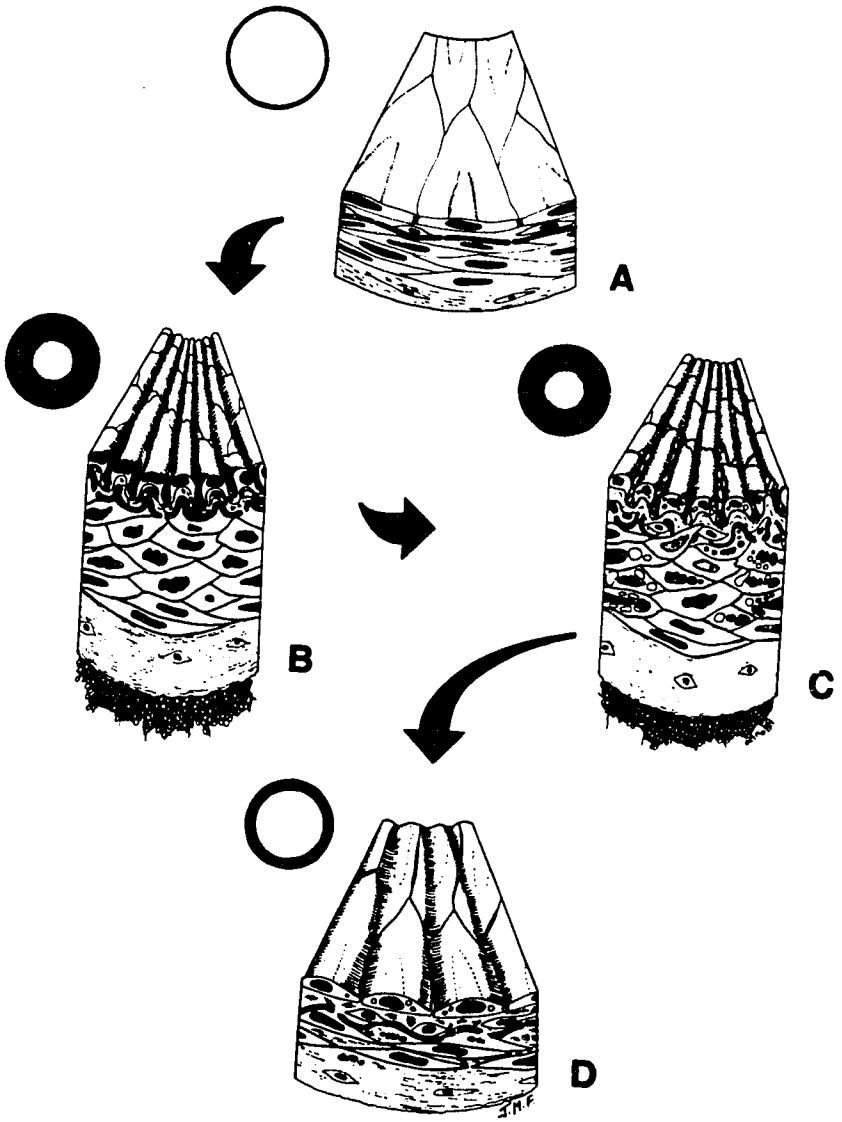

Figure II-5
Arterial wall changes in cerebral vasospasm.
Normal cerebral arterial wall (A). After subarachnoid hemorrhage the artery is coated in blood clot, which over several days induces vasoconstriction associated with medial thickening and corrugation of the internal elastic lamina and intima (B). Sustained constriction leads to functional impairment of the arterial wall associated with ultrastructural damage: endothelial vacuolization, loss of tight junctions, breakage of the internal elastic lamina, and medial myonecrosis (C). Within several weeks most vessels resume normal luminal caliber, although ultrastructural morphological abnormalities persist for a longer period of time (D).

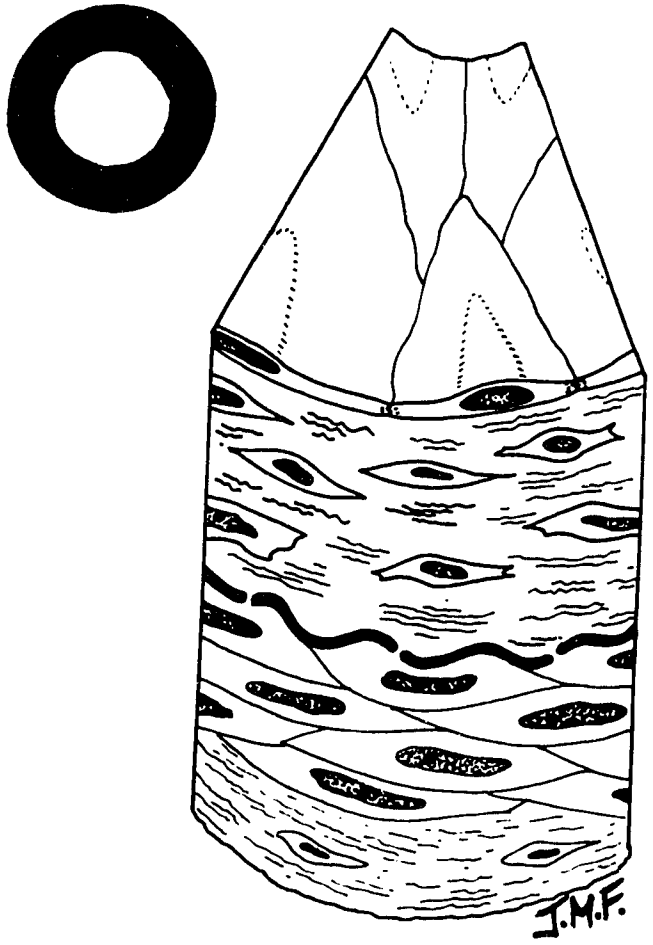

Figure II-6
In aneurysmal subarachnoid hemorrhage, vessel wall injury apparently resulted in intimal fibroplasia and thickening which produced a degree of luminal narrowing in some human autopsy studies.

CHAPTER THREE

EFFECT OF INTRATHECAL FIBRINOLYTIC THERAPY ON SUBARACHNOID CLOT AND CHRONIC VASOSPASM IN A PRIMATE MODEL

SUMMARY

The safety and efficacy of the fibrinolytic agent human recombinant tissue plasminogen activator (rt-PA) in the elimination of subarachnoid clot and prevention of chronic vasospasm (VSP) was evaluated in a blind, randomized, placebo-controlled trial. Twenty-four monkeys were randomized into 2 groups of 12, each of which underwent baseline cerebral angiography and coagulation analysis, followed by right-sided craniectomy and experimental subarachnoid hemorrhage (SAH). An Ommaya reservoir was inserted with its catheter placed into the subarachnoid space. Twenty-four hours later, the rt-PA group received 0.5 mg of rt-PA in 0.5 ml of buffer injected into the reservoir 8 hourly for 3 doses, while the placebo group received the same volume of normal saline. During treatment coagulation studies were repeated. On Day 7 angiography was repeated and the animals sacrificed. One animal, from the placebo group, developed a delayed ischemic neurologic deficit (DIND) on Day 5 after SAH. Moderate to severe VSP (>30% reduction in vessel caliber) was present on Day 7 in the internal carotid and middle cerebral arteries (MCAs) of the placebo group

A version of this chapter has been published.
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Gordon P, Grace M. J Neurosurg 69: 723-735, 1988.

($p<0.01$), while in the rt-PA group only mild narrowing of the anterior cerebral artery was seen. No significant change in coagulation status occurred in either group. All animals in the placebo group had a large amount of subarachnoid clot remaining at sacrifice, but 11 of the 12 animals in the rt-PA group were completely free of clot. The results of electron microscopy of the cerebral arteries correlated with angiography, and there was no histological evidence of brain inflammation associated with the intrathecal use of rt-PA. Intrathecal fibrinolysis with rt-PA is safe and effective in dissolving subarachnoid clot and preventing VSP in the primate model.

INTRODUCTION

Among survivors of aneurysmal subarachnoid hemorrhage (SAH) the leading cause of death and disability is brain ischemia and infarction resulting from a form of arterial narrowing known as cerebral vasospasm (VSP)[17]. It has been shown that the location and volume of subarachnoid hematoma is directly related to the incidence, distribution and severity of the ensuing VSP[4,6,8,11,18,19]. Despite the clear etiologic role of periarterial blood clot in VSP, the precise manner in which the hematoma interacts with the vessel wall in the pathogenesis of VSP is still not completely understood.

A therapeutic approach suggested to reduce the incidence or severity of VSP has been surgical evacuation of the offending subarachnoid hematoma prior to the development of arterial narrowing. Indeed, clinical studies have suggested[25,29,31,35,37,39], and experimental work has

confirmed [13,28], that thorough clot removal will prevent the onset of VSP. However, widespread clinical adoption of this practice has been hampered by the technical difficulties and dangers involved in a total mechanical evacuation of the basal cisterns in the acute stage after SAH. Interest has grown in a nonmechanical and less traumatic method of clearing subarachnoid hematoma using a clot liquefying fibrinolytic agent. Recombinant, human tissue plasminogen activator (rt-PA) is an endogenous fibrinolytic enzyme now being produced through recombinant deoxyribonucleic acid (DNA) techniques in sufficient quantities for laboratory and clinical use [32]. In the present study the safety and efficacy of intrathecally administered rt-PA in lysing and clearing subarachnoid clot and preventing chronic VSP was investigated in a primate model of SAH.

MATERIALS AND METHODS

In Vitro Determination of rt-PA Dosage

The model of SAH employed in this study involves placement of 3 to 5 ml of preclotted, autologous, whole blood into the subarachnoid space of the Cynomolgous monkey. In order to determine the dose of rt-PA necessary to completely lyse this amount of monkey blood clot incubating in a cerebral spinal fluid (CSF)-like solution, the following *in vitro* experiment was performed. Twenty-five milliliters of whole blood was obtained from each of 5 different monkeys; the blood from each monkey was then subdivided into 5 test tubes in aliquots of 5 ml each. Three sets from 3 different animals were incubated for 24 hours at 37 ° C, and the remaining 2 sets were incubated for 48 hours, also at 37 ° C. This

aging of the clots prior to testing with rt-PA was done to ensure completion of the clotting process and to mimic the situation in the SAH model. The aged clots were then placed into new tubes containing 2 milliliters of normal saline. Into separate tubes from each of the 5 sets, rt-PA dissolved in 1 ml of physiologic solution, was injected in incremental doses of 0.25 mg, 0.33 mg, 0.50 mg and 1.00 mg. The remaining tube in each set served as control, receiving 2 ml of saline only. Each vial of rt-PA (Genentech, Inc., South San Francisco, California, USA) contained 50 mg of enzyme in a lyophilized powder that when reconstituted with 50 ml of sterile water yielded a clear, colorless solution with a pH of 7.3 and an osmolarity of 215 mOsm. The specific activity of rt-PA is 500,000 units per milligram.

The tubes were kept in a gently shaking water bath at 37 ° C for 8 hours. The tube contents were then drained onto filter papers and any residual, solid blood clot remaining was collected and weighed (Mettler Instrument AE 163, Greifensee-Zurich, Switzerland).

The minimum dose of rt-PA necessary to completely lyse 5 ml of clotted Cynomolgous blood was 0.5 mg (see results), hence this dose was chosen for injection a total of 3 times into the subarachnoid space of the experimental animals. Repeated administration of rt-PA was intended as allowance for the washout effect of circulating CSF on the concentration of rt-PA delivered into the subarachnoid space.

Randomization and Blinding

This study was designed as a randomized, blinded, placebo-controlled trial (fig. III-1) Twenty-four adult, female Cynomolgous monkeys (Macaca fascicularis) weighing between 2.5 and 4 kg were assigned to one of two groups of 12 by restricted randomization, one group to receive placebo and the other rt-PA. The treatment solution for each individual animal was prepared by an independent person and identified by the monkey number alone. Both the placebo (saline) and rt-PA solution were clear and colorless. In addition, 5 animals from each group were randomly preselected for horseradish peroxidase (HRP) studies and electron microscopy of the cerebral vessels. In these animals HRP was injected through the Ommaya reservoir into the basal cisterns just prior to sacrifice. This was done to assess the ability of substances administered through the reservoir to disseminate throughout the subarachnoid space and contact all major cerebral arteries.

Day 0: Baseline Assessment and Cerebral Angiography

Prior to handling the animals were sedated with ketamine hydrochloride, 6-10 mg/kg intramuscularly. After weighing, the animals were intubated and ventilated with a 2:1 mixture of $N_2O:O_2$, administered by a variable phase animal respirator (Harvard Apparatus, Inc., Millis, Massachusetts), and ventilation rate was adjusted to maintain the $PaCO_2$ near 40 mmHg as ascertained by arterial blood gas measurement. Paralysis was established with gallamine, 2 mg/kg intravenously and analgesia with fentanyl, 1 mcg/kg intravenously. Body temperature was maintained at 37 ° C by heating pad and monitored by rectal

thermometer (Tele-thermometer; Yellow Springs Instrument Co., Yellow Springs, Ohio). Procaine penicillin, 100,000 IU/kg was administered intramuscularly prior to angiography.

Using sterile technique the right axillary artery was dissected under magnification and catheterized with a 7 French, radiopaque, polyethylene catheter (fig. III-2). Approximately 15 ml of blood was drawn from this catheter for coagulation analysis, followed by an additional 5 ml which was allowed to clot in a separate test tube for later use in the creation of the experimental SAH. One citrated tube of blood drawn for coagulation studies was immediately centrifuged at 4 ° C and the supernatant plasma frozen for later batch testing of thrombin time; the euglobulin clot lysis time (ELT) was performed on the euglobulin fraction prepared by dilution and acidification [15]. A separate sample was also collected for later batch testing for fibrin degradation products (FDPs) using the Thrombo-Wellcotest latex slide test (Wellcome Reagents Ltd., Beckenham, England). The catheter was then advanced under fluoroscopic control into the communis artery from which both common carotids originate in the Cynomolgous monkey. The catheter was then connected via a three-way stopcock to a pressure transducer for monitoring of blood pressure (BP) and heart rate (Stratham P23dB pressure transducer; Stratham Instrument Co., Oxnard, California). Constant BP and heart rate recording was done on a Beckman Dynograph R611 eight channel recorder. Angiography was then performed, consisting of one arterial phase, anteroposterior film obtained following injection of 12 ml of iothalamate meglumine at 250 psi via a Cordis Injector (Cordis Corp., Miami, Florida).

Exposure factors were kept constant and a magnification control standard was used for correction to constant magnification.

Operative Procedure: Experimental SAH

Immediately following baseline angiography and under the same anesthetic supplemented with intravenous sodium pentobarbital, 26 mg/kg, and additional gallamine when necessary, all animals underwent a right fronto-temporal craniectomy. To reduce cerebral blood volume and facilitate brain retraction, ventilation was adjusted during the surgical procedure to lower the $PaCO_2$ to between 25 and 35 mmHg. The craniectomy was centered over the pterion and extended to the floors of both the anterior and middle cranial fossae. The dura mater was opened, frontal lobe elevated and temporal lobe retracted posteriorly to expose the ipsilateral optic nerve, internal carotid artery (C4) and adjacent basal cisterns. With the aid of the operating microscope the arachnoid enclosing the basal cisterns and covering the cerebral arteries was incised and dissected, opening the subarachnoid cisterns and exposing the intradural segment of the internal carotid artery, the precommunicating segment of the anterior cerebral artery (A1), and the sphenoidal segment of the middle cerebral artery (MCA). Lilequest's membrane was opened and in some cases it was possible to follow the posterior communicating artery back to the posterior cerebral artery (PCA), and basilar artery (BA). Between 3.5 to 5 ml of previously obtained and now clotted autologous blood was then carefully placed into the subarachnoid space of the cisterns that had been opened, in contact with the cerebral vessels. The dura was closed with interrupted 6-0 silk suture. A fine, perforated

catheter attached to an Ommaya reservoir was inserted through the repaired dural incision into the subarachnoid space adjacent to the subarachnoid clot. The temporalis muscle, subcutaneous fascia and skin were closed in layers using 3-0 silk suture and monofilament polyethylene suture (Dermalon; Davis and Geck, New York, New York), leaving the Ommaya reservoir in a subcutaneous position (fig. III-3). At the completion of the operative procedure paralysis was reversed with intravenous prostigmine 0.07 mg/kg and atropine 0.02 mg/kg, and the animals were ventilated with 100% O_2 until breathing spontaneously. They were extubated upon return of the gag reflex. The animals were returned to their cages where they were fed their usual diet of biscuits, fruit and water.

Day 1: rt-PA and Placebo Administration

Twenty-four hours after cerebral angiography and experimental SAH, the animals began treatment, which consisted of percutaneous puncture of the Ommaya reservoir and injection of 0.5 ml of either placebo (saline), or rt-PA solution containing 0.5 mg of rt-PA. This injection was repeated two more times at eight-hour intervals. Prior to each injection 2 to 3 ml of clear to sanguinous CSF was aspirated from the Ommaya reservoir. Between the second and third injections blood was drawn for repeat coagulation analysis.

Days 2 to 6: Clinical Observation

During this time the animals were monitored closely for the development of any wound complications, general health problems or delayed ischemic neurologic deficits (DINDs). The animals were considered to have a DIND if they developed any left-sided weakness (evident in limb posture and strength) later than 2 days after the

experimental SAH. In the event of a DIND a magnetic resonance imaging (MRI) scan was performed on a Bruker BNT 100 NMR imaging system, obtaining proton images at 100 MHz.

Day 7: Repeat Cerebral Angiography and Sacrifice

Seven days after SAH the animals were sedated, intubated and ventilated under anesthesia as for the baseline assessment, and after re-exposing and catheterizing the right axillary artery, repeat cerebral angiography was carried out. Again arterial blood gases were obtained and ventilation adjusted to maintain a $PaCO_2$ of approximately 40 mmHg. Five randomly pre-selected animals from each group had HRP, 80 mg/kg dissolved in 2 ml of normal saline, injected through the Ommaya reservoir into the subarachnoid space 15 minutes prior to sacrifice. The animals then received a large intravenous dose of pentobarbital, approximately 50 mg/kg, and were sacrificed by exsanguination. Intra-arterial perfusion was performed with a left ventricular cannula with the right atrium opened widely and the descending aorta cross-clamped. Circulating blood was washed out with 0.5 liter of normal saline under 110 mmHg pressure, followed by 0.5 liter perfusion with fixation solution consisting of 2% glutaraldehyde and 2% formaldehyde in Millonig's buffer, 0.12 M, pH 7.4, at 4 ° C.

Pathologic Examination

Following sacrifice the animal's calvarium was removed and the brain along with any remaining subarachnoid clot was taken out and photographed. Gross subarachnoid clot was then separated from the brain and weighed.

In animals who received intrathecal HRP prior to sacrifice the entire circle of Willis and attached basilar and vertebral arteries were dissected free under magnification and incubated in a saturated solution of diaminobenzidine (0.05 M) in Trix-HCL buffer, pH 7.6, and 0.01% H_2O_2. Any HRP reaction products absorbed to the vessel walls stained dark brown, and a record was kept of positively staining vessels.

These same vessels were then placed in a glutaraldehyde-formaldehyde fixative mixture for several days and then divided into segments for scanning and transmission electron microscopy (SEM and TEM respectively). Arterial segments from C4, A1 and the MCA were fixed for one hour in 1% osmium tetroxide in Millonig's buffer, 0.07 M, and then prepared for SEM (Phillips 505 electron microscope; N. V. Phillips Gloeilampenfabrieken, Eindhoven, The Netherlands), and TEM (Phillips, model 410).

The brains from all of the animals were immersed in formalin for one week, and sliced coronally to display the pia-arachnoid and cerebral vessels at the base of the brain, the cerebral cortex and ventricles. Representative sections were made of these areas bilaterally and stained with hematoxylin and eosin (H&E) for light microscopy (LM). Coronal sections of the superior sagittal sinus and associated arachnoid granulations were taken from near the parieto-occipital junction and also stained with H&E for LM.

Radiologic Assessment

Cerebral vessels were measured 4 times with a calibrated optical micrometer, and a mean value obtained. The arteries were measured bilaterally at the following points: extradural internal

carotid artery (C3), intradural internal carotid artery (C4), precommunicating segment of the ACA (A1), sphenoidal segment of the MCA, azygous distal anterior cerebral artery (A2), the first part of the PCA, basilar artery (BA) and vertebral arteries (VA).

This protocol was evaluated and approved by the Animal Ethics Review Committee of the University of Alberta. Care and surgery of the animals were performed according to the standards of the Canadian Council on Animal Care.

All data were coded, entered into a computer, and edited. Data for angiographic vessel caliber change within treatment groups between days 0 and 7 were compared by a paired t-test, and intergroup comparisons were made with a t-test for unpaired variables. Analysis of other measured indices including coagulation studies was done with either a t-test or Fisher's exact test where appropriate. The level of significance for all tests of comparison was $p<0.05$ unless otherwise stated.

RESULTS

Comparisons between the 2 treatment groups showed no significant differences in Day 0 or Day 7 values in body weight, MABP, heart rate, or $PaCO_2$ (Table III-1). Also, there was no significant change in any of these indices within each group between these 2 times. There was no significant difference in volume of clot placed in the creation of the experimental SAH.

In Vitro Determination of rt-PA Dosage

There was no evidence of clot lysis in any of the control tubes. Tubes containing rt-PA showed increasing clot lysis with higher doses of rt-PA complete lysis was obtained in 3 of 5 tubes

containing 1 mg of rt-PA and 2 of 5 tubes containing 0.5 mg of rt-PA (Table III-2, fig. III-4). Clots aged 24 hours versus those aged 48 hours prior to testing did not differ in their response to fibrinolysis. Statistical analysis revealed that while there was no significant difference between 0.5 and 1.0 mg rt-PA on the amount of clot lysis, there was a significant difference between these doses and the lower doses tested. We concluded that the minimum dose of rt-PA required to completely lyse 5 ml of clotted, whole Cynomolgous blood is 0.5 mg, and this was selected as the dose to be used in the *in vivo* primate experiment.

Clinical Status

Two animals in the rt-PA group developed a sanguinous wound leak, one on Day 1 after SAH (during rt-PA administration), and the other on Day 2 after SAH. The fluid appeared to be either blood or bloody CSF. Both leaks stopped spontaneously within 24 hours of onset. It is likely that these leaks represented impaired wound hemostasis secondary to epidural leakage of rt-PA through the surgical defect in the dura mater.

Monkey 12, in the placebo group, died unexpectedly on Day 2 after SAH of an uncertain cause. This animal awoke from surgery with a slight left hemiparesis which worsened the following day and became associated with a decreased level of consciousness. It was found dead the next morning. Day 7 angiography was not possible in this animal.

Monkey 18, also in the placebo group, developed a DIND on day 5 after SAH. This consisted of a left hemiparesis with greater involvement in the upper extremity that persisted until sacrifice.

An MRI scan showed an equivocal increased signal abnormality in the right temporal lobe. None of the other animals in the study developed a clinically detectable, delayed onset, neurologic impairment.

Coagulation Studies

If a plasminogen activator is released into the circulation in concentrations high enough to activate circulating plasminogen, systemic fibrinogenolysis and a bleeding diathesis can occur. Urokinase administered into the ventricles of dogs gains access to the circulation resulting in a systemic fibrinolytic state[30], so coagulation status of our animals was monitored.

Thrombin converts fibrinogen to fibrin directly, and the addition of thrombin to plasma results in clot formation. The time taken for the clot to appear is called the thrombin time, and it is abnormally prolonged if either the concentration of fibrinogen in the plasma sample is low, if the clot is immediately lysed by an excess of plasmin in the sample, or if there is an excess of FDPs. A prolonged thrombin time would therefore reflect systemic activation of the fibrinolytic system. Compared to baseline, there was no significant change in the thrombin times (determined with two concentrations of thrombin) during treatment with either placebo or rt-PA (Table III-3).

The presence of systemic fibrinolysis is also detected with the euglobulin clot lysis time (ELT). The euglobulin fraction of plasma is relatively free of plasmin inhibitors, so that if it is allowed to clot it will then commence spontaneous lysis at a rate dependent upon the concentration of plasminogen activators in the sample.

Since there is normally a very low level of fibrinolytic activity in the circulation the ELT usually takes longer than 90 minutes. An ELT shorter than this is considered abnormal. In this study there was only one recorded ELT less than 90 minutes, and that was from a placebo group animal during treatment who had an ELT of 75 minutes.

Fibrinogenolysis is accompanied by the appearance of fibrin degradation products (FDPs) in plasma; only 2 animals were positive for FDPs, one rt-PA group animal at baseline testing and one placebo group animal during treatment. Both animals had an almost insignificant level of 2 μ g/ml.

These coagulation results indicate that the dose of rt-PA used in this study did not result in systemic activation of the fibrinolytic system.

Extent of Cerebral Vasospasm

Vasospasm was defined as a reduction in vessel caliber of 10% or greater compared to baseline value. In the rt-PA treated group the only significant change in vessel caliber occurred in the right A1 segment where a 14% reduction in vessel caliber was seen ($p<0.01$). In the placebo group a significant reduction in vessel caliber ($p<0.01$) was seen on the right (clot) side in C3 (19% reduction), C4 (33% reduction), and the MCA (51% reduction) (figs. III-5,6,7).

Intergroup comparisons of vessel caliber on Day 0 revealed no significant differences between the placebo and rt-PA group animals; however there were significant differences between these groups on Day 7 in the right C3, C4 and MCA ($p<0.05$).

Considering only the MCA, within the rt-PA group all 12 animals either had no or mild VSP, whereas in the 11 placebo treated animals that survived to Day 7 1 animal had no VSP, 2 animals had mild VSP (11-30% reduction in vessel caliber), 3 animals had moderate VSP (31-50% reduction in vessel caliber), and 5 animals had severe VSP (>50%).

Pathology

Gross Examination

At the time of sacrifice all placebo treated animals had gross subarachnoid clot remaining (fig. III-8); the mean weight of remaining clot was 0.63 gms with a standard deviation (SD) of 0.23. Only one of the rt-PA treated animals had any clot remaining at sacrifice, monkey 7 with 0.050 gms (fig. III-9). The difference between groups was highly significant ($p<0.0001$). None of the animals had epidural, subdural or intracerebral hemorrhages, and none had evidence of ventricular dilatation.

HRP Studies

All animals that received intrathecal HRP prior to sacrifice demonstrated HRP reaction products on the entire circle of Willis and the distal one-half of the BA, indicating that protein molecules administered through the Ommaya reservoir can circulate freely throughout the subarachnoid cisterns and contact the cerebral vessels within the cisterns.

Electron Microscopy

Most arteries from the left side of both treatment groups were normal; in one rt-PA treated animal the left A1 and MCA showed small endothelial convolutions, but were otherwise normal. On SEM almost all right sided vessels from the placebo group animals were

narrowed, showing deep, longitudinal endothelial convolutions and a thickened arterial wall. Thrombus was usually seen adherent to the adventitial surface of the vessel (fig. III-10). On TEM these vessels demonstrated contracted smooth muscle cells, corrugation of the internal elastic lamina, intimal swelling and morphological changes in the endothelial cells.

Of the 5 rt-PA treated animals examined, 3 had normal appearing right sided cerebral arteries on SEM and TEM, and 2 had mild vasospastic changes consisting of shallow endothelial convolutions. The surface of these vessels was invariably free of clot debris and normal appearing.

Light Microscopy

Histological examination of monkey 12, which died unexpectedly on Day 2, was unremarkable. Monkey 18, which developed a DIND on Day 5, showed patchy areas of recent infarction in the right temporal lobe, consisting primarily of eosinophilic neuronal degeneration. None of the other animals showed evidence of cortical or ependymal damage. In 8 of the 12 placebo group animals there were fragments of hematoma adherent to the basal pia-arachnoid membrane on the right side, and in 4 of these there was a concomitant mild to moderate leukocytic meningeal infiltrate. In several cases major cerebral vessels were fortuitously included in the specimen, and there was histological evidence of VSP (fig. III-11).

The basal pia-arachnoid membrane and cerebral vessels of the rt-PA treated animals were, with few exceptions, entirely normal (fig. III-12). Approximately one-half of the animals from each group

the failure of intrathecally administered nimodipine to consistently affect angiographic VSP in a primate model of SAH.

It has been suggested that the beneficial effect of nimodipine might be in the dilatation of smaller arterioles not visualized on angiography, or that the drug may have a direct cerebral protective effect, possibly by inhibiting calcium flux into ischemic neurones. Whatever its mode of action, nimodipine appears to have a definite role in the prevention of cerebral ischemic damage resulting from VSP. Another promising calcium antagonist, nicardipine, is being tested in a multicenter, randomized trial [13] at the time of this writing.

Hemodynamic Therapy

In VSP the cerebral arteries are constricted and rigid so CBF becomes passively dependent on cerebral perfusion pressure and blood viscosity. Realizing that it may be impossible to dilate these spastic vessels, clinicians have sought to improve CBF by increasing the perfusion pressure and lowering blood viscosity. Theoretically it might be possible through a modest improvement in blood flow to raise cerebral perfusion from levels of symptomatic ischemia and impending infarction to a range of asymptomatic oligemia, thus maintaining neuronal viability.

Elevation of perfusion pressure is accomplished with intravascular volume expansion sometimes combined with cardiac inotropic agents to elevate cardiac output and mean blood pressure, while simultaneously reducing raised intracranial pressure. Blood viscosity reduction usually accompanies hemodilutional volume expansion. In patients with heart disease or those in whom

vasopressor agents are required, insertion of a pulmonary artery catheter is required to monitor left-heart filling pressures, as it is not uncommon to induce a degree of congestive heart failure with aggressive hemodynamic therapy. A major danger of induced hypertension is aneurysm rebleeding, so that in most cases this therapy should be reserved for postoperative patients in whom the aneurysm has been secured. Notwithstanding these dangers, hemodynamic therapy is the most effective treatment available at this time for symptomatic VSP, with several uncontrolled trials reporting sustained neurological improvement in 60 to 75% of patients [5,20].

Subarachnoid Clot Removal

Since it has not been possible to pharmacologically dilate vasospastic cerebral vessels successful therapies to date have only been able to modify the effect of the VSP, not prevent or reverse it. Consequently some neurosurgeons have sought to prevent the onset of VSP by surgically removing the subarachnoid clot prior to its deleterious effects on the cerebral vessel wall [41]. These studies suggested that aggressive clot removal carried out at the time of early aneurysm clipping and within 48 hours of SAH reduce the risk of VSP and/or delayed ischemia, and these findings have subsequently been confirmed by Nosko et al [32] and Handa et al [16] in a primate model. Clinical adoption of this practice has been hampered by the technical difficulties and dangers inherent in a thorough, mechanical toilet of the basal subarachnoid cisterns. A less traumatic method of clearing subarachnoid hematoma using the thrombolytic agent recombinant tissue plasminogen activator (rt-PA)

has been investigated in primates [11]. Administered into the basal cisterns beginning 24 hours after experimental SAH, rt-PA was effective in completely lysing the subarachnoid clot in 11 of 12 treatment animals, and it resulted in a significant reduction in angiographic VSP at seven days compared to the placebo treated animals. There was no adverse effect on the animals' coagulation systems nor histologic evidence of meningeal or parenchymal inflammation associated with rt-PA treatment.

Other Treatments

On the premise that VSP may be in part due to free radicals and lipid peroxides generated by the degenerating subarachnoid clot, a potent inhibitor of iron-dependent lipid peroxidation was administered to monkeys after experimental SAH. The 21-aminosteroid U74006F resulted in a modest reduction in the severity of VSP in treated animals compared to placebo treated controls, and there was a suggestion that this drug may also have a cerebral cytoprotective effect [39].

SUMMARY

Our present policy to manage patients with aneurysmal SAH from anterior circulation and technically easy posterior circulation aneurysms is to operate as early as possible to clip the aneurysm and prevent catastrophic rehemorrhage. At operation as much subarachnoid clot as possible is suctioned from the exposed basal cisterns. Hypotension and temporary clips are used only if absolutely necessary. Patients are treated with moderate volume expansion with a mixture of crystalloid and colloid solutions to

increase cardiac output, improve cerebral perfusion and achieve a degree of hemodilution. Intracranial hypertension is treated by CSF drainage via a ventricular or cisternal catheter, ventilation, and osmotherapy if necessary. In the event of delayed ischemia more aggressive hemodynamic therapy is instituted including inotropic support with invasive hemodynamic monitoring. The left-heart filling pressure is incrementally elevated with volume expansion to the point of optimal cardiac output (a pulmonary artery capillary pressure of approximately 18 to 20 mmHg) and systolic BP is raised as high as 200 mmHg in order to reverse the neurologic deficit. Close attention is paid to the oxygenation and acid-base status of the patient, as cardiopulmonary complications of this treatment are common.

Patients who are referred late after SAH who already have angiographic VSP are also treated by prompt aneurysm clipping unless they are comatose or VSP is severe and diffuse. The use of antifibrinolytic agents in almost all patients has been abandoned partly because of the current policy of early surgery and also because of an associated increased risk of delayed ischemic deficits[54]. Their employment would still be considered in a technically difficult posterior circulation aneurysm with a low volume SAH on the initial computerized tomographic scan, when the patient is not being operated acutely.

Although not available for clinical use in Canada at the time of writing, it is felt that calcium antagonists will be useful in preventing delayed ischemic damage in the future, and that the prevention of VSP through thrombolytic clearance of the

subarachnoid clot warrants further investigation. Effective pharmacologic reversal of VSP, if at all possible, may need to await further clarification of the precise biochemical pathogenesis of this disorder.

BIBLIOGRAPHY

1. Allen GS, Ahn HS, Prezosi TJ, et al: Cerebral arterial spasm - A controlled trial of nimodipine in patients with subarachnoid hemorrhage. N Eng J Med 308: 619-624, 1983.
2. Asano T, Tanishima T, Sasaki T, et al: Possible participation of free radical reactions initiated by clot lysis in the pathogenesis of vasospasm after subarachnoid hemorrhage. In Wilkins RH (ed): *Cerebral Arterial Spasm*. Baltimore, Williams and Wilkins, 1980, 190-201.
3. Astrup J, Siesjo BK, Symon L: Thresholds in cerebral ischemia - The ischemic penumbra. Stroke 12: 723-725, 1981 (editorial).
4. Auer LM, Ljunggren B, Brandt L, et al: Acute aneurysm surgery and nimodipine for the prevention of symptomatic vasospasm. In Auer LM (ed): *Timing of Aneurysm Surgery*. Berlin, Walter de Gruyter, 1985, pp 493-502.
5. Awad IA, Carter LP, Spetzler RF, et al: Clinical vasospasm after subarachnoid hemorrhage: response to hypervolemic hemodilution and arterial hypertension. Stroke 18: 365-372, 1987.
6. Dion JE, Gates PC, Fox AJ, et al: Clinical events following neuroangiography: A prospective study. Stroke 18: 997-1004, 1987.
7. Duff TA, Louie J, Feilbach JA, et al: Erythrocytes are essential for development of cerebral vasculopathy resulting from subarachnoid hemorrhage in cats. Stroke 19: 68-72, 1988.

8. Espinosa F, Weir B, Shnitka T: Electron microscopy of simian cerebral arteries after subarachnoid hemorrhage and after the injection of horseradish peroxidase. Neurosurgery 19: 935-945, 1986.

9. Espinosa F, Weir B, Overton T, et al: A randomized placebo-controlled double-blind trial of nimodipine after SAH in monkeys. Part 1: Clinical and radiological findings. J Neurosurg 60: 1167-1175, 1984.

10. Fein JM, Flor WJ, Cohan SL, et al: Sequential changes of vascular ultrastructure in experimental cerebral vasospasm. Myonecrosis of subarachnoid arteries. J Neurosurg 41: 49-58, 1974.

11. Findlay JM, Weir BK, Steinke D, et al: Effect of intrathecal thrombolytic therapy on subarachnoid clot and chronic cerebral vasospasm in a primate model of SAH. J Neurosurg 69: 723-735, 1988.

12. Fisher CM: Clinical syndromes in cerebral thrombosis, hypertensive hemorrhage, and ruptured saccular aneurysm. Clin Neurosurg: 117-147, 1975.

13. Flamm ES, Adams Jr HP, Beck DW et al: Dose-escalation study of intravenous nicardipine in patients with aneurysmal subarachnoid hemorrhage. J Neurosurg 68: 393-400, 1988.

14. Grolimund P, Seiler RW, Aaslid R, et al: Evaluation of cerebrovascular disease by combined extracranial and transcranial Doppler sonography. Experience in 1,039 patients. Stroke 18: 1018-1024, 1987.

15. Grubb RJ Jr, Raichle ME, Eichling JO, et al: Effects of subarachnoid hemorrhage on cerebral blood volume, blood flow and oxygen utilization in humans. J Neurosurg 44: 446-452, 1977.

16. Handa Y, Weir BKA, Nosko M, et al: The effect of timing of clot removal on chronic vasospasm in a primate model. J Neurosurg 67: 558-564, 1987.

17. Hennerici M, Rautenberg W, Schwartz A: Transcranial Doppler ultrasound for the assessment of intracranial flow velocity Part 2. Evaluation of intracranial arterial disease. Surg Neurol 27: 523-532, 1987.

18. Heros RC, Zervas NT, Varsos V: Cerebral vasospasm after subarachnoid hemorrhage: An update. Ann Neurol 14: 599-608, 1983.

19. Hughes JT, Schianchi PM: Cerebral arterial spasm. A histological study at necropsy of the blood vessels in cases of subarachnoid hemorrhage. J Neurosurg 48: 515-525, 1978.

20. Kassell NF, Peerless SJ, Durward QJ, et al: Treatment of ischemic deficits from vasospasm with intravascular volume expansion and induced arterial hypertension. Neurosurgery 11: 337-342, 1982.

21. Kistler JP, Crowell RM, Davis KR, et al: The relation of cerebral vasospasm to the extent and location of subarachnoid blood visualized by CT scan: A prospective study. Neurology (N.Y.) 33: 424-426, 1983.

22. Krueger C, Weir B, Nosko M, et al: Nimodipine and chronic vasospasm in monkeys. Part 2: Pharmacological studies of vessels in spasm. Neurosurgery 16: 137-140, 1985.

23. Lewis PJ, Weir BKA, Nosko MG, et al: Intrathecal nimodipine therapy in a primate model of chronic cerebral vasospasm. Neurosurgery: 22: 492-500, 1988.

24. Ljunggren B, Brandt L, Saveland H, et al: Outcome in 60 consecutive patients treated with early aneurysm operation and intravenous nimodipine. J Neurosurg 58: 864-873, 1984.

25. Martin WRW, Baker RP, Grubb RL, et al: Cerebral blood volume, blood flow, and oxygen metabolism in cerebral ischemia and subarachnoid hemorrhage: an *in vivo* study using positron emission tomography. Acta Neurochir (Wien) 70: 3-9, 1984.

26. Mayberg MR, Hauser OW, Sundt TM Jr: Ultrastructural changes in feline arterial endothelium following subarachnoid hemorrhage. J Neurosurg 48: 49-57, 1978.

27. Mayberg M, Okada T, Bark D: Cerebral arteriopathy and contractile changes after SAH are related to erythrocyte component of whole blood. Stroke 19: 130, 1988 (abstr.)

28. Mee E, Dorrance D, Lowe D, et al: Controlled study of nimodipine in aneurysm patients treated early after subarachnoid hemorrhage. Neurosurgery 22: 484-491, 1988.

29. Nakagomi T, Kassell NF, Sasaki T, et al: Impairment of endothelium-dependent vasodilation induced by acetylcholine and adenosine triphosphate following experimental subarachnoid hemorrhage. Stroke 18: 482-489, 1987.

30. Nakagomi T, Kassell NF, Sasaki T, et al: Effect of hypoxia on the contractile response to KCL, prostaglandin $F_{2\alpha}$ and hemoglobin. J Neurosurg 67: 565-572, 1987.

31. Nosko M, Weir B, Krueger C, et al: Nimodipine and chronic vasospasm in monkeys. Part 1: Clinical and radiological findings. Neurosurgery 16: 129-136, 1985.

32. Nosko M, Weir BKA, Lunt A, et al: Effect of clot removal at 24 hours on chronic vasospasm after SAH in the primate model. J Neurosurg 66: 416-422, 1987.

33. Ohta T, Kajikawa H, Yoshikawa Y, et al: Cerebral vasospasm and hemoglobins: Clinical and experimental studies. In Wilkins RH (ed): *Cerebral Arterial Spasm*. Baltimore, Williams and Wilkins, 1980, pp 166-172.

34. Petruk KC, West M, Mohr G, et al: Nimodipine treatment in poor-grade aneurysm patients. Results of a multicenter double-blind placebo-controlled trial. J Neurosurg 68: 505-517, 1988.

35. Philippon J, Grob R, Dagreou F, et al: Prevention of vasospasm in subarachnoid hemorrhage. A controlled study with nimodipine. Acta Neurochir (Wien) 82: 110-114, 1986.

36. Sasaki T, Asano T, Takakura K, et al: Nature of the vasoactive substance in CSF from patients with subarachnoid hemorrhage. J Neurosurg 60: 1186-1191, 1984.

37. Seiler RW, Grolimund P, Aaslid R, et al: Cerebral vasospasm evaluated by transcranial ultrasound correlated with clinical grade and CT-visualized subarachnoid hemorrhage. J Neurosurg 64: 594-600, 1986.

38. Seiler RW, Grolimund P, Zurbruegg HR, et al: Evaluation of the calcium-antagonist nimodipine for the prevention of vasospasm after aneurysmal subarachnoid hemorrhage. Acta Neurochir (Wien) 85: 7-16, 1987.

39. Steinke DE, Weir BKA, Findlay JM, et al: A trial of the 21-aminosteroid U74006F in a primate model of chronic cerebral vasospasm. Neurosurgery 24:179-186, 1989.

40. Tanabe Y, Sakata K, Yamada H, et al: Cerebral vasospasm and ultrastructural changes in cerebral arterial wall. J Neurosurg 49: 229-238, 1978.

41. Taneda M: Effect of early operation for ruptured aneurysms on prevention of delayed ischemic symptoms. J Neurosurg 57: 622-628, 1982.

42. Vinall PE, Simeone FA: Effects of oxygen and glucose deprivation on vasoactivity in isolated bovine middle cerebral arteries. Stroke 17: 970-975, 1986.

43. Weir B: *Aneurysms Affecting the Nervous System*. Baltimore, Williams and Wilkins, 1987, pp 246-250.

44. Weir B: Antifibrinolytics in subarachnoid hemorrhage. Do they have a role? No. Arch Neurol 44: 116-118, 1987.

45. Wilkins RH: Attempted prevention or treatment of intracranial arterial spasm: a survey. Neurosurgery 6: 198-210, 1980.

46. Wilkins RH: Attempted prevention or treatment of intracranial arterial spasm: an update. Neurosurgery 18: 808-825, 1986.

47. Yamakami I, Isobe K, Yamaura A, et al: Vasospasm and regional cerebral blood flow (rCBF) in patients with ruptured

intracranial aneurysms: serial rCBF studies with the xenon-133 inhalation method. Neurosurgery 13: 394-401, 1983.

48. Yonas H, Wolfson SK Jr, Gur D, et al: Clinical experience with the use of xenon-enhanced CT blood flow mapping in cerebrovascular disease. Stroke 15: 443-450, 1984.

49. Zervas NT, Liszczak TM, Mayberg MR, et al: Cerebrospinal fluid may nourish cerebral vessels through pathways in the adventitia that may be analogous to systemic vasa vasorum. J Neurosurg 56: 475-481, 1982.

CHAPTER TWO

ARTERIAL WALL CHANGES IN CEREBRAL VASOSPASM

SUMMARY

A right-sided subarachnoid hemorrhage (SAH) was created in 12 monkeys. Only the right (clot-side) cerebral arteries developed angiographic vasospasm (VSP), which was maximal 7 days after SAH. Eight animals were sacrificed at this time and the remainder at 14 days. At sacrifice the middle cerebral arteries (MCAs) were harvested, and four normal left (nonclot-side) MCAs were vasoconstricted *in vitro* with prostaglandin $F_{2\alpha}$. All MCAs were studied with scanning and transmission electron microscopy (SEM and TEM). Right MCAs in maximal VSP 7 days from SAH were undistinguishable with SEM from normal arteries vasoconstricted *in vitro*: both groups demonstrated a mean 57% reduction in lumen caliber and a five-fold increase in vessel wall thickness compared to normal, nonvasoconstricted left MCAs. On TEM, however, arteries in SAH induced VSP demonstrated degenerative changes in the tunica intima and media. These changes were still evident at 14 days, despite considerable resolution of VSP. These findings, as well as those from other pathological studies of animal and human cerebral arteries in VSP, suggest that the arterial narrowing and vessel wall thickening seen within several weeks of SAH is due primarily to medial contraction,

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but unlike physiologic vasoconstriction, is associated with degenerative ultrastructural changes in the endothelium and vascular smooth muscle cells which may denote a temporarily irreversible state.

INTRODUCTION

Certain pathological changes have been noted in the walls of cerebral arteries known to be in vasospasm (VSP) following aneurysmal subarachnoid hemorrhage (SAH). It is not known whether these changes reflect damage incurred by prolonged, intense vasoconstriction, or if they represent the direct effect of vasotoxins released by decaying subarachnoid thrombus, or if they result from both processes. Such substances could conceivably injure the tunica intima leading to platelet aggregation, release of mitogens and induction of an inflammatory vessel wall reaction. Some proponents of this latter hypothesis have suggested that the cerebral arterial narrowing seen after SAH is not due primarily to vascular spasm, but instead reflects a thickened, hyperplastic arterial wall. The purpose of this report is to critically review this theory on the basis of our own data from an established model of chronic cerebral VSP in primates as well as a review of published studies on arterial wall changes after SAH.

MATERIALS AND METHODS

Data from 12 female Cynomolgous monkeys (Macaca fascicularis), weighing between 2.3 and 4.5 kg were examined for this report. Details of the procedures for microsurgical exposure of the basal subarachnoid cisterns, induction of SAH, cerebral angiography and animal sacrifice used in our model have been

described elsewhere [18], and will be outlined briefly here. The protocols were evaluated and approved by the University of Alberta's Animal Ethics Review Committee, and experiments were conducted with strict adherence to the standards of the Canadian Council on Animal Care.

After baseline cerebral angiography the animals underwent a right fronto-temporal craniectomy for opening of the basal cisterns, exposure of the cerebral arteries and placement of approximately 4.25 ml of autogenous blood clot into the subarachnoid space. Dura, temporalis muscle and skin were closed, the animals awoken from anesthesia and extubated upon return of the gag reflex. Repeat cerebral angiography and sacrifice by pentobarbital overdose and exsanguination was carried out on the seventh day after SAH in 8 of the animals studied and on the fourteenth day in 4. Intraarterial perfusion was performed with a left ventricular cannula with the right atrium opened widely and the descending aorta cross-clamped. Circulating blood was washed out with 0.5 liters of normal saline under 110 mm Hg pressure. The brains were immediately removed, and any remaining subarachnoid clot noted. Both middle cerebral arteries (MCAs) were dissected from each brain under magnification. The right, clot-side MCA from all of the animals was placed into fixative solution consisting of 2% glutaraldehyde and 2% formaldehyde in Millonig's buffer, 0.12 M, pH 7.4, at 4 ° C. Except for 4 animals sacrificed on day 7 after SAH, the left, nonclot-side MCAs were also placed into fixative. The remaining 4 MCAs were placed into Krebs-Henseleit solution (KHS) (115.0 mM NaCl, 4.7 mM KCl, 2.5 mM CaCl, 1.2 mM $MgCl_2$, 25.0

mM $NaHCO_3$, 1.2 mM KH_2PO_4, 10.0 mM glucose) equilibrated with 95% O_2 and 5% CO_2 at room temperature. Arteries were cut into rings 5 mm long and mounted vertically in a water-jacketed tissue bath containing 10 ml KHS maintained at 37 ± 0.5 ° C and bubbled with 95% O_2 and 5% CO_2. Arterial segments were then contracted with 10^{-5} M prostaglandin $F_{2\alpha}$, and after incubation for 60 minutes in this solution they were removed and fixed in glutaraldehyde-formaldehyde.

To avoid any artifact caused by dissection or cannulation of the arterial segment ends, each segment was cut in half after fixation and only newly cut ends were examined by electron microscopy.

All of the arterial specimens were fixed for 1 hour in 1% osmium tetroxide in Millonig's buffer 0.07 M, and prepared for scanning electronic microscopy (SEM) (Phillips 505 electron microscope; N.V. Phillips Gloeilampenfabrieken, Eindhoven, The Netherlands, and transmission electron microscopy (TEM) (Phillips Model 410).

RESULTS

All 12 monkeys had gross subarachnoid clot remaining in the right Sylvian fissure at sacrifice. Significant VSP (mean ± standard error of the mean decrease in vessel caliber compared to baseline of 48% ± 12%) was noted with day 7 angiography in all right (clot-side) MCAs. Those animals followed 14 days after SAH demonstrated consistently less VSP on their pre-sacrifice angiogram than was present on day 7 (mean ± standard error of the mean decrease in caliber of 21% ± 6% on day 14). Left (nonclot-side)

MCA caliber did not vary significantly from baseline in any of the animals either 7 or 14 days after SAH. No animal developed a delayed ischemic deficit.

Scanning Electron Microscopy

The left, nonclot-side MCAs, which did not show angiographic VSP, also appeared normal on SEM (fig. II-1), with the exception of mild intimal convolutions in several cases. Using the magnification scale on the scanning electron microscope screen and in the micrographs it was possible to measure the lumen diameters. Where the artery assumed an oval shape after fixation the circumference of the vessel was measured and the diameter of a corresponding circle calculated using the formula: circumference = 2 π radius. In a previous study a strong correlation was found between angiographic diameters of monkey arteries and diameters measured using a similar morphometric technique with histological cross-sections[26]. The mean lumen diameter for the normal MCAs was 0.71 mm (SD=0.10), and the mean vessel wall thickness was found to be 0.02 mm (SD=0.005). In contrast, the right, clot-side MCAs harvested 7 days after SAH which demonstrated unequivocal angiographic VSP, showed deep, longitudinal endothelial convolutions and a thickened arterial wall. The mean lumen diameter (measured from the tips of the endothelial convolutions) was 0.32 mm (SD=0.03), and wall thickness 0.11 mm (SD=0.007). Platelets and microthrombi were occasionally seen adherent to the luminal wall along the endothelial furrows. Except for the absence of luminal debris, the SEM appearance of the *in vitro* vasoconstricted, normal, left MCAs was undistinguishable from the

vasospastic arteries. The mean lumen diameter was 0.31 mm (SD=0.03), and wall thickness 0.11 mm (SD=0.03). The right MCAs from animals sacrificed 14 days after SAH had persistent endothelial convolutions, but corresponding to angiography, showed some resolution of VSP. The mean vessel diameter was 0.53 mm (SD=0.20), and wall thickness 0.04 mm (SD=0.002).

Transmission Electron Microscopy

There were no pathological findings in the left, nonclot-side MCAs (fig. II-2). The flattened, tight-junctioned endothelial cells overlay a scanty subendothelium containing some collagen and occasional myointimal cells. The vasospastic right MCAs harvested both 7 and 14 days after SAH revealed ultrastructural abnormalities of varying severity (fig. II-3). The endothelial cells appeared rounded and frequently contained cytoplasmic vacuoles. Tight junctions were disrupted in places. The subendothelium was enlarged and appeared slightly more cellular than in normal vessels, containing myointimal cells. The convoluted internal elastic lamina appeared broken in places, becoming more discontinuous than in normal vessels. The medial smooth muscle cells were contracted, frequently vacuolated and occasionally necrotic. Cellular infiltration was not observed in the medial layer of the vessel wall. The tunica adventitia was thickened, consisting mainly of collagenous fibrous tissue.

The vasoconstricted left MCAs showed similar endothelial and elastic lamina convolutions, rounding of the endothelial cells, and slight expansion of the subendothelial space with focal hypercellularity (fig. II-4). These intimal changes appeared to

represent crowding of normal subendothelial constituents into a smaller volume. Medial smooth muscle cells were contracted but the vessel wall contained none of the degenerative features seen in the vasospastic vessels. Tight junctions were intact and there was no cellular vacuolization or necrosis.

DISCUSSION

Experimental SAH in animals is often followed by an immediate but transitory constriction of the cerebral arteries. Short-term arterial spasm, lasting less than 30 minutes, also occurs after simple mechanical irritation of subarachnoid vessels in animals [3], and is frequently observed during neurosurgical procedures in man. Despite this, "early VSP" has been difficult to document in humans after aneurysmal SAH. Grote and Hassler [22] found no acute VSP in three patients whose aneurysms bled during angiography, and Weir et al [47] did not observe any arterial narrowing in patients who had angiograms performed within 24 hours of the estimated time of SAH. The existence of acute or early VSP in man is therefore uncertain, but it is probable that if it does occur, its clinical importance is negligible.

The relevance of more delayed cerebral arterial narrowing following aneurysmal SAH is not in question. Beginning after several days and peaking in incidence and severity about one week to 10 days after the rupture [47], "late" or "chronic" VSP affects primarily large conducting arteries which course through the subarachnoid cisterns on the ventral surface of the brain. These are the arteries upon which saccular aneurysms arise and consequently those which become coated with thick blood clot after

aneurysm bleeding. The significance of this subarachnoid blood clot in the pathogenesis of chronic VSP has long been appreciated. The most prevalent theory remains that the clot, during its gradual dissolution in the subarachnoid space, liberates one or more potent "spasmogens" which mediate sustained cerebrovascular constriction. Oxygenated hemoglobin (oxyhemoglobin), released from lysing erythrocytes enmeshed in the periarterial thrombus, appears to be particularly important in this process [48]. Indeed, the view that blood clot in some way produces chronic arterial narrowing on the basis of prolonged smooth-muscle constriction on spasm underlies the designation of this disorder as "vasospasm".

A number of observations have led some investigators to question this concept of VSP. Firstly, clinical VSP has thus far proven stubbornly resistant to pharmacologic vasodilatation, suggesting that the narrowing is not physiological vascular constriction. Along these same lines vasospastic vessels obtained from primates after experimental SAH have been shown to have relatively rigid walls compared to controls [5], and markedly diminished *in vitro* contractile responses to agonists such as norepinephrine, 5-hydroxytryptamine, and potassium chloride [5,32]. Secondiy, pathological studies have revealed a number of degenerative and hypertrophic structural changes in vasospastic arterial walls. Finally, it seems likely that the periarterial blood clot elaborates not only vasoconstrictors but also substances injurious to the vessel wall, such as free radicals and lipid peroxides [4]. Consequently a hypothesis has emerged proposing chronic VSP as a vessel injury reaction independent of

vasoconstriction, involving necrosis, edema, leukocytic infiltration, cellular proliferation, and fibrosis within the vessel wall. According to this theory luminal narrowing is due to vessel wall thickening and fibrosis, processes unaffected by vasodilator therapies. Proponents have sought to deemphasize the role of smooth muscle spasm, *per se*, in the genesis of delayed arterial narrowing, and have advocated alternative terms such as "acute proliferative vasculopathy"[33], "post-subarachnoid hemorrhage vasculopathy"[1], "constrictive angiopathy of subarachnoid hemorrhage"[8,41], and "chronic structural narrowing" of cerebral vessels[20]. The development of this theory has generated interest in novel approaches to the prevention and treatment of VSP, such as the use of anti-inflammatory[7], immunosuppressive[34], and antithrombotic[24,40] agents.

Table II-1 summarizes the published studies on pathological arterial wall changes after experimental SAH in animals and after aneurysmal hemorrhage in humans who demonstrated angiographic VSP prior to death or biopsy. Animal studies have emphasized electron microscopic, ultrastructural examination of the cerebral arteries, usually after perfusion fixation, whereas human studies have been histological after immersion fixation. Notably, all but two of these studies found morphological alterations other than simple folding of the internal elastic lamina in vasospastic arteries.

Electron microscopy has frequently revealed early degenerative changes in the endothelial cell layer, including vacuolization, disruption of interendothelial tight junctions, and occasionally endothelial desquamation and luminal microthrombosis. The tunica

intima, like the overlying internal elastic lamina, is frequently convoluted due to contraction of the media. In addition, 17 of the 27 studies reported some degree of intimal thickening variously ascribed to edema, polymorph infiltration, granulation tissue, migration (presumably from the media) and proliferation of smooth muscle cells, or fibroplasia and collagenization. Alksne [2], and others [33] have suggested that cellular proliferation and fibrosis in the intima after SAH may be similar to that seen early in atherogenesis, perhaps indicating a fundamental arterial wall response to various noxious stimuli. Paralleling Russell Ross's "response-to-injury" hypothesis for the pathogenesis of atherosclerosis [36], some workers have speculated that endothelial injury, followed by leukocyte or platelet adherence and release of chemotactic and mitogenic factors, may be important in the intimal reaction observed after SAH [9,31]. Although none of the studies have made a quantitative assessment of intimal thickening and its effect on lumen ize, advocates of the proliferative theory have argued that the relationship is significant. However, as shown in Table II-1, intimal thickening significant enough to be appreciated histologically (ie by light microscopy) is usually not seen in the first week after hemorrhage and is not pronounced until several weeks have passed. Such temporal progression in intimal change has been consistently noted in human studies. Hughes and Scianchi [23], for example, found that in those patients surviving 17 days or less from aneurysm rupture the tunica intima was only slightly swollen, whereas in those surviving longer the intima became the most abnormal component of the arterial wall, showing

concentric thickening with fibroblasts, collagen fibers and foamy macrophages. It is notable that in the 9 autopsies reviewed by Eldevik et al[14], where no specific arterial abnormalities could be found, all of the patients died within 16 days of aneurysm rupture. It would appear that while some degree of intimal thickening may follow severe SAH complicated by arterial narrowing, cerebral ischemia, and death, its delayed appearance does not correlate with the time course of clinical VSP, which begins several days after hemorrhage. Ultrastructural changes in the endothelium demonstrated in animal models precedes any significant hyperplastic intimal changes evident on light microscopy. Seven days after hemorrhage in our primate model, when arteriographic narrowing is maximal, TEM reveals that endothelial cells are swollen, vacuolized and sometimes lose tight junction with neighbors. The subendothelium is slightly edematous and in places becomes hypercellular with myointimal cells, but the large increase in vessel wall thickness is due to medial contraction.

Fein and colleagues[17] were the first to document sequential changes in the tunica media after SAH. Their findings in monkeys, which have been corroborated in other animal models and in 9 of 10 human series, indicated that VSP is often accompanied within the first week of variable degrees of smooth muscle cell necrosis. Also characteristic of vasospastic vessels with narrowed lumen is a markedly thickened media, a change that has been erroneously attributed to either an inflammatory or hypertrophic reaction within this layer of the arterial wall. In 1962 Van Citters et al[46] published a detailed histological analysis of arterial vasoconstriction and vasodilation using small arteries in canine mesentery. In one

representative vessel they found that maximal constriction with a dilute epinephrine solution produced a reduction in lumen diameter of 79% (0.86 to 0.18 mm), while the arterial wall thickness increased over 300% (0.03 to 0.10 mm). The major increase in wall thickness occurred in the media, where the smooth muscle cells became shortened, overlapped and often quite deformed and pleomorphic, particularly near the elastic membrane. The underlying intima and elastic lamina became severely convoluted and slightly thickened, apparently incapable of shortening to the extent that the inner vessel diameter was reduced with vasoconstriction. The authors cautioned against interpreting simple vasoconstriction as evidence of vascular hypertrophy. These findings are in agreement with our own. Cerebral arteries vasoconstricted in vitro demonstrated impressive medial thickening that was indistinguishable from thickening observed in vasospastic arteries 7 days post-hemorrhage. While the lumen diameter decreased approximately 57% the vessel wall thickness increased almost 5 times in both groups. On the other hand, degenerative ultrastructural changes in the endothelium and media were seen only in SAH induced, chronic VSP, and these changes remained at 14 days despite considerable resolution of arterial narrowing and medial thickening.

The surfaces of canine[52] cerebral arteries possess stomas which appear to lead to tunnel-like spaces that connect throughout the tunica adventitia. Similar structures have been identified in monkey arteries in our laboratory[16]. Since cerebral arteries lack a vasa vasorum it has been suggested that these pathways allow cerebrospinal fluid (CSF) to nourish and/or remove wastes from the

arterial wall. After experimental SAH in dogs Liszczak et al[27] found that the most characteristic abnormality accompanying vessel constriction was packing of these adventitial spaces with erythrocytes. Although lysing erythrocytes are highly vasogenic, it has been postulated that blockage of adventitial stomas and intramural CSF circulation may in itself contribute to the pathogenesis of VSP[16,52].

Summarizing these studies provides some insight into the evolution of chronic cerebral VSP in humans (fig. II-5). In our opinion the primary event is vasoconstriction which results in prominent morphological thickening of the tunica media. Although grossly identical to medial thickening seen in normal cerebral arteries vasoconstricted *in vitro*, chronic VSP does not appear to represent simple physiological vascular contraction. Vasospastic vessels resist vasodilation *in vivo* and respond minimally to vasoactive agents *in vitro*[5]. In addition, chronic VSP is accompanied by degenerative changes in the smooth muscle cells of the tunica media. Irrigating exposed feline basilar arteries with either serotonin or calcium gluconate, Yoshioka et al[51] found that vasoconstriction longer than 10 hours was both pharmacologically irreversible and associated with myonecrotic changes on electron microscopy. Fujii and Fujistsu[21] found that incubation of cultured arterial smooth muscle cells with oxyhemoglobin (OxyHb) for 24 hours resulted in irreversible progressive contraction of the cells and ultrastructural changes resembling myonecrosis. It seems likely, therefore, that prolonged constriction in response to OxyHb and/or its vasoactive byproducts such as lipid peroxides or

prostaglandins induces a temporarily irreversible state associated with ultrastructural myonecrotic change. Although this pathophysiologic process has not yet been fully elucidated, it is known that in the majority of cases VSP spontaneously resolves within several weeks of its onset[19]. This relaxation argues against one suggestion that early medial fibrosis accounts for the noncontractile, rigid arterial walls associated with clinical VSP[5]. On the other hand, in rare instances cerebral VSP leads to a persistent arterial stenosis[37]. As originally suggested by Conway and McDonald[10] it is likely that this is due to prominent intimal thickening (fig. II-6). Some degree of intimal injury associated with ultrastructural alterations is probably inevitable in cerebral VSP, due either to hypoxia related to prolonged vasoconstriction or the release of toxic substances from the decaying periarterial clot. Why some vessels go on to develop a significant hypertrophic intimal reaction is not known, but it may be a nonspecific pathologic response related to the severity of the vessel wall injury. But again arguing against "structural" theories of VSP, the onset of appreciable intimal thickening, when it does occur, is too late to correspond with the luminal narrowing of clinical VSP beginning several days after SAH. In addition, the complete absence of intimal thickening on histological examination of some arteries in VSP[14] emphasizes that they are not a necessary ingredient or product of the arterial narrowing.

BIBLIOGRAPHY

1. Alksne JF, Branson PJ, Bailey M: Modification of experimental post-subarachnoid hemorrhage vasculopathy with intracisternal plasmin. Neurosurgery 23: 335-337, 1988.
2. Alksne JF, Branson PJ: A comparison of intimal proliferation in experimental subarachnoid hemorrhage and atherosclerosis. Angiology 27: 712-720, 1976.
3. Arutiunov AI, Baron MA, Majorova NA: The role of mechanical factors in the pathogenesis of short-term and prolonged spasm of the cerebral arteries. J Neurosurg 40: 459-472, 1974.
4. Asano T, Tanishima T, Sasaki T, Sano K: Possible participation of free radical reactions initiated by clot lysis in the pathogenesis of vasospasm after subarachnoid hemorrhage, in Wilkins RH (ed): *Cerebral Arterial Spasm*. Baltimore, Williams & Wilkins, 1980, pp 190-201.
5. Bevan JA, Bevan RD, Frazee JG: Functional arterial changes in chronic cerebrovasospasm in monkeys: An *in vitro* assessment of the contribution to arterial narrowing. Stroke 18: 472-481, 1987.
6. Bevan JA, Bevan RD, Frazee JG: Experimental chronic cerebrovascular spasm in the monkey: An assessment of the functional changes in the cerebral arteries and their protection by diltiazem. Am J Cardiol 56: 15H-20H, 1985.
7. Chyatte D, Fode NC, Nichols DA, Sundt TM Jr: Preliminary report: Effects of high dose methylprednisolone on delayed cerebral ischemia in patients at high risk for vasospasm after

aneurysmal subarachnoid hemorrhage. Neurosurgery 21: 157-160, 1987.

8. Clower BR, Smith RR, Haining JL, Lockard J: Constrictive endarteropathy following experimental subarachnoid hemorrhage. Stroke 12: 501-508, 1981.

9. Clower BR, Yoshioka J, Honma T, Smith RR: Blood platelets and early intimal changes in cerebral arteries following experimental subarachnoid hemorrhage, in Wilkins RH (ed): *Cerebral Vasospasm*. New York, Raven Press, 1988, pp 335-342.

10. Conway LW, McDonald LW: Structural changes of the intradural arteries following subarachnoid hemorrhage. J Neurosurg 37: 715-723, 1972.

11. Crompton MR: The pathogenesis of cerebral infarction following the rupture of cerebral berry aneurysms. Brain 87: 491-510, 1964.

12. Duff TA, Scott G, Feilbach JA: Ultrastructural evidence of arterial denervation following experimental subarachnoid hemorrhage. J Neurosurg 64: 292-297, 1986.

13. Duff TA, Louie J, Feilbach JA, Scott G: Erythrocytes are essential for development of cerebral vasculopathy resulting from subarachnoid hemorrhage in cats. Stroke 19: 68-72, 1988.

14. Eldevik, OP, Kristiansen K, Torvik A: Subarachnoid hemorrhage and cerebrovascular spasm. Morphological study of intracranial arteries based on animal experiments and human autopsies. J Neurosurg 55: 869-876, 1981.

had evidence of red blood cell accumulation in the arachnoid granulations.

DISCUSSION

As early as 1958 it was suggested that early operation after aneurysm rupture for surgical evacuation of the subarachnoid clot might be a means of alleviating VSP, although the authors of these remarks seemed to feel that this would be the result of freeing the vessels from mechanical distortion and compression caused by the adjacent thrombus [16]. Since that time our concept of VSP has changed considerably. The major contemporary theory regarding the pathogenesis of VSP is that the periarterial blood clot, during its gradual degradation, releases certain vasoactive substances that cause sustained vascular smooth-muscle spasm, as well as vasotoxic substances that injure the vessel wall, impairing its ability to synthesize endogenous vasodilatory substances and inciting an inflammatory vessel wall reaction [17]. Although a number of clot-derived "spasmogens" have been postulated [43] in the genesis of VSP, there is now a great deal of information implicating oxyhemoglobin (OxyHb). Liberated during the process of hemolysis, the time course of OxyHb release into the periarterial clot from erythrocytes appears to follow the time course of angiographic and clinical VSP [12]. It is speculated that through the oxidative conversion of OxyHb to methemoglobin free radicals are produced, which in turn can generate vasoconstrictive and vasotoxic lipid peroxides and prostaglandins [3]. In addition, it has been suggested that the periarterial clot may prevent CSF circulation from reaching the adventitial surface and "rete vasorum" of the

vessel wall, thereby interfering with normal vessel wall nutrition and oxygenation [7,46]. While unproven, vessel wall hypoxia may play a role in the pathogenesis of VSP [10,26,42]. With these modern pathogenetic theories in mind, and discouraged by the ineffectiveness of vasodilator therapies for VSP, neurosurgeons have recently returned to the concept of early subarachnoid clot removal. This is based upon the premise that the onset of VSP can be prevented through the removal of the clot prior to its elaboration of vasospastic and vasotoxic substances.

A number of neurosurgeons, mostly Japanese, have reported their experience with surgical subarachnoid clot removal at the time of early aneurysm clipping [24,25,29,31,35,37,39]. These studies indicate that aggressive clot removal, sometimes combined with post-operative cisternal drainage, reduces the risk of VSP and/or DIND. In most of these reports surgery was performed within 3 days of SAH, and Suzuki et al [37] felt that surgery with clot evacuation done later than 48 hours from hemorrhage was not effective in preventing VSP. However, even in these optimistic early reports important drawbacks to this approach were evident, such as severe brain swelling and cerebral hemorrhage related to retraction of an already damaged brain [29], and the need for multiple craniotomies in some patients in order to thoroughly cleanse the cisterns [illegible] addition, the retrospective and nonrandom nature of these studies must be considered when analyzing the results.

Notwithstanding these concerns, the basic tenet of these clinical reports has recently been validated in our laboratory. Nosko et al [28], demonstrated in a blind, randomized study that

complete subarachnoid clot removal 24 hours after SAH prevents VSP in the primate model of SAH. In an extension of this work, Handa et al [13], showed in the same model that surgical removal of the clot 48 hours after SAH, but not later than this, prevented the occurrence of significant VSP.

Applying this knowledge to the clinical situation remains problematic. Extensive surgical removal of an experimentally placed clot in the primate model is far easier than the task of removing a comparable amount of blood spread throughout the basal cisterns of patients in the acute stage after SAH. Indeed, most North American neurosurgeons have not adopted the practice of extensive clot removal at the time of aneurysm clipping; the advantage conferred by surgical clot removal has not been considered worth the risk entailed in the procedure [5]. The authors have therefore grown interested in a simpler, nonmechanical method of subarachnoid clot elimination employing the fibrinolytic system.

The fibrinolytic system refers to a set of enzymes in the circulation that when activated lead to fibrin breakdown, or "fibrinolysis", and hence dissolution of the blood clot. The active fibrinolytic enzyme is plasmin, the activated product of the proenzyme plasminogen. Plasminogen, a normal blood component which circulates in plasma in concentrations of 10 to 20 mg/100 ml [41], has a high affinity for fibrin and is tightly adsorbed onto fibrin polymers as they form in the process of coagulation. It is this fibrin-bound plasminogen that is primarily responsible for clot lysis when it is activated by the enzymatic splitting of an

arginine-valine bond [1]. The most important endogenous plasminogen activator is tissue plasminogen activator (t-PA). Tissue plasminogen activator is synthesized by vascular endothelium, and its release is triggered by vessel injury and occlusion [21]. It is therefore released on demand at the site of vessel injury and thrombus formation, and because it has a strong affinity for fibrin (like plasminogen), it quickly binds to fibrin polymers. It then proceeds to hydrolyse and activate the nearby fibrin-bound plasminogen into plasmin, which in turn digests the fibrin framework of the thrombus and dissolves the blood clot [1,41].

Since sufficient plasminogen for clot lysis is adsorbed into the thrombus during coagulation [1], the rate-limiting factor in the process of fibrinolysis is the availability of plasminogen activators. Therapeutic advantage has been taken of this through the administration of certain exogenous plasminogen activators such as urokinase and streptokinase into the blood stream to hasten intravascular clot dissolution. The high affinity of t-PA for fibrin polymers and selective activation of fibrin-bound plasminogen rather than circulating plasminogen largely avoids the problem of systemic fibrinogenolysis often seen with urokinase and streptokinase. This "clot-selective" activity of t-PA, along with its lack of antigenicity make it a superior thrombolytic agent [41].

Fibrinolytic (or thrombolytic; the terms are interchangeable) agents have been used almost exclusively in the intravascular space for thromboembolic disease, but another area where they might be useful is in the elimination of intracranial extravascular blood clots, specifically subarachnoid hematomas. Normally the CSF is deficient

in t-PA and therefore possesses scant fibrinolytic activity[34,38,44], although this activity may increase slightly after SAH with the release of t-PA from irritated and damaged small meningeal vessels[9,14]. With increasing awareness that VSP is the result of prolonged contact between thick subarachnoid clot and the vessel wall, and the demonstration that timely clot removal will prevent VSP, the augmentation of the meager thrombolytic activity of the CSF with an intrathecally administered thrombolytic agent seems a promising treatment to examine. Pertaining to this is the work of Peterson et al[33]. They injected 100,000 units of streptokinase and 25,000 units of streptodornase into the cisterna magna of cats one hour after injection of 4 to 5 ml of subarachnoid blood, and found this resulted in the complete lysis and disappearance of the clot when the cats were sacrificed after three hours. When cats injected with the enzyme alone were allowed to live 24 hours, however, a significant and diffuse meningo-encephalitic response was found, correlating with the poor clinical condition of the animals. These findings suggest an inflammatory effect of streptokinase and streptodornase. Alksne et al[2] found that the administration of a single intrathecal dose of 100 units of plasmin reduced the severity of a post-SAH vasculopathy in pigs. Ten days after SAH, TEM demonstrated a significant reduction in intimal proliferation and medial smooth muscle necrosis compared to the saline-injected controls. Finally, there have been several preliminary, uncontrolled studies from Japan on the use of ventriculo-cisternal irrigation with solutions containing urokinase after SAH[36,45], but it is difficult to draw any firm conclusions from these publications.

The primate model of SAH at the University of Alberta appears to closely mimic the human condition. It has been amply shown that craniectomy and arachnoid dissection alone does not provoke VSP in this model [13,22,27,28] so that sham-operated animals are no longer included in current protocols. Sustained VSP is seen on angiography and by EM in over 90% of animals, peaking in incidence at the end of the first week after experimental SAH. Approximately 1 in 20 animals develops a DIND accompanied by computerized tomographic, MRI and pathological evidence of cerebral infarction [13,22,27,28]. A particular advantage conferred by the Cynomolgous model in the present study is the similarity in the coagulation and fibrinolytic systems between this species and man. Using an *in vitro* I^{125}-fibrinogen labelled plasma clot lysis test it has been shown that human t-PA has virtually identical activity in man, the Cynomolgous macaque and chimpanzee (91, 94, and 90% lysis at 4 hours, respectively) [23]. The activity of t-PA is somewhat variable among other primate species, and substantially less in other mammals [20].

In the present study we first established *in vitro* a dose of rt-PA effective in completely lysing 5 ml of clotted, whole Cynomolgous blood, the maximal volume used to induce experimental SAH in our model. This dosage, given 3 times through an Ommaya reservoir into the basal cisterns one day after SAH, was effective in completely lysing the subarachnoid hematoma in 11 of 12 rt-PA treated animals and significantly reduced the incidence and magnitude of VSP compared to controls. Intrathecal rt-PA in the dosage employed had no apparent adverse effect on the animals

neurologic condition or on the histological appearance of the cerebral cortex, pia-arachnoid or ependyma. Coagulation analyses indicated that the intrathecal dose of rt-PA used did not result in systemic fibrinolysis. Temporary wound bleeding was noted in 2 rt-PA treated animals, probably due to epidural leakage of the enzyme through a non-water-tight dural closure. Horseradish peroxidase studies in this experiment indicate that protein molecules injected through the Ommaya reservoir circulate freely in the subarachnoid cisterns, contacting all major vessels including those in the posterior fossa, and that dissemination of the enzyme rt-PA through the CSF may allow for lysis of more diffuse subarachnoid hematomas, such as are frequently seen after severe aneurysmal SAH.

Intrathecal thrombolysis with rt-PA is safe and extremely efficacious in dissolving subarachnoid clot and preventing VSP in the primate model. Intrathecal thrombolytic therapy with rt-PA following early surgery for aneurysm clipping may come to play an important role in the prevention of VSP.

BIBLIOGRAPHY

1. Alkjaersig N, Fletcher AP, Sherry S: The mechanism of clot dissolution by plasmin. J Clin Invest 38: 1086-1095, 1959.
2. Alksne JF, Branson PJ, Bailey M: Modification of experimental post-subarachnoid hemorrhage vasculopathy with intracisternal plasmin. Neurosurgery 19: 20-25, 1986.
3. Asano T, Tanishima T, Sasaki T, Sano S: Possible participation of free radical reactions initiated by clot lysis in the pathogenesis of vasospasm after subarachnoid hemorrhage. In Wilkins RH (ed): *Cerebral Arterial Spasm*. Baltimore, Williams and Wilkins, 1980, pp 190-201.
4. Bell BA, Kendall BE, Symon L: Computed tomography in aneurysmal subarachnoid hemorrhage. J Neurol Neurosurg and Psychiatry 43: 522-524, 1980.
5. Drake CG: Management of cerebral aneurysm. Stroke 12: 273-283, 1981.
6. Eisentrout C, Tomsick TA, Tew JM: Computed tomography findings as a prognostic factor in subarachnoid hemorrhage from ruptured cerebral aneurysms. Stroke 11: 124, 1980 (Abstract).
7. Espinosa F, Weir B, Shnitka T: Electron microscopy of simian cerebral arteries after subarachnoid hemorrhage and after the injection of horseradish peroxidase. Neurosurgery 19: 935-945, 1986.
8. Fisher CM, Kistler JP, Davis JM: Relation of cerebral vasospasm to subarachnoid hemorrhage visualized by computed tomographic scanning. Neurosurgery 6: 1-9, 1980.

9. Fostad H, Kok P, Algers G: Fibrinolytic activity of cerebral tissue after experimental subarachnoid hemorrhage: Inhibitory effect of tranexamic acid (AMCA). Acta Neurol (Scandinav) 64: 29-46, 1981.

10. Furchgott RF: Metabolic factors that influence contractility of vascular smooth muscle. Bull NY Acad Med 42: 996-1006, 1966.

11. Gurusinghe NT, Richardson AE: The value of computerized tomography in aneurysmal subarachnoid hemorrhage: A concept of the CT score. J Neurosurg 60: 763-770, 1984.

12. Handa H, Osaka K, Okamoto S: Breakdown products of erythrocytes as a cause of cerebral vasospasm. In Wilkins RH (ed): *Cerebral Arterial Spasm*. Baltimore, Williams and Wilkins, 1980, 158-165.

13. Handa Y, Weir BKA, Nosko M, et al: The effect of timing of clot removal on chronic vasospasm in a primate model. J Neurosurg 67: 558-564, 1987.

14. Hindersin P, Endler S: Alterations of coagulation and fibrinolysis in cerebrospinal fluid in subarachnoid haemorrhage. J Neurosurg Sci 30: 183-186, 1986.

15. Hougie C: Methods, in Williams WJ, Bentler E, Ersler AJ, Lichtman MA (eds): *Hematology*, ed 3. New York: McGraw-Hill, 1983, pp 1167-1670.

16. Johnson R, Potter JF, Reid RG: Arterial spasm in subarachnoid hemorrhage: Mechanical considerations. J Neurol Neurosurg Psychiatry 21: 68, 1958 (Abstract).

17. Kassell NF, Sasaki T, Colohan ART, Nazar G: Cerebral vasospasm following aneurysmal subarachnoid hemorrhage. Stroke 16: 562-572, 1985.

18. Kistler JP, Crowell RM, Davis KR, Heros R, Ojemann RG, Zervas T, Fisher CM: The relation of cerebral vasospasm to the extent and location of subarachnoid blood visualized by CT scan: A prospective study. Neurology (N.Y.) 33: 424-426, 1983.

19. Knuckey NW, Fox RA, Surveyor I, Stokes BAR: Early cerebral blood flow and computerized tomography in predicting ischemia after cerebral aneurysm rupture. J Neurosurg 62: 850-855, 1985.

20. Korninger C, Collen D: Studies on the specific fibrinolytic effect of human extrinsic (tissue-type) plasminogen activator in human blood and in various animal species *in vitro*. Thromb Haemostas 46: 561-565, 1981.

21. Kwaan HC: The role of fibrinolysis in disease processes. Semin Thromb Hemost 10: 71-79, 1984.

22. Lewis PJ, Weir BKA, Nosko MG, et al: Intrathecal nimodipine therapy in a primate model of chronic cerebral vasospasm. Neurosurgery 22: 492-500, 1988.

23. Lijnen HR, Marafina BJ, Collen D: *In vitro* fibrinolytic activity of recombinant tissue-type plasminogen activator in the plasma of various primate species. Thromb Haemostas 52: 308-310, 1984.

24. Ljunggren B, Brandt L, Kagstrom E, Sundbarg G: Results of early operations for ruptured aneurysms. J Neurosurg 54: 473-479, 1981.

25. Mizukami M, Kawase T, Usami T, Tazawa T: Prevention of vasospasm by early operation with removal of subarachnoid blood. Neurosurgery 10: 301-307, 1982.

26. Nakagomi T, Kassell NF, Sasaki T, et al: Effect of hypoxia on the contractile response to KCL, prostaglandin $F2_{\alpha}$ and hemoglobin. J Neurosurg 67: 565-572, 1987.

27. Nosko M, Weir B, Krueger C, et al: Nimodipine and chronic vasospasm in monkeys: Part 1: Clinical and radiological findings. Neurosurgery 16: 129-136, 1985.

28. Nosko M, Weir BKA, Lunt A, et al: Effect of clot removal at 24 hours on chronic vasospasm after SAH in the primate model. J Neurosurg 66: 416-422, 1987.

29. Ohta H, Ito Z, Yasui N, Suzuki A: Extensive evacuation of subarachnoid clot for prevention of vasospasm - Effective or not? Acta Neurochir 63: 111-116, 1982.

30. Pang D, Sclabassi RJ, Horton JA: Lysis of intraventricular blood clot with urokinase in a canine model: Part 2. *In vivo* safety study of intraventricular kinase. Neurosurgery 19: 547-552, 1986.

31. Pasqualin A, Da Pian R: An analysis of vasospasm following early surgery for intracranial aneurysms. Acta Neurochir 63: 153-159, 1982.

32. Pennica D, Holmes WE, Kohr WJ, et al: Cloning and expression of human tissue-type plasminogen activator cDNA in E. coli. Nature 301: 213-221, 1983.

33. Peterson EW, Choo SH, Lewis AJ, et al: Lysis of blood clot and experimental treatment of subarachnoid hemorrhage. In Wilkins RH (ed): *Cerebral Arterial Spasm*. Baltimore, Williams and Wilkins, 1980, pp 625-627.

34. Porter JM, Acinapura AJ, Kapp JP, Silver D: Fibrinolytic activity of the spinal fluid and meninges. Surg Forum 17: 425-427, 1966.

35. Saito I, Ueda Y, Sano K: Significance of vasospasm in the treatment of ruptured intracranial aneurysms. J Neurosurg 47: 412-429, 1977.

36. Shiobara R, Kawase T, Ebato K, Miyahara Y: "Scavengery surgery" for subarachnoid hemorrhage (II) Continuous ventriculo-cisternal perfusion using artificial cerebrospinal fluid with urokinase. In Auer L (ed): *Timing of Aneurysm Surgery*. Berlin, de Gruyter, 1985, pp 365-372.

37. Suzuki J, Onuma T, Yoshimoto T: Results of early operations on cerebral aneurysms. Surg Neurol 11: 407-412, 1979.

38. Takashima S, Koga M, Tanaka K: Fibrinolytic activity of human brain and cerebrospinal fluid. Br J Exp Path 50: 553-539, 1969.

39. Taneda M: Effect of early operation for ruptured aneurysms on prevention of delayed ischemic symptoms. J Neurosurg 57: 622-628, 1982.

40. Verstraete M, Collen D: Thrombolytic therapy in the 80s. Blood 67: 1529-1541, 1986.

41. Verstraete M: New thrombolytic drugs in acute myocardial infarction: Theoretical and practical considerations. Circulation 76 (suppl II): 31-38, 1987.

42. Vinall PE, Simeone FA: Effects of oxygen and glucose deprivation on vasoactivity in isolated bovine middle cerebral arteries. Stroke 17: 970-975, 1986.

43. Weir B: *Aneurysms Affecting the Nervous System*. Baltimore, Williams and Wilkins, 1987, pp 518-522.

44. Wu KKY, Jacobsen CD, Hoak JC: Plasminogen in normal and abnormal human cerebrospinal fluid. Arch Neurol 28: 64-66, 1973.

45. Yoshida Y, Ueki S, Takahashi A: Intrathecal irrigation with urokinase in ruptured cerebral aneurysm cases. Basic study and clinical application. Neurol Med Chir (Tokyo) 24: 989-997, 1985 (Japanese).

46. Zervas NT, Liszczak TM, Mayberg MR, Black PMcL: Cerebrospinal fluid may nourish cerebral vessels through pathways in the adventitia that may be analogous to systemic vasa vasorum. J Neurosurg 56: 475-481, 1982.

Table III-1
Values for measured indices in the 2 animal groups*

Parameter	Day 0		Day 7	
	Placebo Group	rt-PA Group	Placebo Group†	rt-PA Group
No. of Monkeys	12	12	11	12
Body Weight (kg)	3.42±0.45	3.21±0.22	3.20±0.20	3.09±0.16
MABP (mmHg)	100±5	100±5	100±5	99±6
Heart Rate (min^{-1})	127±1.4	123±9	124±5	123±5
$PaCO_2$ (mmHg)	40±3	38±4	39±3	38±4
Vessel Caliber (mm)				
C3-R	1.57±0.20	1.54±0.23	1.28±0.19	1.57±0.19
C3-L	1.63±0.17	1.56±0.20	1.64±0.22	1.55±0.17
C4-R	1.30±0.12	1.32±0.09	0.86±0.34	1.29±0.13
C4-L	1.35±0.15	1.32±0.17	1.32±0.10	1.32±0.12
A1-R	0.67±0.36	0.92±0.14	0.48±0.52	0.79±0.14
A1-L	0.91±0.19	0.89±0.18	0.82±0.28	0.88±0.18
A2	0.95±0.11	0.99±0.12	0.92±0.09	0.98±0.08
MCA-R	1.14±0.17	1.00±0.15	0.54±0.31	0.96±0.18
MCA-L	1.01±0.13	1.04±0.13	1.01±0.08	1.02±0.12
PCA-R	0.89±0.01	1.01±0.19	0.95±0.15	0.76±0.12
PCA-L	0.80±0.05	0.75±0.15	0.83±0.21	0.71±0.11
BA	1.29±0.22	1.25±0.18	1.25±0.19	1.20±0.17
VA-R	1.07±0.16	1.05±0.25	1.05±0.14	0.95±0.16
VA-L	1.16±0.20	0.99±0.20	1.10±0.16	0.98±0.18
Volume of SAH (ml)	4.67±0.25	4.38±0.23		
Weight of Remaining Clot (gm)			0.630±0.230	0.004±0.010

*Values are means ± standard deviations

MABP = Mean arterial blood pressure; C3 = extradural internal carotid artery; C4 = intradural internal carotid artery; A1 = precommunicating segment of anterior cerebral artery; A2 = distal azygous anterior cerebral artery; MCA = sphenoidal segment of middle cerebral artery; PCA = posterior cerebral artery; BA = basilar artery; VA = vertebral artery; SAH = subarachnoid hemorrhage

†One of the 12 placebo group animals died on Day 2

Table III-2
In Vitro Clot Lysis Test*

Monkey No.†	Control	rt-PA (mg)			
		1	0.5	.33	.25
1	1.05	0	0	.04	.20
2	.97	.03	0	.09	.48
3	.81	.04	.08	.15	.37
4	1.21	0	.09	.12	.60
5	1.03	0	.11	.18	.22

*Weight (gms) of 5 ml whole blood clot remaining after 8 hour incubation at 37°C with 0.5 ml saline (control) and increasing doses of rt-PA in 0.5 ml buffer.

†Blood samples from monkeys 1-3 aged 24 hours, and from 4 & 5 aged 48 hours prior to in vitro testing.

Table III-3
Coagulation Studies

Parameter	Day 0		Day 1*	
	Placebo Group	rt-PA Group	Placebo Group	rt-PA Group
Thrombin Time (sec)				
5.5 U/ml	12.0±1.2	11.0±1.8	12.8±1.9	12.7±1.0
1.8 U/ml	24.5±3.5	20.9±4.7	24.9±3.3	24.2±2.2
ELT less than 90 min (no. samples)	0	0	1 (75 min)	0
FDP positive (no. samples)	0	1 (2 μg/ml)	1 (2 μg/ml)	0

*Samples taken during intrathecal treatment

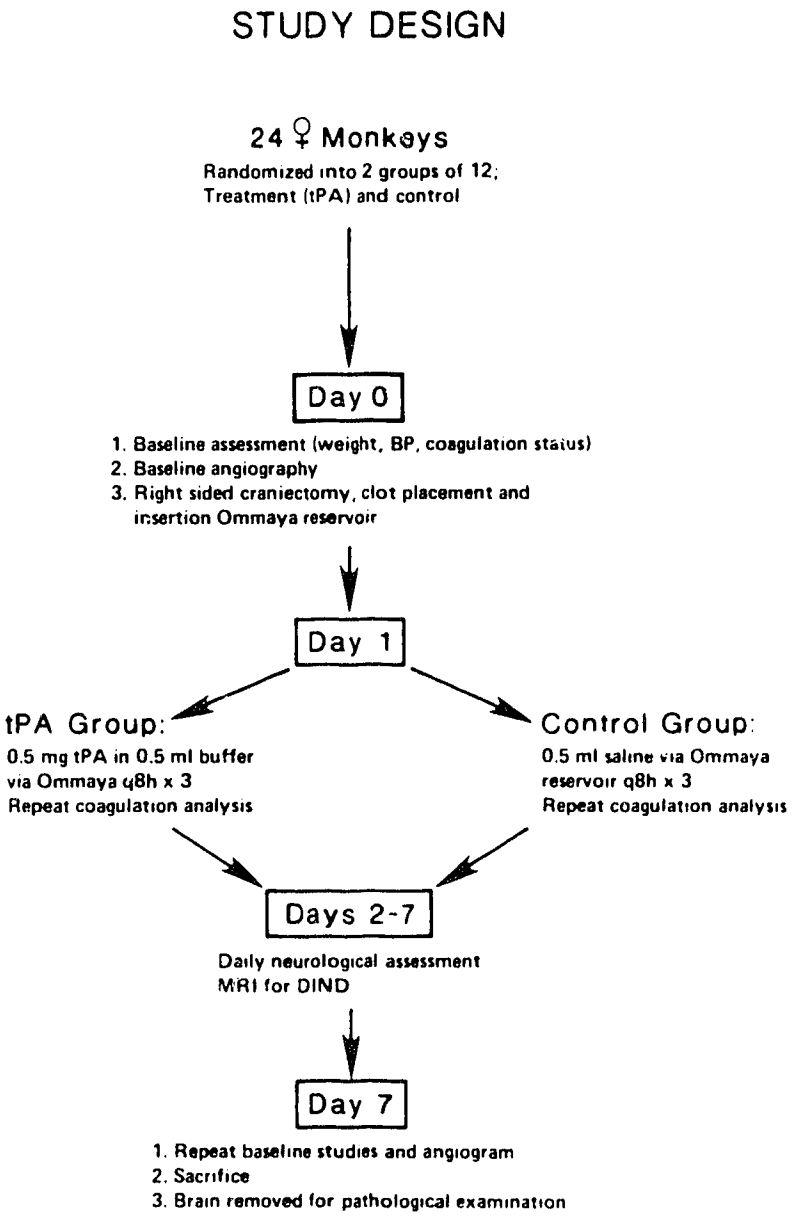

Figure III-1
Study design for the randomized, blinded, placebo-controlled trial of intrathecal rt-PA suspension after unilateral subarachnoid hemorrhage in the primate model.

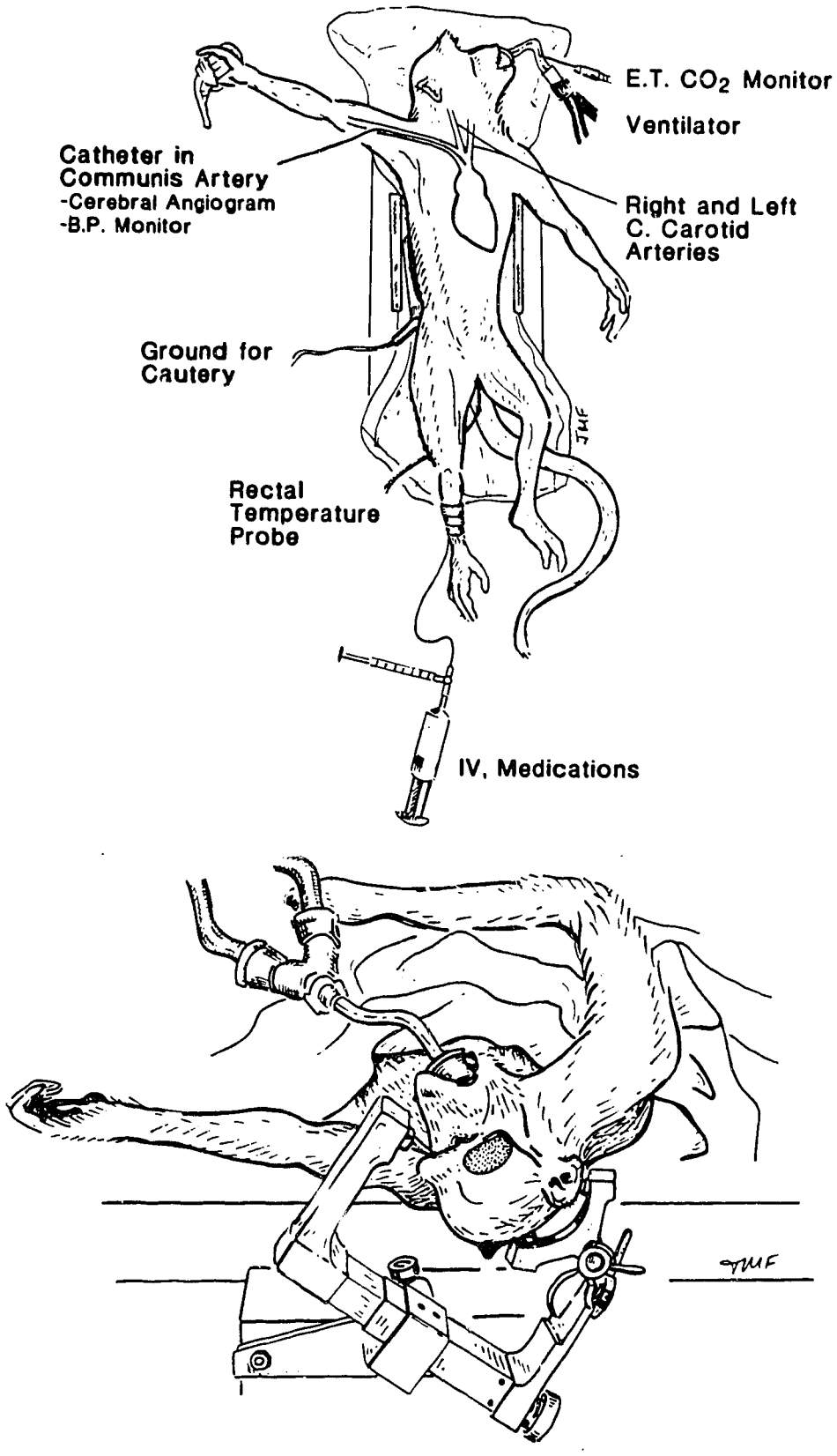

Figure III-2
Monkey positioning for cerebral angiography (A), and right fronto-temporal craniectomy (B).

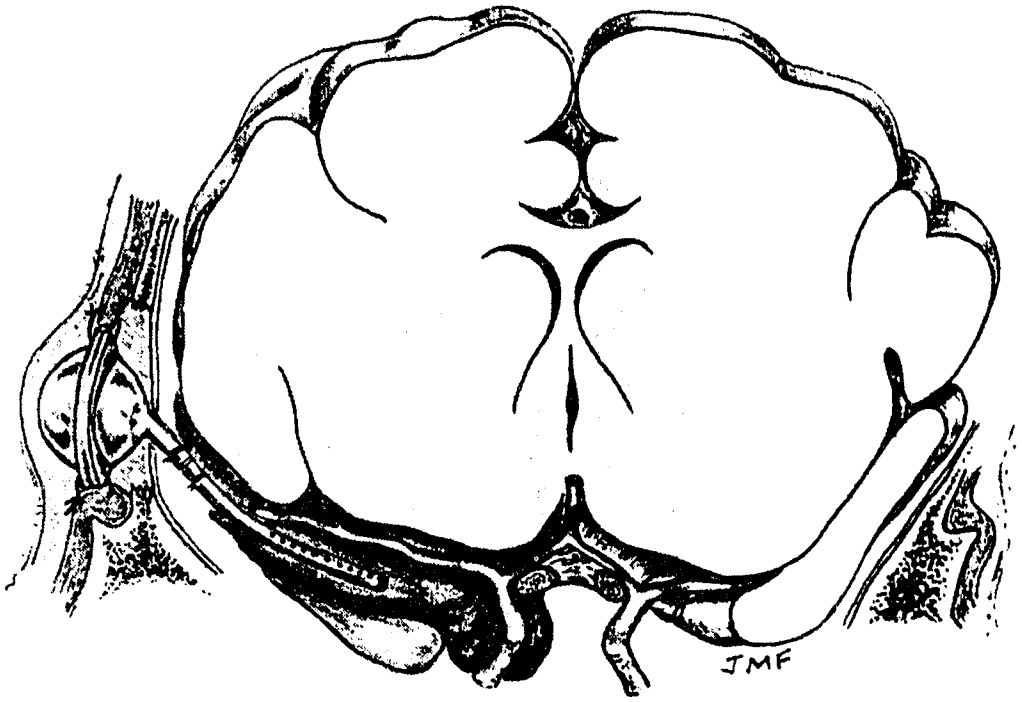

Figure III-3
Diagram of experimental subarachnoid hemorrhage and placement of Ommaya reservoir.

Figure III-4
Greater *in vitro* lysis is achieved with an incrementally larger dose of rt-PA. On the left is a 5 ml monkey blood clot incubated in saline alone.

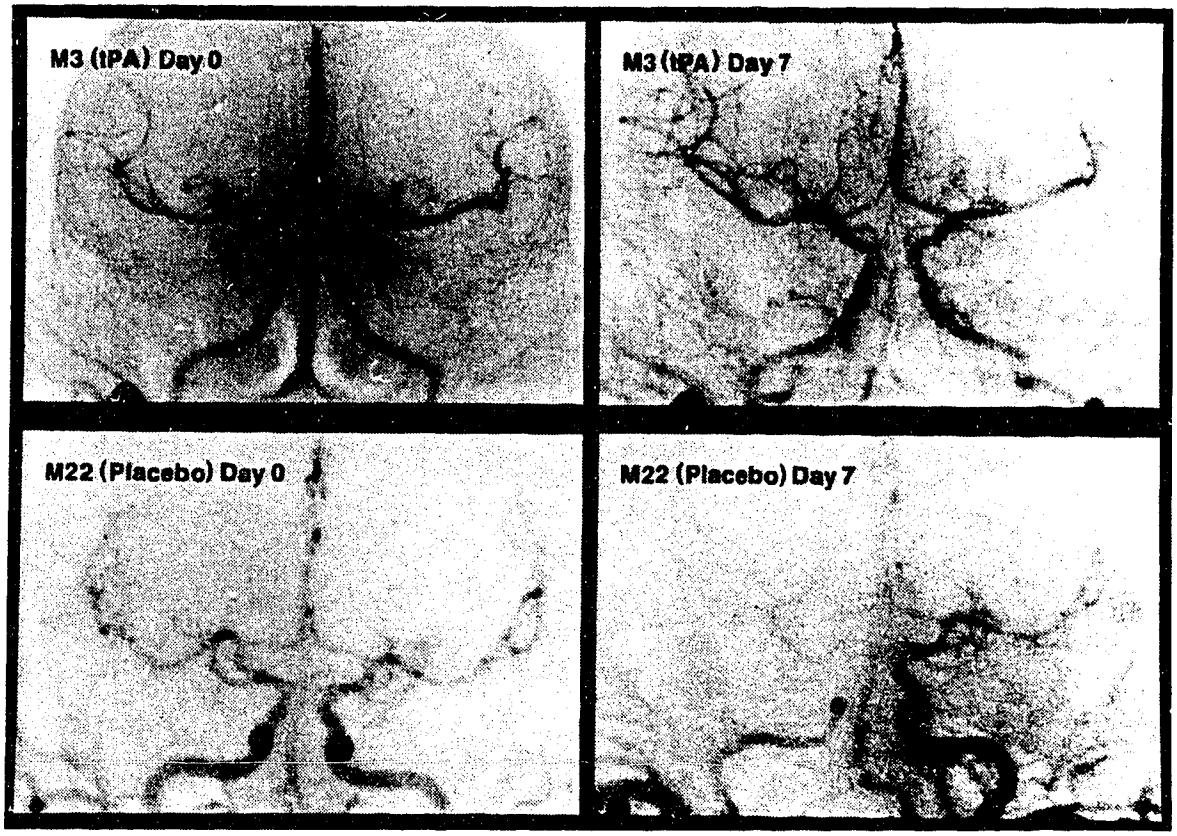

Figure III-5
Day 0 (pre-SAH) and day 7 (post-SAH) cerebral angiograms of Monkeys 3 (rt-PA group, upper panel), and 22 (placebo group, lower panel). Monkey 3 shows no evidence of vasospasm in the right middle cerebral artery (double arrows), right precommunicating anterior cerebral artery (single arrow) or right intradural internal carotid artery (opposing arrows) on day 7, while Monkey 22 has severe vasospasm on the right side on day 7, with only faint opacification of the precommunicating anterior cerebral artery.

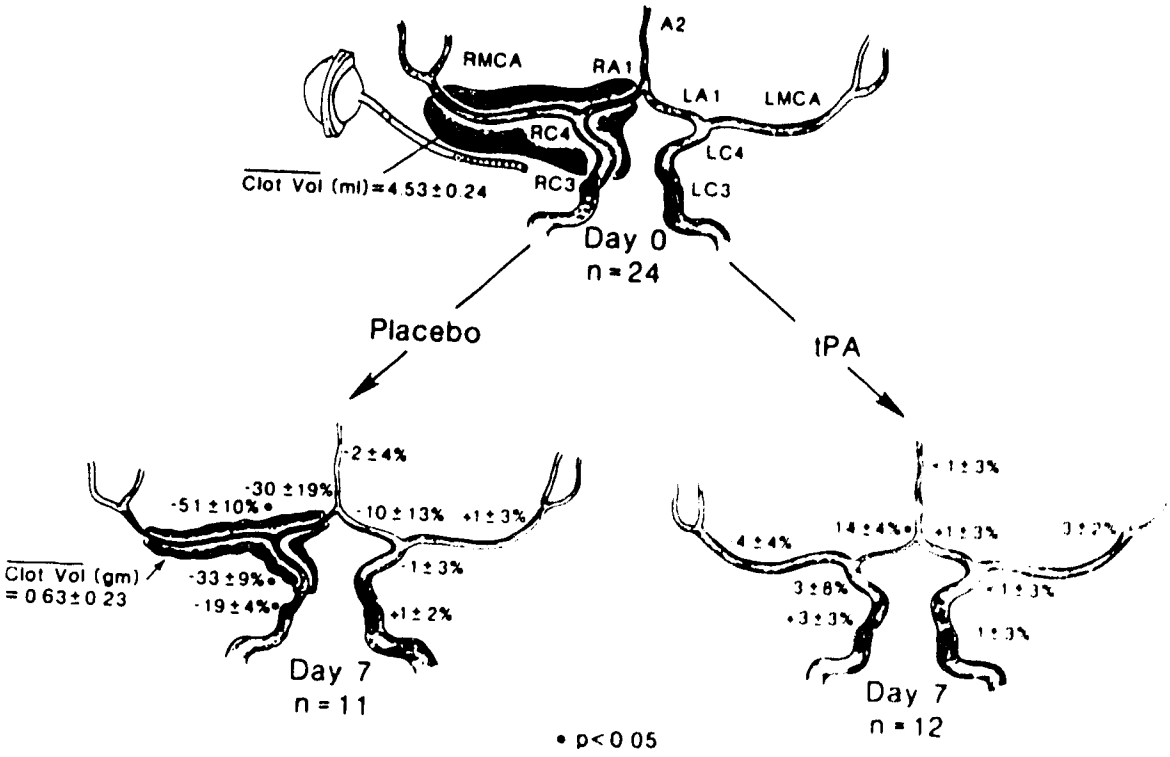

Figure III-6
Illustration depicting percentage change from baseline in the cerebral vessel diameters as well as the change in clot weight in the two treatment groups. Percentages indicate mean ± standard error of the means. C3 = Extradural internal carotid artery; C4 = Intradural internal carotid artery; A1 = precommunicating segment of the anterior cerebral artery; MCA = sphenoidal segment of middle cerebral artery; A2 = distal azygous anterior cerebral artery; PCA = posterior cerebral artery.

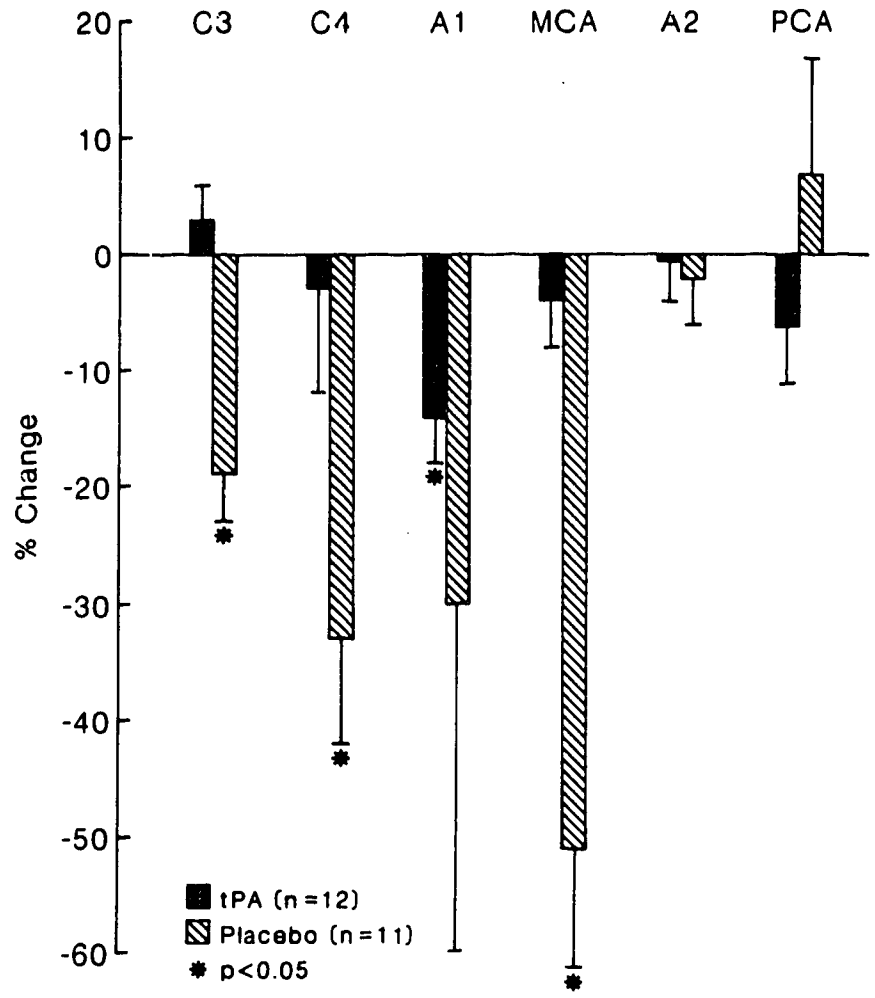

Figure III-7
Bar graph depicting the percentage change from baseline in the right-sided cerebral vessel diameters in the two groups. Percentages indicate means ± standard error of the means. C3 = Extradural internal carotid artery; C4 = Intradural internal carotid artery; A1 = precommunicating segment of the anterior cerebral artery; MCA = sphenoidal segment of middle cerebral artery; A2 = distal azygous anterior cerebral artery; PCA = posterior cerebral artery.

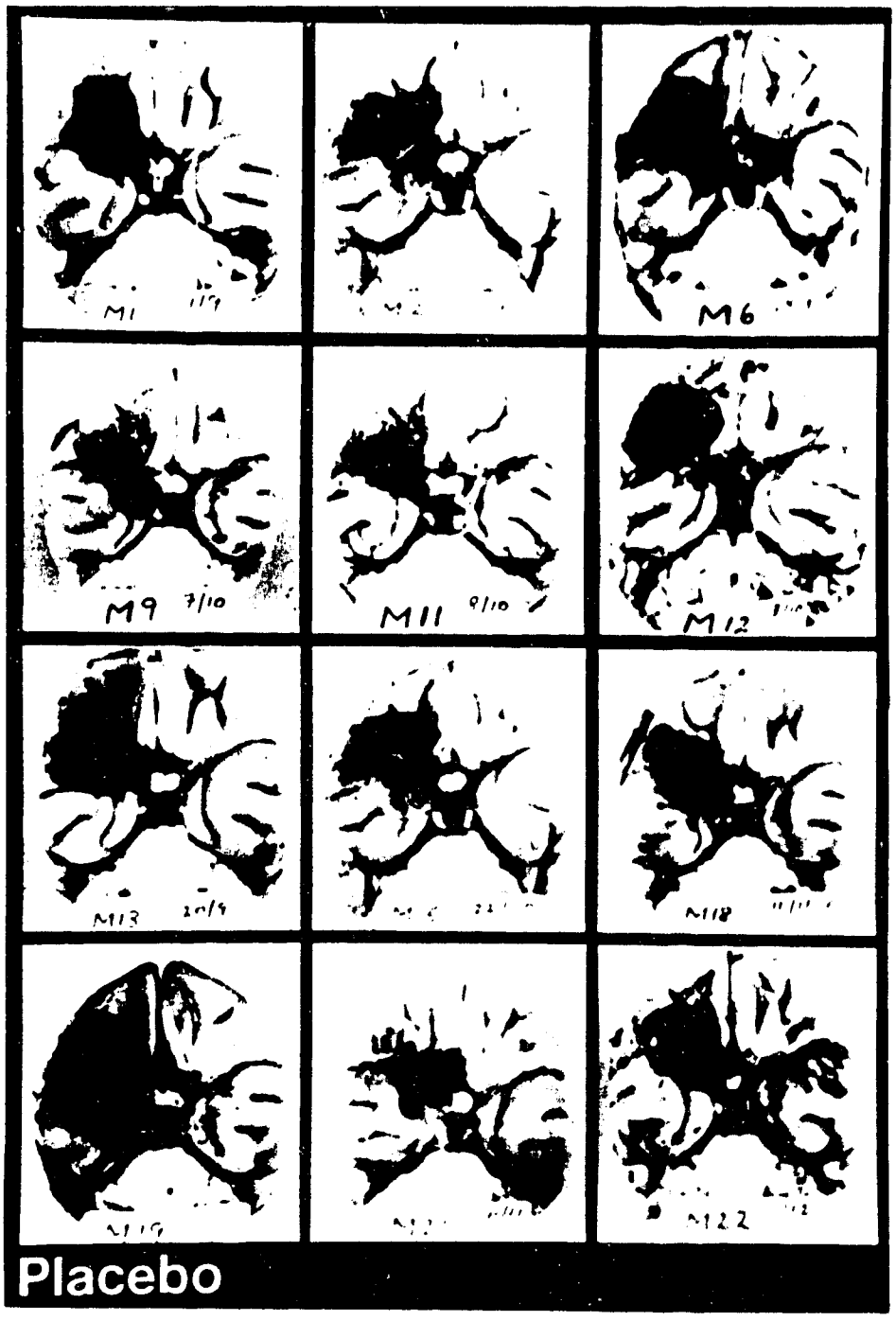

Figure III-8
Ventral aspect of placebo group brains with remaining subarachnoid blood.

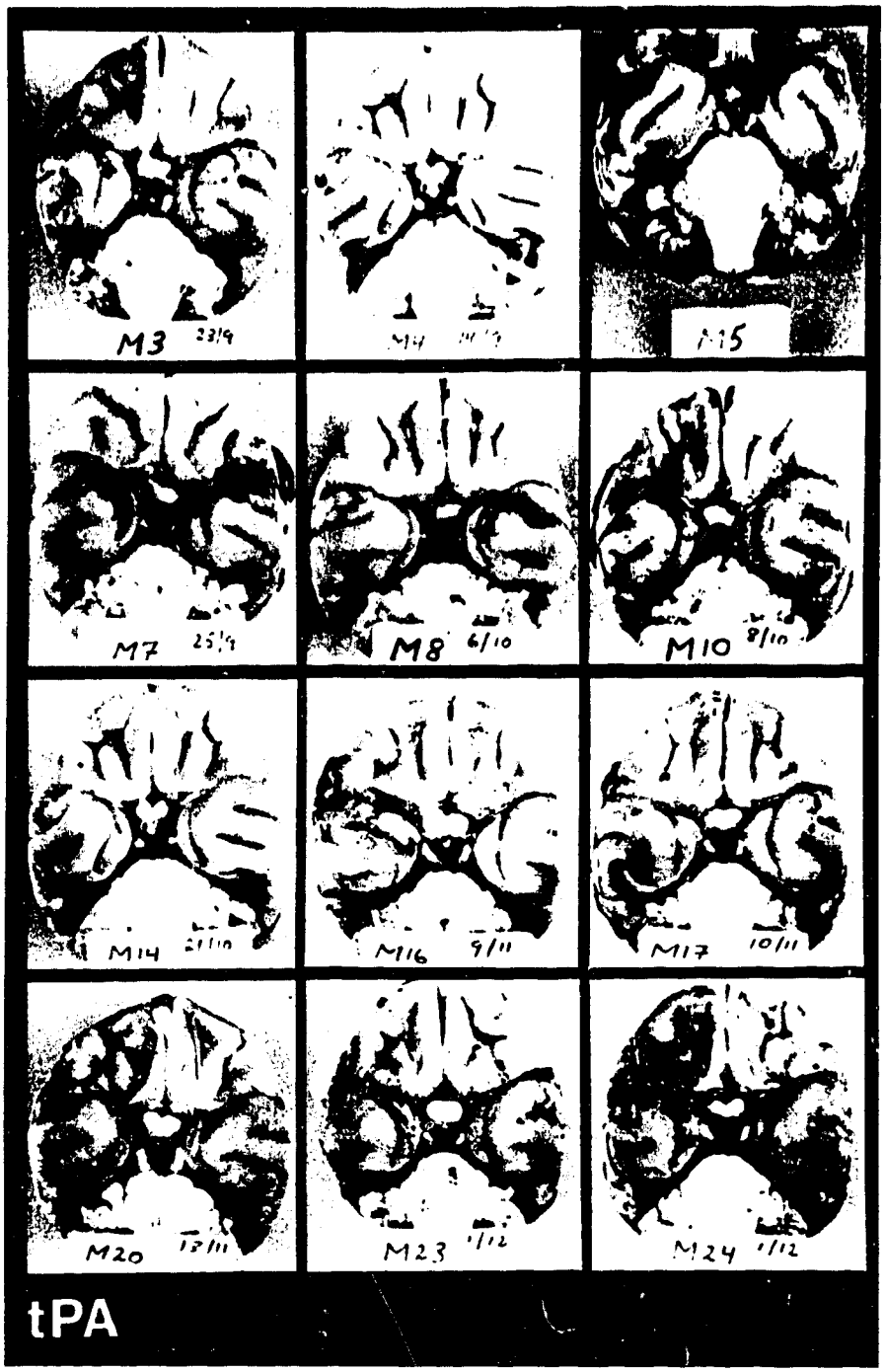

Figure III-9
Ventral aspect of rt-PA group brains free of gross subarachnoid clot.

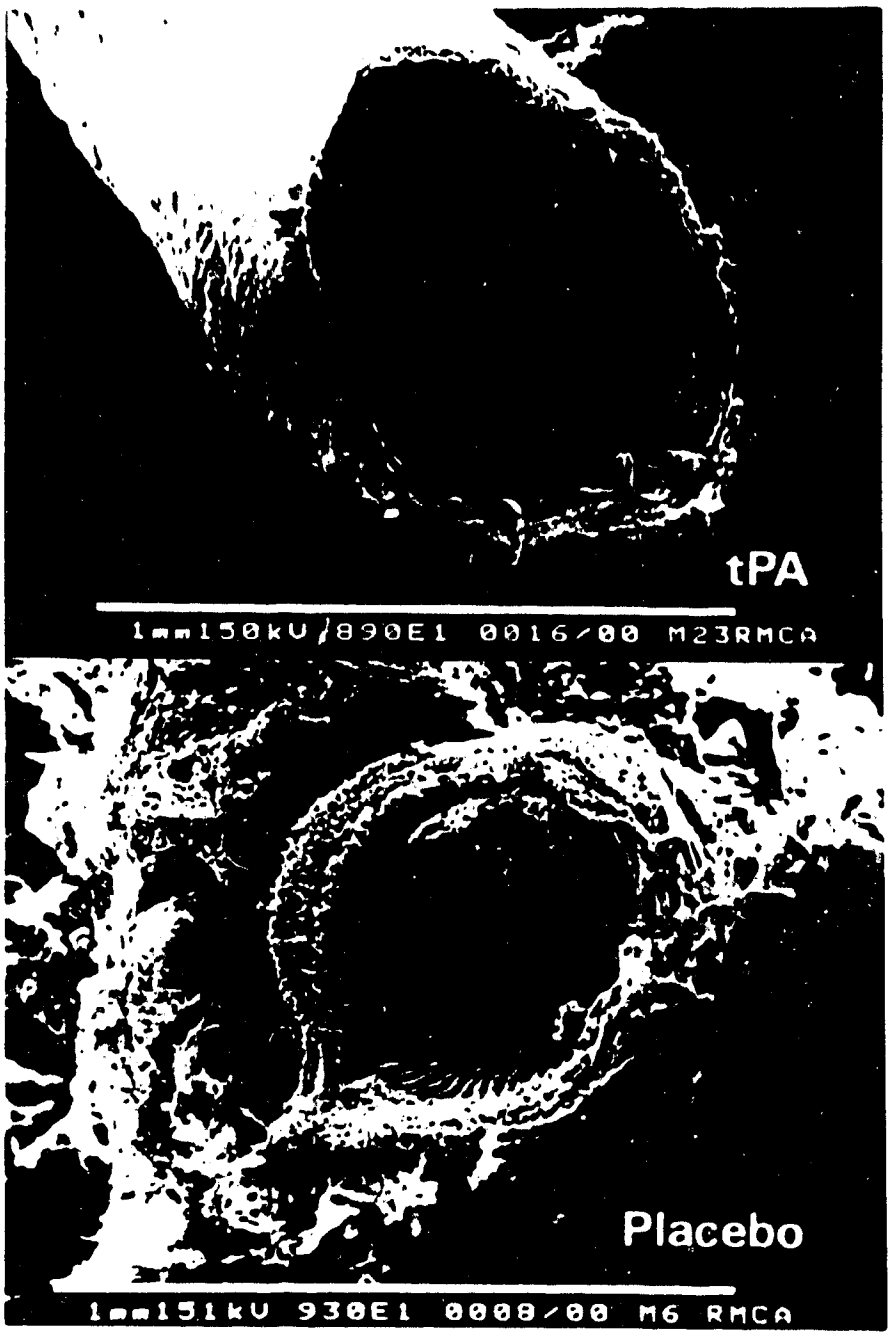

Figure III-10
Scanning electron micrographs of the luminal and adventitial surface of the right middle cerebral arteries (MCAs) from Monkey 23 (tPA group, upper) and Monkey 6 (placebo group, lower). Magnification factors and scales are shown at the bottom of each picture. The upper MCA is normal, while the lower MCA shows luminal narrowing, intimal corrugations, a thickened vessel wall and thrombus adherent to the adventitial surface.

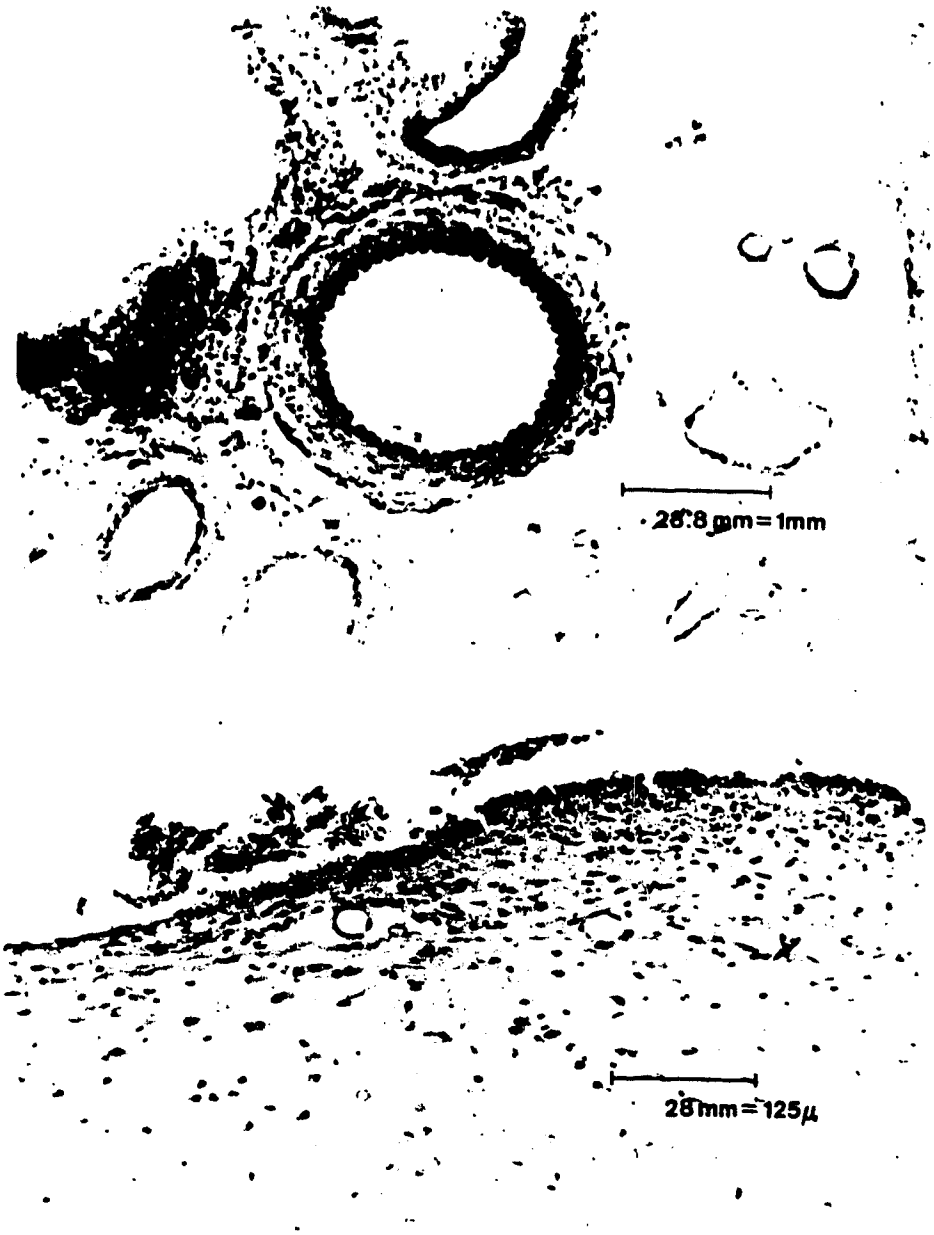

Figure III-11
Photomicrographs from Monkey 13 (placebo group), showing vasospastic right internal carotid artery and adherent thrombus in the basal subarachnoid space (upper micrograph), and a crust of blood clot attached to the pia-arachnoid membrane of the basal hypothalamus (lower micrograph). H&E.

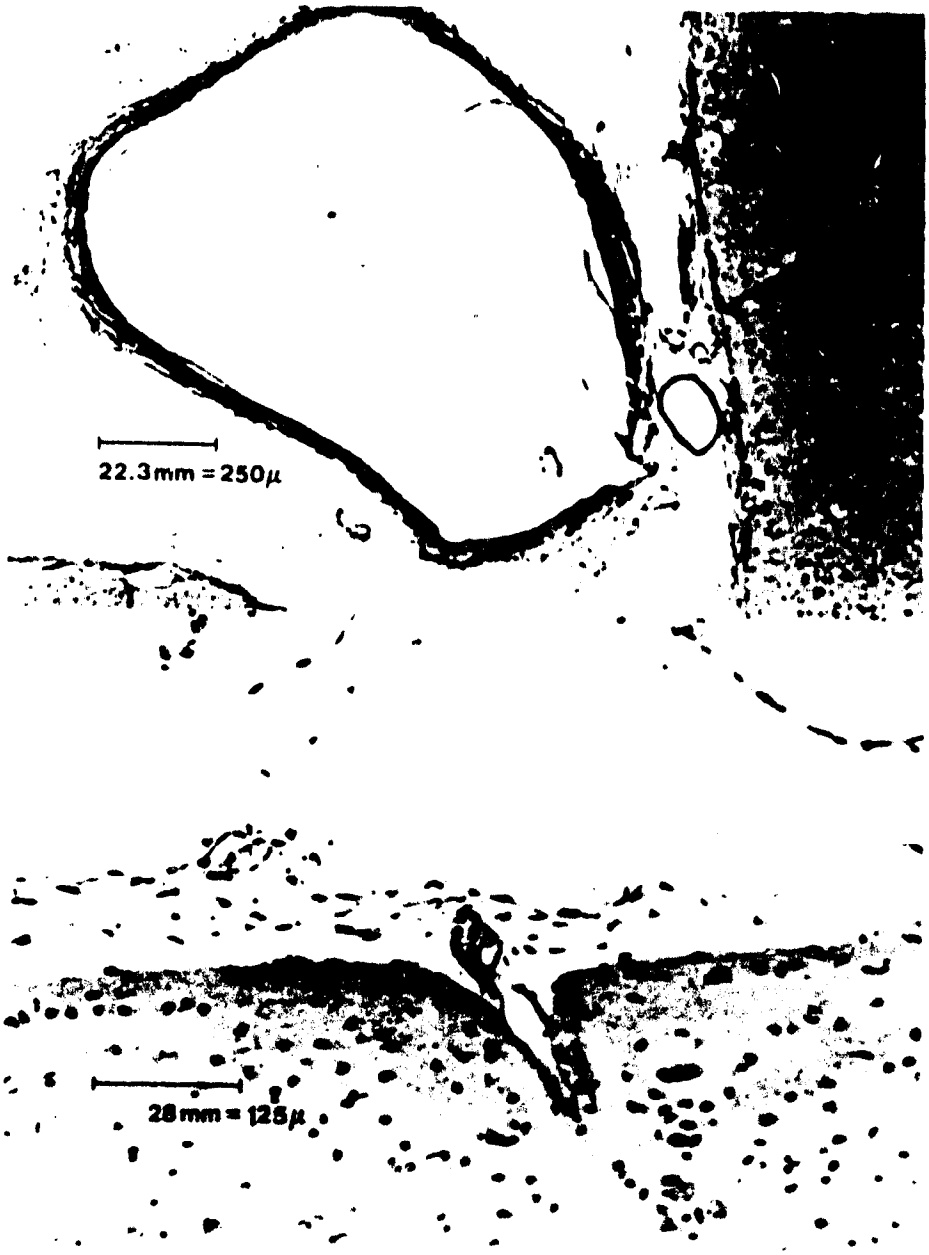

Figure III-12
Photomicrographs from Monkey 14 (rt-PA group), showing normal, non-spastic right internal carotid artery in the basal subarachnoid space (upper micrograph), and normal pia-arachnoid over the basal hypothalamus (lower micrograph). H&E.

CHAPTER FOUR

SAFETY AND EFFICACY OF INTRATHECAL FIBRINOLYTIC THERAPY IN A PRIMATE MODEL OF CEREBRAL VASOSPASM

SUMMARY

To test the safety of a large intrathecal dose of human recombinant tissue plasminogen activator (rt-PA), 6 Cynomolgous monkeys were given 10 mg of rt-PA (mean, 2.7 mg/kg) through an Ommaya reservoir after craniectomy and dissection of the basal cisterns. Bleeding occurred briefly at the incision in 2 animals; otherwise, the clinical condition of all 6 remained normal throughout the postoperative period. Systemic fibrinolysis did not occur, and gross and microscopic examination of the brain and meninges revealed no abnormality.

Next, we evaluated the efficacy of unilateral administration of rt-PA suspension (0.5 mg) plus slow-release gel rt-PA (1.25 mg) in clearing a bilateral subarachnoid clot and preventing vasospasm (VSP) in a randomized, placebo-controlled trial. Sixteen monkeys were divided randomly into 2 groups, each of which underwent baseline cerebral angiography, followed by left then right-sided frontotemporal craniectomy and induction of subarachnoid hemorrhage (SAH). Before closure on the right rt-PA or an equal volume of placebo was injected into the subarachnoid space. Seven days later angiography was repeated and the animals were killed

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under anaesthesia for necropsy. One in the placebo group developed a cerebral infarction on day 5. In the placebo group significant VSP occurred in all major right and left-sided anterior cerebral vessels ($p < 0.01$). No VSP occurred in the rt-PA treated animals. Whereas gross subarachnoid clot was found in all of the placebo animals (mean clot weight 1.13 gm), only a small fragment of clot was found in a single rt-PA treated animal. The gel had no adverse microscopic effects on the brain. Intrathecal fibrinolysis with rt-PA is safe and effective in preventing VSP in primates.

INTRODUCTION

We have recently shown in a controlled trial that intrathecal administration of the fibrinolytic agent human recombinant tissue plasminogen activator (rt-PA) is effective in lysing a unilateral subarachnoid hematoma and preventing vasospasm (VSP) in primates[2]. The dose of rt-PA used in that study (1.5 mg over 24 hours) did not cause systemic fibrinolysis or damage to the brain. In the first part of this report a higher dose of rt-PA (10 mg over 24 hours) was administered intrathecally to 6 monkeys postoperatively who were then followed for the development of any adverse effects. In the second part we investigated the efficacy of a single, unilateral, cisternal injection of both rt-PA suspension (0.5 mg in 0.5 ml) and a slow-release formulation of gel rt-PA (1.25 mg in 0.5 ml) in lysing a diffuse, bilateral subarachnoid hematoma and preventing VSP in primates.

MATERIALS AND METHODS

Details of procedures for microsurgical exposure of the basal subarachnoid cisterns, induction of subarachnoid hemorrhage (SAH), cerebral angiography, and animal sacrifice used in our primate model have been published elsewhere [2,3,11], and will only be outlined here. The protocol was evaluated and approved by the University of Alberta's Animal Ethics Review Committee, and experiments were conducted with strict adherence to the standards of the Canadian Council on Animal Care.

SAFETY STUDY

Six female Cynomolgous monkeys (Macaca fascicularis) weighing between 3.5 and 4 kg (mean, 3.71 kg) were studied. After sedation with ketamine hydrochloride (6-10 mg/kg, injected intramuscularly), blood was drawn from a peripheral vein for baseline coagulation analysis. One tube of citrated blood was centrifuged immediately at 4 ° C. The supernatant plasma was frozen for later batch-testing of thrombin time and euglobulin clot-lysis time (ELT) with a method [4] in which ELT <90 minutes indicates systemic activation of the fibrinolysis system. A separate sample was collected to test for fibrin degradation products (FDPs) with the Thrombo-Wellcotest latex slide test (Wellcome Reagents Ltd., Beckenham, England).

The animals then underwent a right frontotemporal craniectomy under general anesthesia for opening of the basal cisterns and exposure of the ipsilateral cerebral vessels. In 2 monkeys a small portion (3 mm) of the inferior frontal lobe was resected with electrocautery, and in 2 others the posterior communicating artery

was coagulated and divided. Subarachnoid hematomas were not placed in this group of monkeys. After dural closure an Ommaya reservoir was placed over the temporalis muscle and its catheter was advanced through dura into the subarachnoid space.

At 24 and 36 h postoperatively, 5 mg of rt-PA (Genentech Inc., South San Francisco, CA) was injected percutaneously into the Ommaya reservoir in 0.5 ml of physiologic solution. Two hours after the second injection, blood was drawn for repeat coagulation analysis.

The animals were observed closely for the development of wound complications or neurologic deficits until the 4th postoperative day, when they were injected intravenously with a large bolus of sodium pentobarbital, approximately 50 mg/kg, and exsanguinated. A cannula was inserted into the left ventricle, and intra-arterial perfusion was performed with the atrium opened widely and the ascending aorta cross-clamped; circulating blood was washed out with 0.5 L of normal saline under 110 mmHg pressure. This was followed by perfusion with 0.5 L of fixation solution consisting of 2% glutaraldehyde and 2% formaldehyde in Millonig's buffer, 0.12 M, pH 7.4 at 4 ° C.

The brain was removed and photographed. The middle cerebral arteries (MCAs) were excised and cut into segments, which were fixed for 1 hour in 1% osmium tetroxide in Millonig's buffer, 0.07 M, and prepared for scanning electron microscopy (SEM) with a Phillips model 505 microscope (N.V. Phillips Gloeilampenfabrieken, Eindhoven, The Netherlands). The brains were immersed in formalin for 1 wk, then sliced coronally to display the pia-arachnoid

and vessels at the base of the brain, the cerebral cortex, and ventricles. Representative sections were made of these areas bilaterally and stained with hematoxylin and eosin (H&E) for light microscopy (LM).

Bilateral Subarachnoid Clot Study

This was designed as a randomized, placebo-controlled trial (fig. IV-1). Because slow-release gel containing rt-PA is visibly distinguishable from gel alone (placebo), treatment could not be administered blindly, but reading of the angiograms and LM was done in a blinded fashion. Sixteen adult, female Cynomolgous monkeys weighing between 3.0 and 4.0 kg (mean, 3.45 kg) were assigned to one of two groups of 8 by restricted randomization, one group to receive rt-PA and the other placebo.

After baseline cerebral angiography the animals underwent a left frontotemporal craniectomy for opening of the basal cisterns, exposure of the cerebral vessels and placement of 3 ml of autologous blood clot into the subarachnoid space. After closure the animal's head was turned and the procedure repeated on the right side. After clot placement and prior to dural closure on the right the treatment group had 0.5 mg of rt-PA suspended in 0.5 ml physiologic solution followed by 1.25 mg of rt-PA suspended in a slow-release gel formulation injected into the subarachnoid space in the vicinity of the Sylvian fissure (total dose 1.75 mg). The gel is a specially formulated hyaluronidase preparation (Genentech Inc., South San Francisco, CA) which liquifies over a several day period and continuously releases rt-PA molecules into the cerebrospinal fluid (CSF). The placebo group received an injection of 0.5 ml of

physiological solution alone followed by 0.5 ml of gel vehicle without rt-PA.

The animals were then followed for the development of any wound complications and delayed neurological deficits. In the event of the latter a computerized tomographic scan of the head was obtained.

Seven days after bilateral clot placement and unilateral (right-sided) rt-PA administration the animals underwent repeat cerebral angiography and were killed by exsanguination. The brains were removed and photographed, and any remaining subarachnoid clot weighed. After formalin fixation the brains were then sectioned and stained with H&E for LM.

Radiologic Assessment

Cerebral vessels were measured 4 times with a calibrated optical micrometer, and a mean value obtained. The arteries were measured bilaterally at the following points: extradural internal carotid artery (C3), intradural internal carotid artery (C4), precommunicating segment of the ACA (A1), sphenoidal segment of the MCA, azygous distal anterior cerebral artery (A2), the first part of the PCA, and basilar artery (BA).

All data were coded, entered into a computer, and edited. Data for angiographic vessel caliber change within treatment groups between days 0 and 7 were compared by a paired t-test, and intergroup comparisons were made with t-test for unpaired variables. Analysis of other measured indices including coagulation studies was done with either a t-test or Fisher's exact test where

appropriate. The level of significance for all tests of comparison was $p<0.05$ unless otherwise stated.

RESULTS

Safety Study

Five of the monkeys, including the 2 that had undergone division of the posterior communicating artery, remained neurologically intact throughout the study period. The sixth animal (which had undergone cortical resection), was withdrawn and anorexic. In 2 monkeys wound hemorrhages developed during rt-PA administration and persisted for 24 hours.

Plasma thrombin times, determined with two concentrations, were unchanged by intrathecal rt-PA. ELT was $\geq$ 120 minutes in all specimens withdrawn before or during rt-PA administration, and FDPs were not detected in any samples.

On gross examination the brain and meninges of all 6 animals appeared normal, and on LM the brain, ependyma, and basal meninges appeared histologically normal. Sections of tissue from the monkeys which had undergone coagulation and division of cerebral arteries revealed no SAH, and tissue from those which underwent cortical resection contained only minute patches of intracerebral hemorrhage at the resection site. Scanning electron microscopy of segments of middle cerebral vessels from 5 monkeys revealed no abnormalities, and in the sixth animal minor narrowing of the arterial lumen and thickening of the vessel wall, consistent with mild VSP, was found.

Bilateral Subarachnoid Clot Study

Comparisons between the 2 treatment groups showed no significant differences in day 0 or day 7 values for body weight, mean arterial blood pressure, heart rate or $PaCO_2$ (Table IV-1). Also, there was no significant change in any of these parameters within each group on these 2 days.

Clinical Status

Significant but transient (48 hours) facial edema occurred in 3 rt-PA treated animals and 1 placebo treated animal. There were no instances of incisional bleeding.

Monkey 13 in the placebo group developed a left hemiparesis on day 5 that worsened over the next 2 days until sacrifice. A computerized tomographic scan on day 6 demonstrated cerebral infarction in the distribution of the right MCA (fig. IV-2).

Extent of Cerebral Vasospasm

Vasospasm was defined as a reduction in vessel caliber of 10% or greater compared to baseline value. Comparison of the cerebral angiograms taken on day 7 to baseline angiograms within each group by paired t-test revealed significant reductions (VSP) in the following vessels in the placebo group: right and left C3, right and left C4, right and left A1 and right and left MCA ($p<0.01$). No significant narrowing occurred in any vessel from animals in the rt-PA group, although a mild reduction in caliber (less than 10%) was noted in the MCA's (not significant) (figs. IV-3,4,5).

Because of significant differences in several of the vessel calibers at baseline between the 2 treatment groups (C4, A1 and A2), intergroup comparisons for angiograms taken on day 7

required adjustment by analysis of covariance. There remained, however, significant differences between rt-PA and placebo groups for the right C4, right and left A1, and right and left MCA ($p<0.05$). In each instance the value for the placebo group was less than that for the rt-PA group.

Gross Pathology

At sacrifice all placebo treated animals had gross subarachnoid clot remaining (mean weight, 1.13 g; SD, 0.07), while only one of the rt-PA treated animals had a fragment of clot left (0.01 g) (fig. IV-6). This difference was highly significant ($p<0.001$). There was no gel remaining in any animal. There was a moderate sized left temporal lobe intracerebral hemorrhage (5 mm diameter) in monkey 7 of the placebo group, contiguous with operative site. There were no epidural or subdural hematomas, and no evidence of hydrocephalus in any animal at brain cutting.

Microscopic Pathology

Examination of the brain of monkey 13, which had developed delayed left hemiplegia, revealed a cerebral infarction in the right frontoparietal junctional area. Most (7 of 8) of the placebo treated animals had subarachnoid blood clot attached to the basal meninges, and several had modest leptomeningeal inflammatory infiltrates and evidence of arterial VSP. These findings were not present in the rt-PA treated animals. A single animal in each group had petechial hemorrhages in the temporal cortex near the sylvian fissure.

Scanning electron microscopy done in a limited number of placebo treated monkeys demonstrated the typical changes of VSP,

namely marked vascular constriction associated with a degree of vessel wall thickening (fig. IV-7).

DISCUSSION

Cerebral ischemia is one of the major causes of death and disability in survivors of aneurysmal SAH, and while a number of factors influence its development and severity, the most important is delayed arterial narrowing known as cerebral VSP. The incidence, distribution, and severity of VSP are correlated to the location and volume of blood deposited in the basal subarachnoid cisterns by the ruptured aneurysm [6]. Over a period of days the erythrocytes of the blood clot lyse and release vasoconstrictive and vasotoxic substances which permeate the walls of cerebral arteries and induce muscular spasm and vessel wall damage [1,9,16]. Our understanding of the precise biochemical interaction between the blood clot and the vessel wall is presently incomplete, and unfortunately all attempts to prevent or reverse VSP consistently with various pharmacological agents have failed [17,18].

It has been suggested in clinical trials and proven in primate experiments that timely and thorough surgical evacuation of the cisternal blood clot (within 48 hours of SAH) will prevent VSP [3,10,11]. However, this is a hazardous and often impossible procedure after significant subarachnoid bleeding. We have previously shown that instillation of thrombolytic agent rt-PA into the subarachnoid space is effective in lysing an ipsilateral blood clot and preventing VSP in primates [2]. In that study the total dose of rt-PA was 1.5 mg, which was selected on the basis of tests *in vitro*, when injected into the basal cisterns via an Ommaya reservoir

(0.5 mg 3 times in 24 hours), it eliminated a mean clot volume of 4.38 ml and significantly reduced both the incidence and severity of VSP compared to placebo treated controls. Further, that dose of rt-PA appeared to have no adverse effects on the brain and was not followed by systemic fibrinolysis.

To test the safety of intrathecal rt-PA further a much higher dose of enzyme was given to 6 animals in the first part of this study. However even a total dose of 10 mg of rt-PA (mean, 2.7 mg/kg) did not activate systemic fibrinolysis or have inflammatory or toxic effects on the brain. We have estimated that lysis of a large subarachnoid clot, of about 50 ml, in humans requires 15 mg of rt-PA, or approximately 0.21 mg/kg for a 70-kg adult. In comparison, the dose of rt-PA given intravenously for acute myocardial infarction in an adult of that weight is 100 mg, or approximately 1.40 mg/kg. The safety of intrathecal rt-PA can be attributed to the relative specificity of this serine protease [8,15], and it concurs with reports of rt-PA given to rabbits [5]. In contrast to these findings, a combination of streptokinase and streptodornase, while effective in clearing subarachnoid hematoma in cats, produced a diffuse meningoencephalitis by 24 hours [13], and urokinase instilled into the ventricles of dogs after induction of intraventricular hemorrhage caused systemic fibrinolysis [12]. Here, and in our earlier study, subarachnoid or cerebral bleeding did not occur after dissection, coagulation and small cortical resections; we believe the incisional hemorrhages seen in a total of 4 animals was due to epidural leakage of the enzyme along the Ommaya catheter.

In the second part of this investigation we wished to test the efficacy of a single, unilateral, intraoperative administration of rt-PA on a more diffuse SAH. Bilateral craniectomies were done and blood clot placed on the left and then right sides (3 ml per side), in contact with the major cerebral vessels. A combination of liquid rt-PA suspension and slow-release gel rt-PA (total dose 1.75 mg) was injected into the right Sylvian cistern of the active treatment group monkeys prior to final closure. The same volumes of vehicle alone were administered to the placebo group. One animal in the placebo group developed delayed cerebral infarction in the distribution of the right MCA. Whereas all of the animals in the placebo group had a large volume of subarachnoid clot remaining at 7 days, 7 of the 8 treated animals had no clot. Significant VSP occurred in the anterior cerebral vessels in the placebo group, while no significant narrowing was observed in the rt-PA treated animals. Histological examination of the brains revealed no adverse effect of the gel vehicle, and incisional hemorrhages did not occur in the rt-PA treated animals, probably due to the better dural closure possible without an Ommaya catheter.

Thus far the intrathecal administration of rt-PA has proven safe in the primate model. Furthermore, it appears that unilateral administration of rt-PA in the form of suspension and a slow-release gel will effect lysis of a diffuse, bilateral subarachnoid clot and prevent VSP on both sides of the anterior cerebral circulation. It is possible that in the future intrathecal fibrinolysis with rt-PA could be used in combination with early aneurysm clipping to prevent VSP.

BIBLIOGRAPHY

1. Duff TA, Louie J, Feilbach JA, Scott G: Erythrocytes are essential for development of cerebral vasculopathy resulting from subarachnoid hemorrhage in cats. Stroke 19: 68-72, 1988.
2. Findlay JM, Weir BKA, Steinke D, Tanabe T, Gordon P, Grace M: Effect of intrathecal thrombolytic therapy on subarachnoid clot and chronic vasospasm in a primate model of SAH. J Neurosurg 69: 723-735, 1988.
3. Handa Y, Weir BKA, Nosko M, Mosewich R, Tsuji T, Grace M: The effect of timing of clot removal on chronic vasospasm in a primate model. J Neurosurg 67: 558-564, 1987.
4. Hougie C: Methods, in Williams WJ, Bentler E, Ersler AJ, Lichtman MA (eds): *Hematology*, ed 3. New York: McGraw-Hill, 1983, pp 1167-1670.
5. Kaufman HH, Schochet S, Koss W, Herschberger J, Bernstein D: Efficacy and safety of tissue plasminogen activator. Neurosurgery 20: 403-407, 1987.
6. Kistler JP, Crowell RM, Davis KR, Heros R, Ojemann RG, Zervas T, Fisher CM: The relation of cerebral vasospasm to the extent and location of subarachnoid blood visualized by CT scan: A prospective study. Neurology (N.Y.) 33: 424-426, 1983.
7. Lijnen HR, Marafina BJ, Collen D: *In vitro* fibrinolytic activity of recombinant tissue-type plasminogen activator in the plasma of various primate species. Thromb Haemost 52: 308-310, 1984.

8. Ludrook PA: Thrombolytic therapy with t-PA. Cardiovasc Med, February 1986, 37-44.

9. Mayberg M, Okada T, Bark D: Cerebral arteriopathy and contractile changes after SAH are related to erythrocyte component of whole blood. Stroke 19: 130, 1988 (abstr.)

10. Mizukami M, Kawase T, Usami T, Tazawa T: Prevention of vasospasm by early operation with removal of subarachnoid blood. Neurosurgery 10, 301-307, 1982.

11. Nosko M, Weir BKA, Lunt A, Grace M, Allen P, Mielke B: Effect of clot removal at 24 hours on chronic vasospasm after SAH in the primate model. J Neurosurg 66: 416-422, 1987.

12. Pang D, Sclabassi RJ, Horton JA: Lysis of intraventricular blood clot with urokinase in a canine model: Part 2. *In vivo* safety study of intraventricular urokinase. Neurosurgery 19: 547-552, 1986.

13. Peterson EW, Choo SH, Lewis AJ, Lach B, Bormanis J: Lysis of blood clot and experimental treatment of subarachnoid hemorrhage, in Wilkins RH (ed): *Cerebral Arterial Spasm*. Baltimore, Williams & Wilkins, 1980, pp 625-627.

14. Taneda M: Effect of early operation for ruptured aneurysms on prevention of delayed ischemic symptoms. J Neurosurg 57: 622-628, 1982.

15. Verstraete M, Collen D: Thrombolytic therapy in the '80s. Blood 67: 1529-1541, 1986.

16. Weir B: *Aneurysms Affecting the Nervous System*. Baltimore, Williams & Wilkins, 1987, pp 518-522.

begins immediately and reaches a plateau from 2 to 10 days after SAH, depending upon the size of the hemorrhage. It is hemolysis which liberates spasmogenic OxyHb in highest concentration around the subarachnoid arteries. Larger hemorrhages are associated with a more delayed (typically 7-11 days post-SAH) and much higher peak concentration in this pigment in the CSF, correlating with a higher risk of VSP[88]. It is likely that there is an as of yet undefined OxyHb concentration and exposure time necessary for VSP to occur. The second mechanism is phagocytosis by macrophages, which occurs in the leptomeninges irritated by the hemorrhage, in the arachnoid granulations which become engorged with erythrocytes, and within the subarachnoid clot by migrating phagocytes. These macrophages, along with arachnoid lining cells and choroid plexus epithelium, appear to be the site of Hb metabolism to bilirubin, which is also released into the CSF and may play a role in the genesis of VSP[21]. CSF xanthochromia resulting from hemolysis in the subarachnoid space is quite variable in duration, ranging from several to over 20 days. This time is dependent on a number of factors, but prominent among these is the volume of SAH. Direct passage of red blood cells across arachnoid villi into the venous circulation is of lesser significance in erythrocyte clearance from CSF. Normally, and in contrast to the situation with intravascular thrombi, the rate of spontaneous fibrinolysis in the CSF is too slow to substantially augment intact erythrocyte removal from the basal cisterns.

Intrathecal Fibrinolytic Treatment after Aneurysmal SAH

While intrathecal fibrinolysis after SAH is a new direction in managing patients with ruptured aneurysms, systemic anti-fibrinolytic therapy is not. Mullan et al[61] observed that the plasminogen and t-PA inactivator epsilon aminocaprioc acid prolonged the duration of intraluminal clots in femoral arteries of dogs and reasoned that through the same mechanisms this agent might prevent dissolution of a thrombus sealing the rent in an aneurysm. Indeed, antifibrinolytic therapy has subsequently been shown to reduce the rate of rehemorrhage after aneurysm rupture, but the beneficial effect on outcome is negated by a concomitant increase in the incidence of VSP and delayed cerebral ischemia[44,92]. One explanation for this is that inhibition of already limited CSF fibrinolytic activity further delays clearance of vasogenic, hemolysing erythrocytes from the basal cisterns. Because of this risk, and because of the more widespread adoption of early aneurysm clipping to prevent rebleeding, antifibrinolytic agents no longer have a major role in the management of aneurysmal SAH[97].

Provided the ruptured aneurysm has been secured surgically it would be desirable to empty the basal cisterns of hemolysing erythrocytes in order to prevent clot-mediated VSP. Because of the technical difficulties and hazards of an aggressive surgical evacuation of diffuse subarachnoid clot, interest has grown in accomplishing this task enzymatically with a fibrinolytic agent. The literature on use of thrombolytic agents for intravascular thromboembolic disease, including stroke, is extensive[19,56,57,81].

In addition, urokinase has been used to lyse intracerebral and intraventricular hematomas in various experimental models [63,71]. The concept of intrathecal fibrinolytic treatment after SAH is to augment the normally limited FA of CSF and promote rapid digestion of the fibrin structure of the subarachnoid thrombus. Disruption and dissolution of the hematoma, aided by the mechanical pulsatile force of the brain, could allow clearance of previously entrapped erythrocytes by bulk CSF circulation or CSF drainage prior to their hemolysis and release of OxyHb in high concentrations near the subarachnoid arteries (fig. V-6).

Kennady appears to have been the first to instill a fibrinolytic agent into the subarachnoid space [46]. He found that the addition of "fibrinolysin" to an intrathecal irrigation system increased the efficiency of red blood cell removal from the CSF after experimental SAH in dogs. Peterson et al [74] lysed an experimental subarachnoid clot in cats with a combination of streptokinase and streptodornase injected into the cisterna magna. They found that while this effectively cleared the hematoma it unfortunately produced a diffuse meningoencephalitis. Alksne et al injected plasmin into the cisterna magna of pigs one hour after the second injection of blood in a two hemorrhage model of experimental SAH and found that this reduced the severity of SAH-induced histological changes in cerebral arteries compared to controls [4]. In an extension of this work these same workers have reported that delaying plasmin injection 2, 4 or 6 days after the second hemorrhage results in a progressive increase in the extent and severity of intimal proliferation [5], a

presumed marker of VSP. Cerebral angiography was not done in these studies. Some Japanese investigators have infused urokinase into the ventricles of patients after SAH, with indeterminate results [80].

Our own work began with intrathecally administered rt-PA suspension into the basal cisterns of monkeys after experimental SAH. In a blinded, randomized, placebo-controlled trial it was found that 0.5 mg of rt-PA (a dose chosen on the basis of *in vitro* studies) injected into the subarachnoid space 3 times over 24 hours beginning 24 hours after SAH uniformly lysed a mean clot volume of 4.38 ml and virtually eliminated VSP measured at 7 days in the 12 treatment animals [26]. In comparison, the 12 placebo animals all had a large volume of subarachnoid clot remaining and significant VSP in the clot-side cerebral vessels on day 7. Intrathecal rt-PA did not alter the coagulation profiles of the animals nor did it have any adverse effect on the microscopic appearance of the brain or meninges. A subsequent safety study using a much higher dose of intrathecal rt-PA, 10 mg, also failed to show any adverse effect of the enzyme on neurologic condition of the animals, coagulation status or appearance of the brain on light microscopy [27]. Two animals in this study and 2 in the previous study developed temporary wound hemorrhages that were felt due to epidural leakage of the enzyme along the Ommaya catheter.

We then evaluated the efficacy of unilateral intraoperative administration of rt-PA suspension (0.5 mg) plus sustained-release gel rt-PA (1.25 mg) in clearing a bilateral 6 ml subarachnoid clot and preventing VSP in a randomized, placebo-controlled trial with 8

monkeys in each group[27]. As in the original study, 1 placebo animal developed a delayed cerebral infarct. There were no incisional hemorrhages. In the placebo group significant VSP occurred in all major right and left-sided cerebral arteries and gross clot was found in the subarachnoid space in all animals. No significant VSP occurred in the treatment group and a single animal had a small fragment of clot left at 7 days. The development of the sustained-release gel rt-PA proved a significant advance because it obviated the need for an Ommaya reservoir and postoperative intrathecal injections.

In the study reported here a dosage study was performed using gel rt-PA alone. It was found that 0.5 and 0.75 mg of rt-PA administered at the time of experimental SAH was effective in preventing VSP. Mild to moderate VSP was observed in all of the anterior cerebral vessels in the 0.125 and 0.25 mg groups on day 7. With an increasing dose of rt-PA there was a progressive decline in amount of clot remaining at 7 days. Two of the 4 animals from the 0.5 mg group were free of clot, as were all of those in the 0.75 mg group. It is noteworthy that the amount of VSP and clot remaining in the smallest dosage groups (0.125 and 0.25 mg) in this study was still significantly less than in the group of 12 nontreatment control animals reported previously (a mean 51, 33 and 19% reduction in vessel caliber for the MCA, C4 and C3 respectively, and a mean remaining clot weight of 0.63 gm after a 4.67 ml volume SAH)[26]. This suggests that even partial clot lysis is effective in reducing the severity of VSP in our model.

We remain hopeful that intrathecal thrombolysis with rt-PA may prove to be effective preventative therapy for VSP after aneurysmal SAH. *It will be necessary to employ it in conjunction with early aneurysm clipping*, and to avoid the promotion of intracerebral hemorrhage it should probably not be used in patients with significant cortical disruption due to either the primary hemorrhage or surgery. In a recent Swedish study spanning 34 months[40], such early surgery was possible in 71% of 121 patients surviving transport to a neurosurgical center, and a similar percentage of these patients had thick localized or diffuse clot confined to the subarachnoid spaces. We are currently at work in our laboratory to determine the time interval after experimental SAH during which administration of rt-PA remains effective in significantly reducing the incidence of VSP.

BIBLIOGRAPHY

1. Adams JE, Prawirohardjo S: Fate of red blood cells injected into cerebrospinal fluid pathways. Neurology 9: 561-564, 1959.
2. Alkjaersig N, Fletcher AP, Sherry S: The mechanism of clot dissolution by plasmin. J Clin Invest 38: 1086-1095, 1959.
3. Alksne JF, Lovings ET: The role of the arachnoid villus in the removal of red cells from the subarachnoid space. An electron microscopic study in the dog. J Neurosurg 36: 192-200, 1972.
4. Alksne JF, Branson PJ, Bailey N: Modification of experimental post-subarachnoid hemorrhage vasculopathy with intracisternal plasmin. Neurosurgery 19: 20-25, 1986.
5. Alksne JF, Branson PJ, Bailey N: Modification of experimental post-subarachnoid hemorrhage vasculopathy with intracisternal plasmin. Neurosurgery 23: 335-337, 1988.
6. Alpers BJ, Forster FM: The reparative processes in subarachnoid hemorrhage. J Neurol Pathol Exp Neurol 4: 262-268, 1945.
7. Anderson M, Matthews KB, Stuart J: Coagulation and fibrinolytic activity of the cerebrospinal fluid. J Clin Pathol 31: 488-492, 1978.
8. Aoki N, Harpel PC: Inhibitors of the fibrinolytic enzyme system. Semin Thromb Hemostasis 10: 24-41, 1984.
9. Asano T, Tanishima T, Sasaki T, et al: Possible participation of free radical reactions initiated by clot lysis in the pathogenesis of vasospasm after subarachnoid hemorrhage. In:

Wilkins RH, ed. *Cerebral Arterial Spasm*. Baltimore: Williams & Wilkins, 1980: 190-201.

10. Astrup T, Mullertz S: The fibrin plate method for estimating fibrinolytic activity. Arch Biochem Biophys 40: 346-351, 1952.
11. Bachmann F, Kruithof IEKO: Tissue plasminogen activator: Chemical and physiological aspects. Semin Thrombo Hemostasis 10: 6-17, 1984.
12. Barrows LJ, Hunter FT, Banker BQ: The nature and clinical significance of pigments in the cerebrospinal fluid. Brain 78: 59-80, 1955.
13. Barabas A: The effect of CSF on blood clotting. Min Neurochir 14: 314-323, 1970.
14. Bauer JD: Hemoglobin, porphyrin, and iron metabolism. In: Kaplan LA, Pesce AJ, eds. *Clinical Chemistry Theory Analysis and Correlation*. St. Louis: C. V. Mosby Co, 1984: 613-651.
15. Boullin DJ, Tagari P, Du Boulay G, et al: The role of hemoglobin in the etiology of cerebral vasospasm. An *in vivo* study of baboons. J Neurosurg 59: 231-236, 1983.
16. Bloomfield SM, Sonntag VKH: Delayed cerebral vasospasm after uncomplicated operation for an unruptured aneurysm. Case report. Neurosurgery 17: 792-796, 1985.
17. Castellino FJ: Biochemistry of human plasminogen. Semin Thromb Hemostasis 10: 18-23, 1984.
18. Collen D: On the regulation and control of fibrinolysis: Edward Kowalski Memorial Lecture. Thromb Haemost 43: 77-89, 1980.

19. del Zoppo GJ: Thrombolytic therapy and cerebrovascular disease. Stroke 19: 1174-1179, 1988.

20. Duff TA, Louie J, Feilbach JA, et al: Erythrocytes are essential for development of cerebral vasculopathy resulting from subarachnoid hemorrhage in cats. Stroke 19: 68-72, 1988.

21. Duff TA, Feilbach JA, Yusuf Q, et al: Bilirubin and the induction of intracranial arterial spasm. J Neurosurg 69: 593-598, 1988.

22. Ellington E, Margolis G: Block of arachnoid villus by subarachnoid hemorrhage. J Neurosurg 30: 651-657, 1969.

23. Espinosa F, Weir B, Shnitka T: Electron microscopy of simian cerebral arteries after subarachnoid hemorrhage and after the injection of horseradish peroxidase. Neurosurgery 19: 935-945, 1986.

24. Espinosa F, Weir B, Shnitka T, et al: A randomized placebo-controlled double-blind trial of nimodipine after SAH in monkeys. Part 2: Pathological findings. J Neurosurg 60: 1176-1185, 1984.

25. Espinosa F, Weir B, Overton T, et al: A randomized placebo-controlled double-blind trial of nimodipine after SAH in monkeys. Part 1: Clinical and radiological findings. J Neurosurg 60: 1167-1175, 1984.

26. Findlay JM, Weir BK, Steinke D, et al: Effect of intrathecal thrombolytic therapy on subarachnoid clot and chronic vasospasm in a primate model of SAH. J Neurosurg 69: 723-735, 1988.

27. Findlay JM, Weir BKA, Gordon P, et al: Safety and efficacy of intrathecal thrombolytic therapy in a primate model of cerebral vasospasm. Neurosurgery 24: 491-498, 1989.

28. Findlay JM, Weir BKA: Subarachnoid and intracerebral hemorrhage. In: Weinstein PR, Faden AI, eds. *Protection of the Brain from Ischemia*. Baltimore: Williams & Wilkins, 1989.

29. Fisher CM, Kistler JP, Davis JM: Relation of cerebral vasospasm to subarachnoid hemorrhage visualized by computed tomographic scanning. Neurosurgery 6: 1-9, 1980.

30. Fodstad H, Kok P, Algers G: Fibrinolytic activity of cerebral tissue after experimental subarachnoid hemorrhage: Inhibitory effect of tranexamic acid (AMCA). Acta Neurol Scandinav 64: 29-64, 1981.

31. Fodstad H, Nilsson IM: Coagulation and fibrinolysis in blood and cerebrospinal fluid after aneurysmal subarachnoid hemorrhage: Effect of tranexamic acid (AMCA). Acta Neurochir (Wien) 56: 25-38, 1981.

32. Froin G: Les hemorragies sous-arachnoidiennes et le mecanisme de l'hematolyse en general, Paris, G. Steinheil 1904.

33. Fujii S, Fujitsu K: Experimental vasospasm in cultured arterial smooth-muscle cells. Part 1: Contractile and ultrastructural changes caused by oxyhemoglobin. J Neurosurg 69: 92-97, 1988.

34. Gomez DJ, Potts G, Deonarine V: Arachnoid granulations of the sheep. Structural and ultrastructural changes with varying pressure differences. Arch Neurol 30: 169-175, 1974.

35. Guggiari M, Dagreou F, Rivierez M, et al: Prediction of cerebral vasospasm. Value of Fibrinogen Degradation Products (FDP) in the cerebro-spinal fluid (CSF) for prediction of vasospasm following subarachnoid hemorrhage due to a ruptured aneurysm. Acta Neurochir 73: 25-33, 1984.

36. Hammes EM Jr: Reaction of the meninges to blood. Arch Neurol Psychiatry 52: 505-514, 1944.

37. Handa Y, Weir BKA, Nosko M, et al: The effect of timing of clot removal on chronic vasospasm in a primate model. J Neurosurg 67: 558-564, 1987.

38. Hara H, Nosko M, Weir B: Cerebral perivascular nerves in subarachnoid hemorrhage. A histochemical and immunohistochemical study. J Neurosurg 65: 531-539, 1986.

39. Hassler O, Fodstad H: Fibrinolytic activity in the walls of cerebral saccular aneurysms. Acta Neurochir 37: 49-55, 1977.

40. Hillman J, Von Essen C, Leszniewski W, et al: Significance of "ultra-early" rebleeding in subarachnoid hemorrhage. J Neurosurg 68: 901-907, 1988.

41. Hindersin P, Endler S: Alterations of coagulation and fibrinolysis in cerebrospinal fluid in subarachnoid hemorrhage. J Neurosurg Sci 30: 183-186, 1986.

42. Hindersin P, Heidrich R, Endler S: Hemostasis in cerebrospinal fluid. Basic concept of antifibrinolytic therapy of subarachnoid hemorrhage. Acta Neurochir Suppl 34, 1984.

43. Kanamaru K, Waga S, Kojima T, et al: Endothelium-dependent relaxation of canine basilar arteries. Part 2: Inhibition by

hemoglobin and cerebrospinal fluid from patients with aneurysmal subarachnoid hemorrhage. Stroke 18: 938-943, 1987.

44. Kassell NF, Torner JC, Adams HP, Jr: Antifibrinolytic therapy in the acute period following aneurysmal subarachnoid hemorrhage. Preliminary observations from the cooperative aneurysm study. J Neurosurg 61: 225-230, 1984.

45. Kasuya H, Shimizu T, Okada T, et al: Activation of the coagulation system in the subarachnoid space after subarachnoid hemorrhage: Serial measurement of fibrinopeptide A and bradykinin of cerebrospinal fluid and plasma in patients after subarachnoid hemorrhage. Acta Neurochir (Wien) 91: 120-125, 1988.

46. Kennady JC: Investigations of the early fate and removal of subarachnoid blood. Pacif Med Surg 75: 163-168, 1967.

47. Kida S, Yamashima T, Kubota T, et al: A light and electron microscopic and immunohistochemical study of human arachnoid villi. J Neurosurg 69: 429-435, 1988.

48. Kistler JP, Crowell RM, Davis KR, et al: The relation of cerebral vasospasm to the extent and location of subarachnoid blood visualized by CT scan: A prospective study. Neurology (N.Y.) 33: 424-426, 1983.

49. Kondziolka D, Bernstein M, Spiegel SM, et al: Symptomatic arterial luminal narrowing presenting months after subarachnoid hemorrhage and aneurysm clipping. J Neurosurg 69: 494-499, 1988.

50. Krueger C, Weir B, Nosko M, et al: Nimodipine and chronic vasospasm in monkeys: Part 2. Pharmacological studies of vessels in spasm. Neurosurgery 16: 137-140, 1985.

51. Kun-Yu Wu K, Jacobsen CD, Hoak JC: Plasminogen in normal and abnormal human cerebrospinal fluid. Arch Neurol 28: 64-66, 1973.

52. Kwaan HC: The role of fibrinolysis in disease processes. Semin Thromb Hemostasis 10: 71-79, 1984.

53. Kwak R, Niizuma H, Ohi T, et al: Angiographic study of cerebral vasospasm following rupture of intracranial aneurysms. Part 1: Timing of appearance. Surg Neurol 11: 257-262, 1979.

54. Lellum GR, Irvine TW Jr, Zervas NT: Cerebral vasoactivity of heme proteins *in vitro*. J Neurosurg 56: 777-783, 1982.

55. Lewis PJ, Weir BKA, Nosko MG, et al: Intrathecal nimodipine therapy in a primate model of chronic cerebral vasospasm. Neurosurgery 22: 492-500, 1988.

56. Marder VJ, Sherry S: Thrombolytic therapy: Current status. First of two parts. N Eng J Med 318: 1512-1520, 1988.

57. Marder VJ, Sherry S: Thrombolytic therapy: Current status. Second of two parts. N Eng J Med 318: 1585-1595, 1988.

58. Matthews WF, Frommeyer WB Jr: The *in vitro* behavior of erythrocytes in human cerebrospinal fluid. J Lab Clin Med 45: 508-515, 1955.

59. Maurice-Williams RS, Gordon YB, Sykes A: Monitoring fibrinolytic activity in the cerebrospinal fluid after aneurysmal

subarachnoid hemorrhage: a guide to the risk of rebleeding. J Neurol Neurosurg Psychiatry 43: 175-181, 1980.

60. Mayberg M, Akada T, Bark D: Cerebral arteriopathy and contractile changes after SAH are related to erythrocyte component of whole blood. Stroke 19: 130, 1988. (Abstr.)

61. Mullan S, Beckman F, Vailati G, et al: An experimental approach to the problem of cerebral aneurysm. J Neurosurg 21: 838-845, 1964.

62. Mullertz S: Fibrinolysis: An overview. Semin Thromb Hemostasis 10: 1-5, 1984.

63. Narayan RK, Narayan TM, Katz DA, et al: Lysis of intracranial hematomas with urokinase in a rabbit model. J Neurosurg 62: 580-586, 1985.

64. Nosko M, Schulz R, Weir B, et al: Effect of vasospasm on levels of prostacyclin and thromboxane A_2 in cerebral arteries of the monkey. Neurosurgery 22: 45-50, 1988.

65. Nosko M, Weir B, Krueger C, et al: Nimodipine and chronic vasospasm in monkeys, Part 1. Clinical and radiological findings. Neurosurgery 16: 129-136, 1985.

66. Nosko M, Weir BKA, Lundt A, et al: Effect of clot removal at 24 hours on chronic vasospasm after SAH in the primate model. J Neurosurg 66: 416-422, 1987.

67. Ohta T, Kajikawa H, Yoshikawa Y, et al: Cerebral vasospasm and hemoglobins: Clinical and experimental studies. In: Wilkins RH, ed. *Cerebral Arterial Spasm*. Baltimore: Williams & Wilkins, 1980: 166-172.

68. Okada Y, Tsuchiya T, Tada T, et al: Experimental study on plasminogen activator and trypsin inhibitor system in the cerebrospinal fluid and brain in cases of trauma and shock. Brain Nerve 18: 784, 1966.

69. Okamoto S, Handa H, Handa Y: Hemolysate-induced release of prostaglandinlike substances from the canine cerebral arteries. Surg Neurol 23: 421-424, 1985.

70. Osaka K: Prolonged vasospasm produced by the breakdown products of erythrocytes. J Neurosurg 47: 403-411, 1977.

71. Pang D, Sclabassi RJ, Horton JA: Lysis of intraventricular blood clot with urokinase in a canine model: Part 3. Effects of intraventricular urokinase on clot lysis and post hemorrhagic hydrocephalus. Neurosurgery 19: 553-572, 1986.

72. Paoletti P, Gaetani P, Grignani G, et al: CSF leukotriene C_4 following subarachnoid hemorrhage. J Neurosurg 69: 488-493, 1988.

73. Pennica D, Holmes WE, Kohr WJ, et al: Cloning and expression of human tissue-type plasminogen activator cDNA in E-Coli. Nature 301: 214-221, 1983.

74. Peterson EW, Choo SH, Lewis AJ, et al: Lysis of blood clot and experimental treatment of subarachnoid hemorrhage. In: Wilkins RH, ed. *Cerebral Arterial Spasm*. Baltimore: Williams & Wilkins, 1980: 625-627.

75. Pimstone NR, Tenhunen R, Seitz P, et al: The enzymatic degradation of hemoglobin to biopigments by macrophages. J Exp Med 133: 1264-1268, 1971.

76. Porter JM, Acinapura AJ, Capp JP, et al. Fibrinolysis in the central nervous system. Neurology 19: 47-52, 1969.

77. Porter JM, Acinapura AJ, Capp JP, et al: Fibrinolytic activity in the spinal fluid and meninges. Surg Forum 17: 425-427, 1966.

78. Roost K, Pimstone NR, Diamond I, et al: The formation of cerebrospinal fluid xanthochromia after subarachnoid hemorrhage. Enzymatic conversion of hemoglobin to bilirubin by the arachnoid and choroid plexus. Neurology 22: 973-977, 1972.

79. Seifert V, Stolke D, Kunz U, et al: Influence of blood volume on cerebrospinal fluid levels of arachidonic acid metabolites after subarachnoid hemorrhage: Experimental study on the pathogenesis of cerebral vasospasm. Neurosurgery 23: 313-321, 1988.

80. Shiobara R, Kawase T, Ebato K, et al: "Scavengery surgery" for subarachnoid hemorrhage (II) continuous ventriculo-cisternal perfusion using artificial cerebrospinal fluid with urokinase. In: Auer L, ed. *Timing of Aneurysm Surgery*. Berlin: de Gruyter, 1985: 365-372.

81. Sloan MA: Thrombolysis and stroke. Past and future. Arch Neurol 44: 748-768, 1987.

82. Steinke DE, Weir BKA, Findlay JM, et al: A trial of the 21-aminosteroid U74006F in a primate model of chronic cerebral vasospasm. Neurosurgery 24: 179-186, 1989.

83. Steinmetz H, Grote E: The fibrinolytic activity of cerebrospinal fluid after subarachnoid hemorrhage. Neurol Res 5: 59-65, 1983.

84. Taneda M: Effect of early operation for ruptured aneurysms on prevention of delayed ischemic symptoms. J Neurosurg 57: 622-628, 1982.

85. Takashima S, Koga M, Tanaka K: Fibrinolytic activity of human brain and cerebrospinal fluid. Br J Exp Path 50: 533-539, 1969.

86. Toda N, Shimizu K, Ohta T: Mechanism of cerebral arterial contraction induced by blood constituents. J Neurosurg 53: 312-322, 1980.

87. Todd AS: Localization of fibrinolytic activity in tissues. Brit Med Bull 20: 210-219, 1964.

88. Tourtellotte WW, Metz LN, Bryan ER, et al: Spontaneous subarachnoid hemorrhage: Factors affecting the cerebral spinal fluid. Neurology 14: 301-306, 1964.

89. Tovi D, Nilsson IM, Thulin CA: Fibrinolytic activity of the cerebrospinal fluid after subarachnoid hemorrhage. Acta Neurol Scandinav 49: 1-9, 1973.

90. Van Hinsberg VWM, Bertina RM, van Wijgaarden A, et al: Activated protein C decreases plasminogen activator-inhibitory activity in endothelial cell-condition medium. Blood 65: 444-451, 1985.

91. Vermeulen M, van Vliet HHDM, Lindsay KW, et al: Source of fibrin/fibrinogen degradation products in the CSF after subarachnoid hemorrhage. J Neurosurg 63: 573-577, 1985.

92. Vermeulen M, Lindsay KW, Murray GD, et al: Antifibrinolytic treatment in subarachnoid hemorrhage. N Engl J Med 311: 432-437, 1984.

93. Verstraete M, Collen D: Thrombolytic therapy in the 80's. Blood 67: 1529-1541, 1986.

94. Verstraete M: New thrombolytic drugs in acute myocardial infarction: Theoretical and practical considerations. Circulation 76 (Suppl II): 31-38, 1987.

95. Weir B, Grace M, Hanson J, et al: Time course of vasospasm in man. J Neurosurg 48: 173-178, 1978.

96. Weir B: *Aneurysms Affecting the Nervous System*. Baltimore: Williams & Wilkins, 1987: 246-250.

97. Weir B: Antifibrinolytics in subarachnoid hemorrhage. Do they have a role? No. Arch Neurol 44: 116-118, 1987.

98. White RP: Overview of the pharmacology of vasospasm. In: Wilkins RH, ed. *Cerebral Arterial Spasm*. Baltimore: Williams & Wilkins, 1988: 229-236.

99. Zervas NT, Liszczak TM, Mayberg MR, et al: Cerebral spinal fluid may nourish cerebral vessels through pathways in the adventitia that may be analogous to systemic vasa vasorum. J Neurosurg 56: 475-481, 1982.

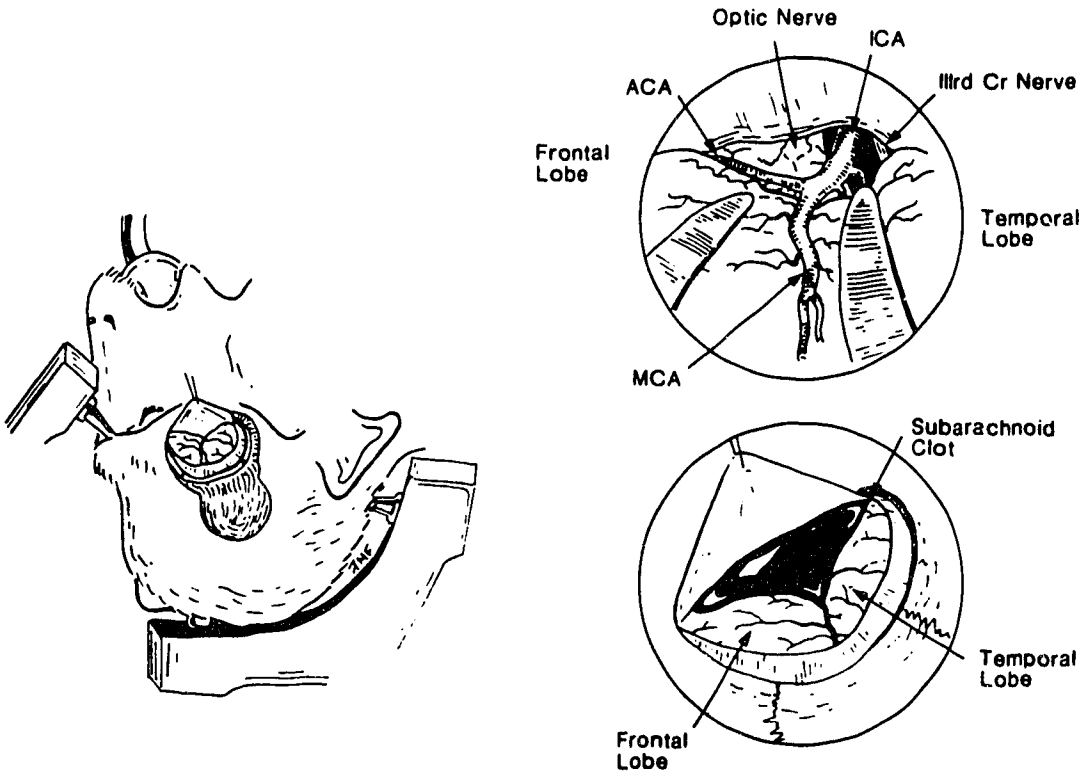
Table V-1
Change in clot (right)-side vessel caliber and remaining clot in the dosage groups*

	Dosage Group †			
Vessel ¶	0.125 mg	0.25 mg	0.5 mg	0.75 mg
C3	-14 ± 16	-11 ± 9	-1 ± 14	+1 ± 4
C4	-20 ± 20	-22 ± 13 (P=0.043)	+1 ± 18	-1 ± 7
A1	-30 ± 34	-38 ± 19	-7 ± 18	+15 ± 15
MCA	-22 ± 21	-25 ± 13 (P=0.035)	-6 ± 15	+4 ± 9
Weight of Remaining Clot (gms)	0.2250	0.1375	0.0675	0
(mean ± SD)	± 0.03	± 0.12	± 0.12	

*Percentage change in vessel caliber (mm) day 7 compared to day 0 for right (clot)-sided cerebral arteries. Percentages indicate means ± standard deviations. Where the change is significant, the P value is shown in parentheses.

† 4 animals in each group.

¶ C3 = extradural internal carotid artery; C4 = intradural internal carotid artery; A1 = precommunicating segment of anterior cerebral artery; MCA = sphenoidal segment of middle cerebral artery



PRIMATE MODEL OF SAH AND VSP

Figure V-1
Primate model of subarachnoid hemorrhage and chronic cerebral vasospasm. With the animals under anesthesia and controlled ventilation a right frontotemporal craniectomy is performed (left). After arachnoid is dissected free of the major anterior cerebral vessels (upper right) the subarachnoid space is filled with autogenous arterial blood clot (lower right).

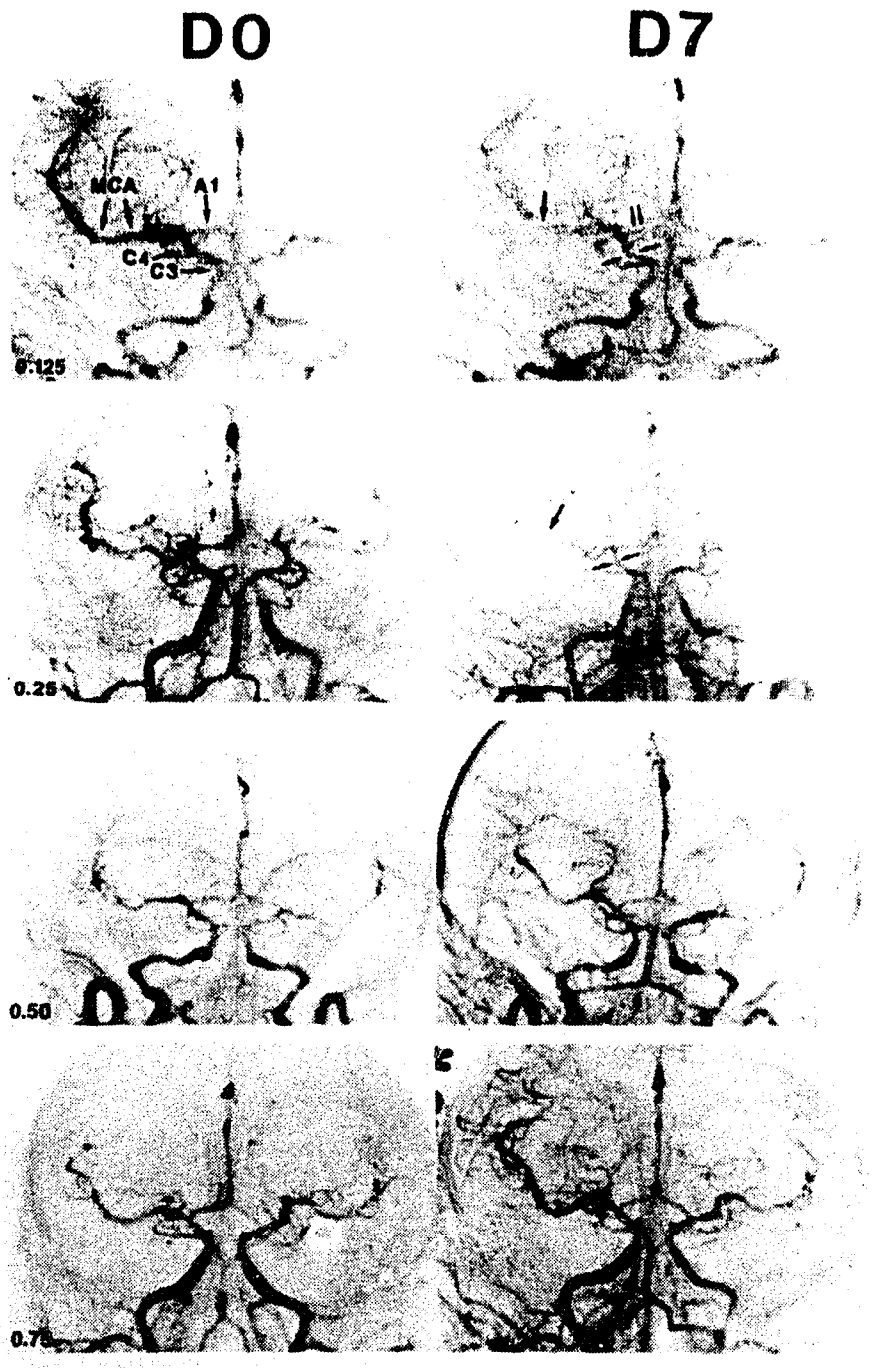


Figure V-2
Examples of day 0 (pre-SAH, left) and day 7 (post-SAH, right) anteroposterior cerebral angiograms from a monkey in each dosage group. The right (clot-side) middle cerebral (MCA), proximal anterior cerebral (A1), extradural internal carotid (C3) and intradural internal carotid (C4) arteries are labeled in the day 0 angiogram of the 0.125 mg dosage group example. Note the presence of vasospasm in these vessels after 7 days in the 0.125 mg and 0.25 mg examples (arrows), in contrast to the normal appearing day 7 angiograms in the 0.50 and 0.75 mg group examples.

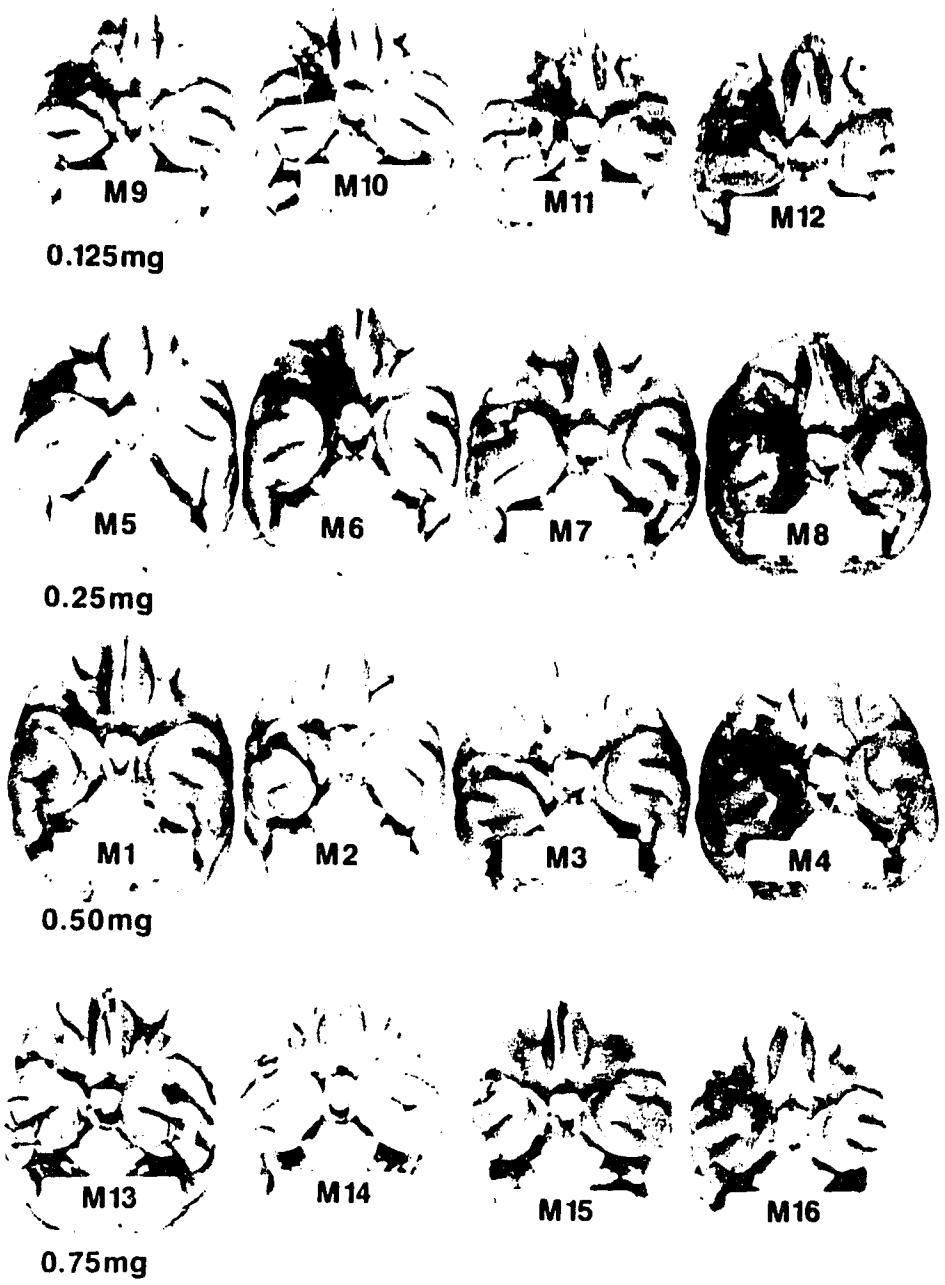


Figure V-3
All eight monkeys in the 0.125 and 0.25 mg dosage groups had gross subarachnoid blood clot remaining on the ventral surface of the brain at necropsy (Monkeys 5 to 12), and some clot was present in 2 animals in the 0.50 mg group (Monkeys 2 and 4). All 4 monkeys in the 0.75 mg group were free of clot (Monkeys 13 to 16).

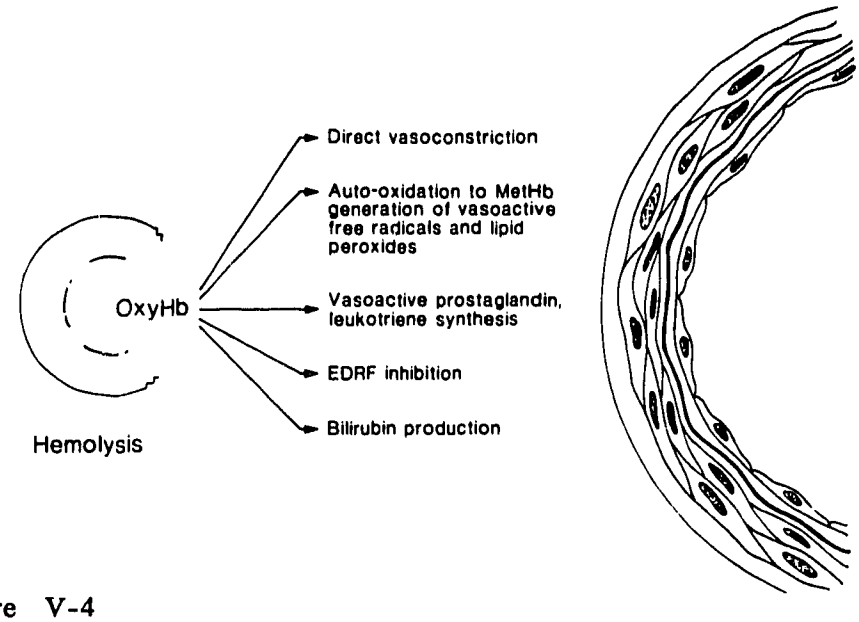


Figure V-4
Hemolyzing erythrocytes entrapped in periarterial subarachnoid clot expose the external cerebral arterial wall to a high concentration of oxyhemoglobin (OxyHb), which is the principal mediator of vasospasm. A number of mechanisms have been proposed to explain the effect of OxyHb on the vessel wall, including: 1. a direct vasoconstrictive activity, 2. the generation of vasoactive and vasotoxic free radical species following auto-oxidation of OxyHb to methemoglobin (MetHb), 3. the generation of vasoactive prostaglandins or leukotrienes, and 4. the production of bilirubin, which may also be vasogenic (see text for references).

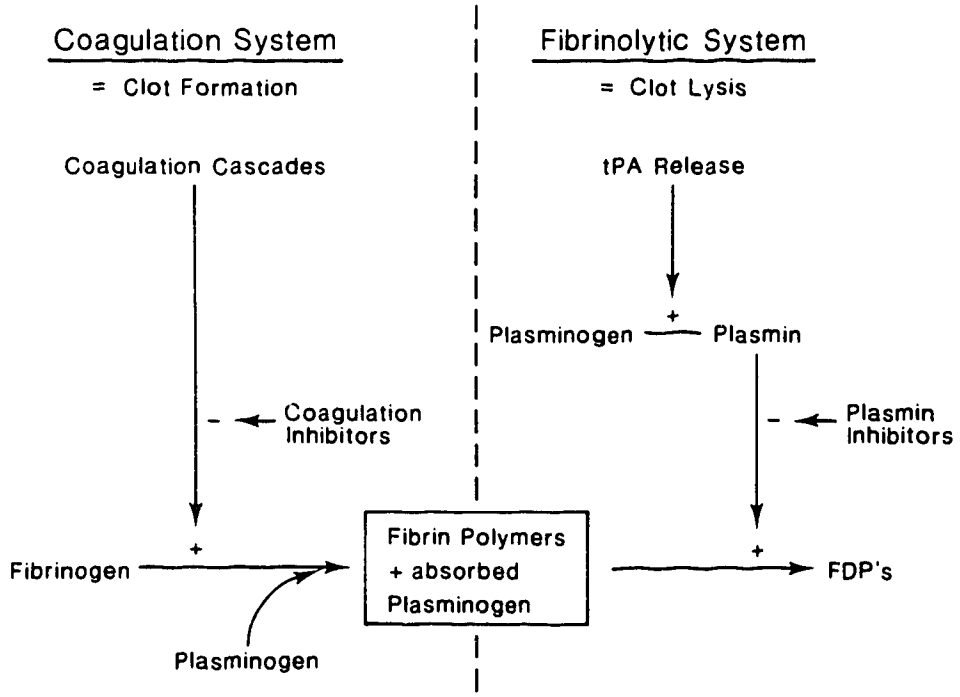


Figure V-5
A comparison of the coagulation (left) and fibrinolytic (right) systems.

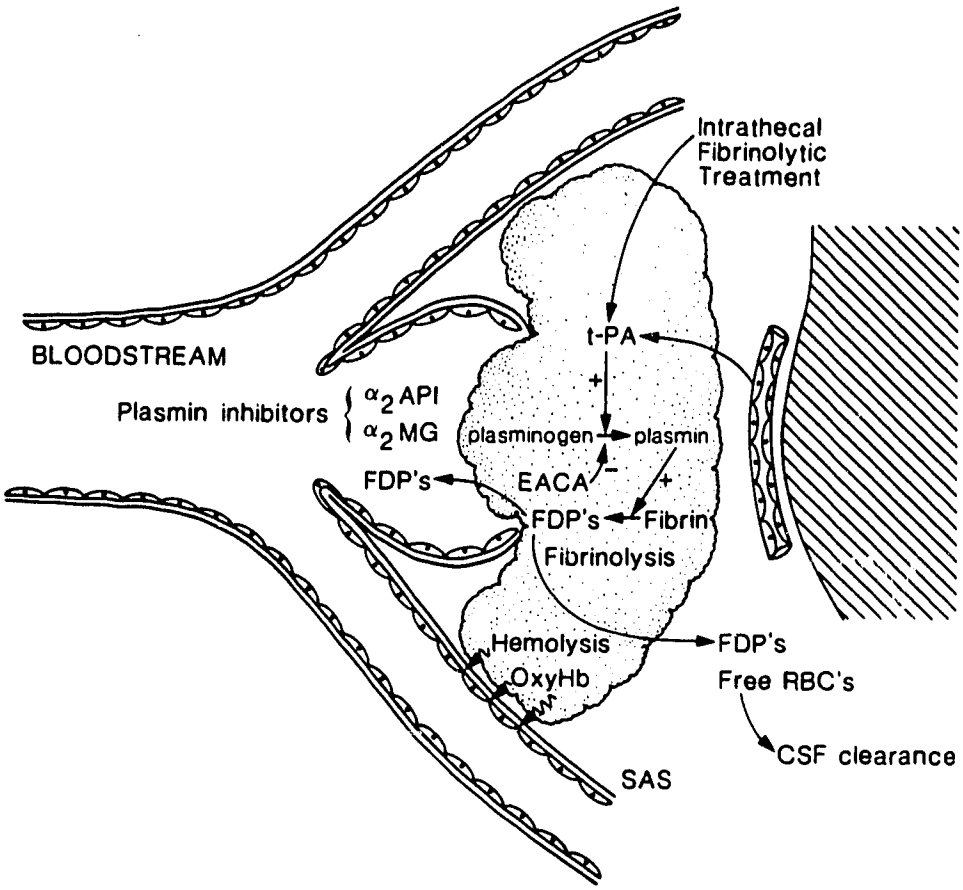


Fig. V-6
Fibrinolysis in the subarachnoid space (SAS) is mediated by tissue plasminogen activator (t-PA) released mainly from endothelial cells of irritated blood vessels in the leptomeninges. T-PA binds to fibrin and converts plasminogen into plasmin, which in turn proceeds to split fibrin into various degradation products (FDPs). Plasmin inhibitors, including α2-antiplasmin (α2-API) and α2-macroglobulin (α2-MG), are absent in the cerebrospinal fluid. Antifibrinolytic agents, such as epsilon aminocaproic acid (EACA), inhibit plasmin formation and hence retard fibrinolysis. The goal of intrathecal fibrinolytic treatment is to augment t-PA supplies to the subarachnoid clot and promote rapid fibrinolysis, which would allow clearance of red blood cells (RBCs) from the basal cisterns prior to their hemolysis, release of oxyhemoglobin (OxyHb), and vasogenic effect on the cerebral arterial wall. In order to prevent aneurysmal rebleeding, the aneurysm neck will have to be clipped prior to fibrinolytic treatment.

CHAPTER SIX

THE EFFECT OF TIMING OF INTRATHECAL FIBRINOLYTIC THERAPY ON CEREBRAL VASOSPASM IN A PRIMATE MODEL OF SUBARACHNOID HEMORRHAGE

SUMMARY

The effect of intrathecal tissue plasminogen activator administered at times from 0 to 72 hours after subarachnoid hemorrhage (SAH) on the development of cerebral vasospasm (VSP) in primates was examined. Thirty monkeys were randomized into one of 5 equal groups: a control group which underwent SAH alone, and 0-, 24-, 48- and 72-hour treatment groups which received 0.75 mg of tissue plasminogen activator at those times after baseline cerebral angiography and right-sided SAH. Seven days later angiography was repeated and the animals sacrificed. One animal in the 72-hour group developed a delayed ischemic deficit on day 7 after SAH. In the control and 72-hour groups significant VSP occurred in most of the major, right-sided cerebral arteries ($p<0.05$), but no significant VSP developed in the 0-, 24- and 48-hour groups. While a large subarachnoid clot remained in the control animals, most clot had been cleared in all treatment groups. Clearance of subarachnoid hematoma with intrathecal tissue plasminogen activator within 72 hours of SAH is effective in preventing VSP in primates.

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INTRODUCTION

Intrathecally administered human recombinant tissue plasminogen activator (rt-PA) promotes rapid, safe clearance of subarachnoid hematoma in primates and prevents the development of VSP [4,5,6]. The present study was designed to evaluate the effect of intrathecal rt-PA administration at various times from 0 to 72 hours after SAH on the development of VSP, in order to determine whether there is a critical time after which this treatment is ineffective.

MATERIALS AND METHODS

Details of the procedures for microsurgical exposure of the basal subarachnoid cisterns, induction of SAH, cerebral angiography and animal sacrifice used in our primate model have been published previously [4], and will be outlined here. The protocol was evaluated and approved by the University of Alberta's Animal Ethics Review Committee, and experiments were conducted with strict adherence to the standards of the Canadian Council on Animal Care.

Thirty female Cynomolgous monkeys (Macaca fascicularis), weighing between 3.0 and 4.5 kg were divided into 5 groups of 6 (fig. VI-1). All groups underwent experimental SAH. The first group, which served as control, received no fibrinolytic treatment. The remaining groups received 0.75 mg of intrathecal rt-PA at the following times: at the time of SAH (0-hour group), 24 hours after SAH (24-hour group), 48 hours after SAH (48-hour group), and 72 hours after SAH (72-hour group). The rt-PA was administered either directly intraoperatively (0-hour group) or via a

subarachnoid catheter connected to a subcutaneous injection portal (all other groups) into the region of the Sylvian fissure. The rt-PA was suspended in a sustained-release hyaluronidase gel preparation (1 mg/ml), which goes into solution and releases rt-PA over a 1 to 2 day period (Genentech Inc., South San Francisco, CA, USA).

After sedation (ketamine hydrochloride, 10 mg/kg, intramuscular) and endotracheal intubation the animals underwent baseline cerebral angiography. Ventilation was adjusted to maintain the $PaCO_2$ at or near 40 mmHg as ascertained by continuous end-tidal CO_2 monitoring (Patient Monitor 78356A, Hewlett Packard, Federal Republic of Germany) and checked by arterial blood gas measurement. Paralysis (gallamine, 2 mg/kg, intravenous) and general anesthesia (fentanyl, 1 mg/kg, and sodium pentobarbital 26 mg/kg, intravenous) was then established, and the $PaCO_2$ was lowered to approximately 30 mmHg by adjusting ventilation. The animals' heads were placed in three-point pin fixation and a right fronto-temporal craniectomy was performed. With the aid of an operating microscope the right basal subarachnoid cisterns were opened and the cerebral vessels dissected free of arachnoid. All animals had 4.25 ml of autogenous blood clot injected into the right subarachnoid cisterns in contact with exposed cerebral arteries. The 0-hour group of animals then had rt-PA injected intraoperatively. Dura was closed, and with the exception of the control and 0-hour groups, a subarachnoid catheter was inserted with its tip in the Sylvian fissure. Temporalis muscle was closed, followed by skin, with the subarachnoid catheter terminating at an injection portal left in the subcutaneous space.

At 24, 48 and 72 hours after experimental SAH, monkeys from appropriate groups were sedated (ketamine hydrochloride, 5 mg/kg, intramuscular) and injected via the subcutaneous portal with 0.75 mg of gel rt-PA. The control and 0-hour groups underwent no further intervention. All animals were followed for the development of any complications or delayed neurological deficits.

Seven days after SAH all animals underwent repeat cerebral angiography and were killed by pentobarbital overdose (pentobarbital, 50 mg/kg, intravenous) and exsanguination. The brains were removed and photographed, and any remaining subarachnoid clot removed and weighed.

At the conclusion of the experiments all baseline and day 7 angiogram pairs had their identities covered and were pooled and shuffled so that they could be examined in a blinded fashion. Cerebral vessels were measured 4 times with a calibrated optical micrometer, and a mean value obtained. The arteries on each of the baseline and day 7 pair of angiograms were measured bilaterally at a similar point on the following vessels: extradural internal carotid artery (C3), intradural internal carotid artery (C4), precommunicating segment of the anterior cerebral artery (A1), sphenoidal segment of the middle cerebral artery (MCA), azygous distal anterior cerebral artery, the first part of the posterior cerebral artery (PCA), and the basilar artery (BA).

All data were coded, entered into a computer and edited. Data for angiographic vessel caliber change within treatment groups between baseline and day 7 were compared by a paired t-test, and intergroup comparisons were made with an analysis of variance. If

17. Wilkins RH: Attempted prevention or treatment of intracranial arterial spasm: a survey. Neurosurgery 6: 198-210, 1980.

18. Wilkins RH: Attempted prevention or treatment of intracranial arterial spasm: an update. Neurosurgery 18: 808-825, 1986.

Table IV-1
Values for measured indices in the 2 animal groups*

Parameter	Day 0		Day 7	
	Placebo Group	rt-PA Group	Placebo Group	rt-PA Group
No. of Monkeys	8	8	8	8
Body Weight (kg)	3.46 ± 0.19	3.45 ± 0.30	3.43 ± 0.24	3.37 ± 0.28
MABP (mmHg)	104 ± 7	106 ± 9	109 ± 6	104 ± 8
Heart Rate (min^{-1})	114 ± 7	108 ± 5	101 ± 6	106 ± 4
$PaCO_2$ (mmHg)	40 ± 5	40 ± 5	39.5 ± 5	39.5 ± 5
Volume of SAH (ml)	6	6	-	-
Weight of Remaining Clot (gm)	-	-	1.13 ± .07	0.01 ± .02
Vessel Caliber (mm)				
C3-R	2.02 ± .06	1.79 ± 0.33	1.81 ± 0.17	1.82 ± 0.33
C3-L	1.98 ± 0.10	1.77 ± 0.37	1.29 ± 0.60	1.82 ± 0.31
C4-R	1.73 ± 0.29	1.38 ± 0.32	1.05 ± 0.27	1.41 ± 0.30
C4-L	1.72 ± 0.23	1.36 ± 0.35	0.85 ± 0.45	1.40 ± 0.30
A1-R	0.89 ± 0.05	0.76 ± 0.19	0.53 ± 0.17	0.77 ± 0.18
A1-L	0.93 ± 0.08	0.75 ± 0.16	0.53 ± 0.21	0.75 ± 0.11
A2	1.10 ± 0.20	0.91 ± 0.11	1.02 ± 0.16	0.95 ± 0.12
MCA-R	0.98 ± 0.08	0.90 ± 0.24	0.38 ± 0.29	0.85 ± 0.14
MCA-L	1.02 ± 0.11	0.96 ± 0.11	0.29 ± 0.27	0.89 ± 0.10
PCA-R	1.02 ± 0.10	1.02 ± 0.09	0.80 ± 0.30	1.07 ± 0.05
PCA-L	1.01 ± 0.10	1.03 ± 0.11	0.97 ± 0.10	1.05 ± 0.13
BA	1.43 ± 0.14	1.30 ± 0.12	1.31 ± 0.24	1.33 ± 0.12

*Values are means ± standard deviations

MABP = Mean arterial blood pressure; C3 = extradural internal carotid artery; C4 = intradural internal carotid artery; A1 = precommunicating segment of anterior cerebral artery; A2 = distal azygous anterior cerebral artery; MCA = sphenoidal segment of middle cerebral artery; PCA = posterior cerebral artery; BA = basilar artery; VA = vertebral artery; SAH = subarachnoid hemorrhage

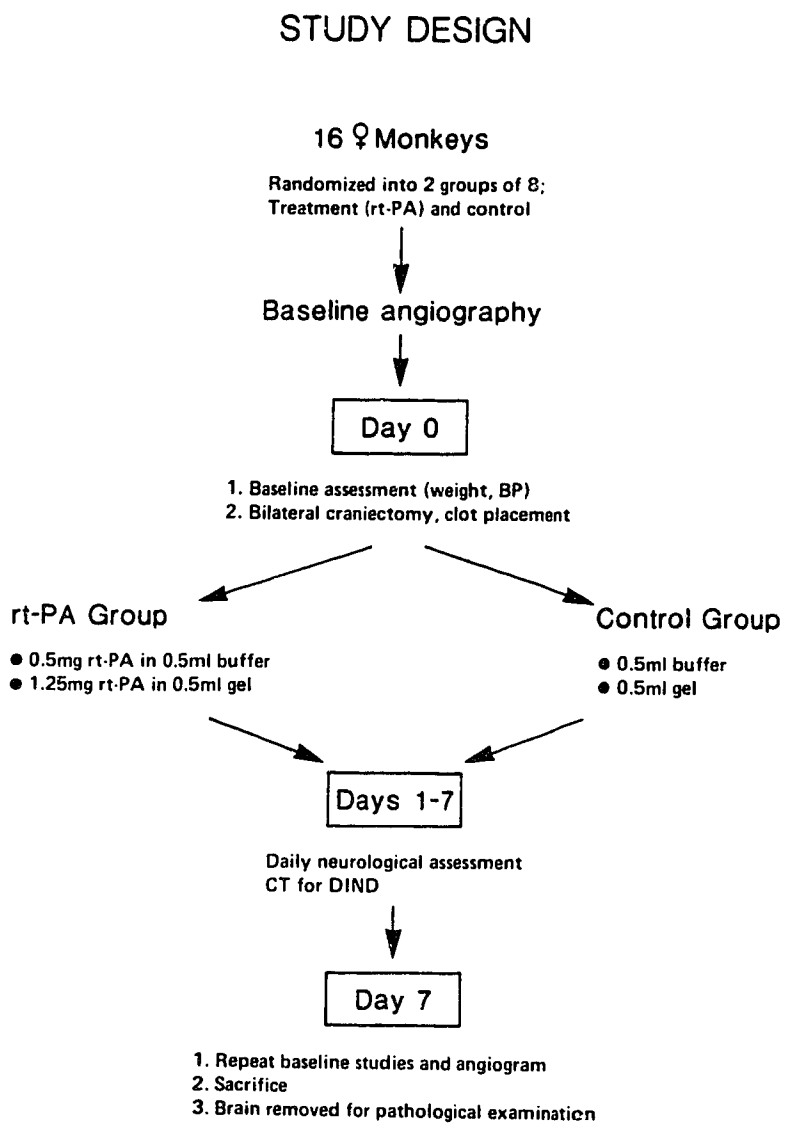

Figure IV-1
Study design of randomized, placebo-controlled trial of unilateral, intraoperative administration of rt-PA suspension plus sustained-release gel after a bilateral subarachnoid hemorrhage in the primate model.

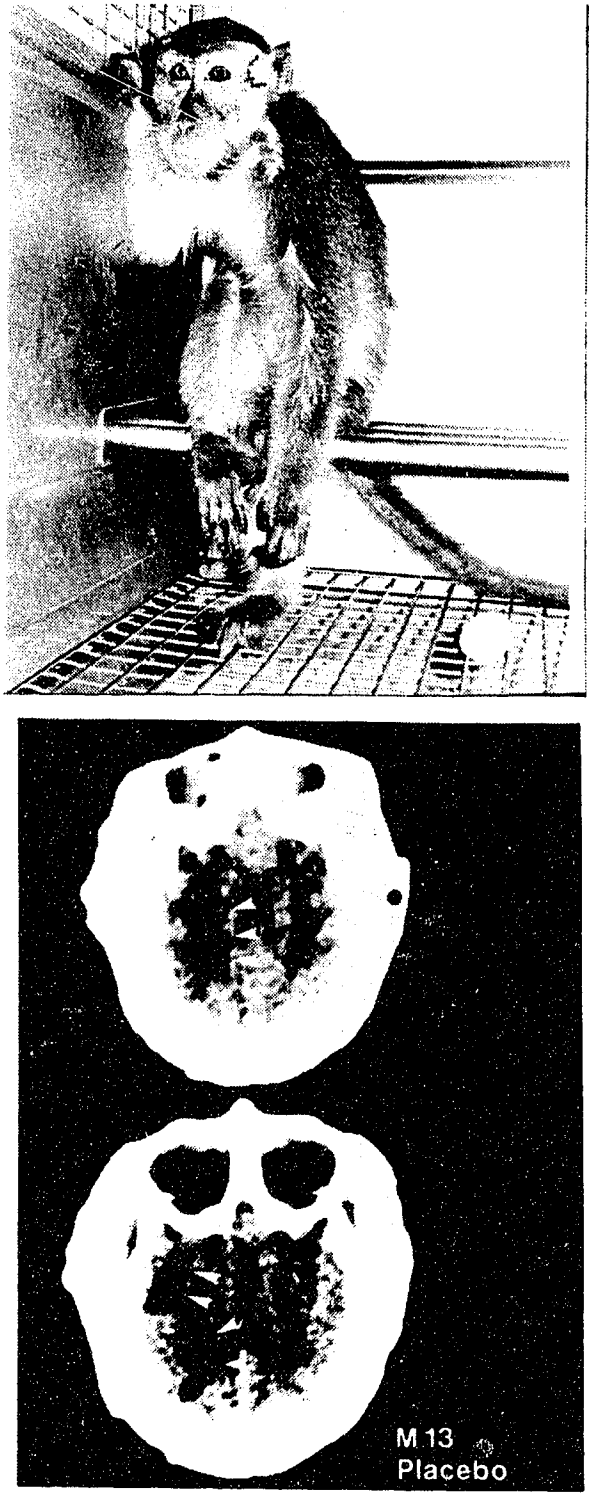

Figure IV-2
Monkey 13 (placebo group) developed a left hemiparesis on day 5 after subarachnoid hemorrhage (A); a computerized tomographic scan of monkey 13 on day 6 demonstrated cerebral edema in the right frontal and parietal lobes (arrows) (B).

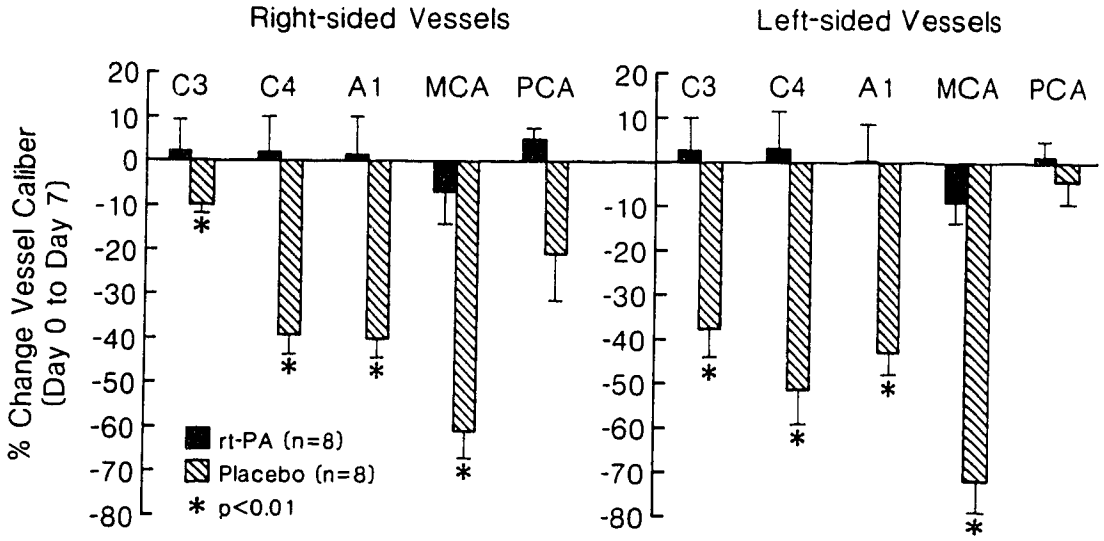

Figure IV-3
Bar graph depicting the percentage change from baseline in the cerebral vessel diameters on the right and left sides in the two treatment groups. Percentages indicate means ± standard error of the means. C3 = extradural internal carotid artery; C4 = intradural internal carotid artery; A1 = precommunicating segment of the anterior cerebral artery; MCA = sphenoidal segment of middle cerebral artery; PCA = posterior cerebral artery.

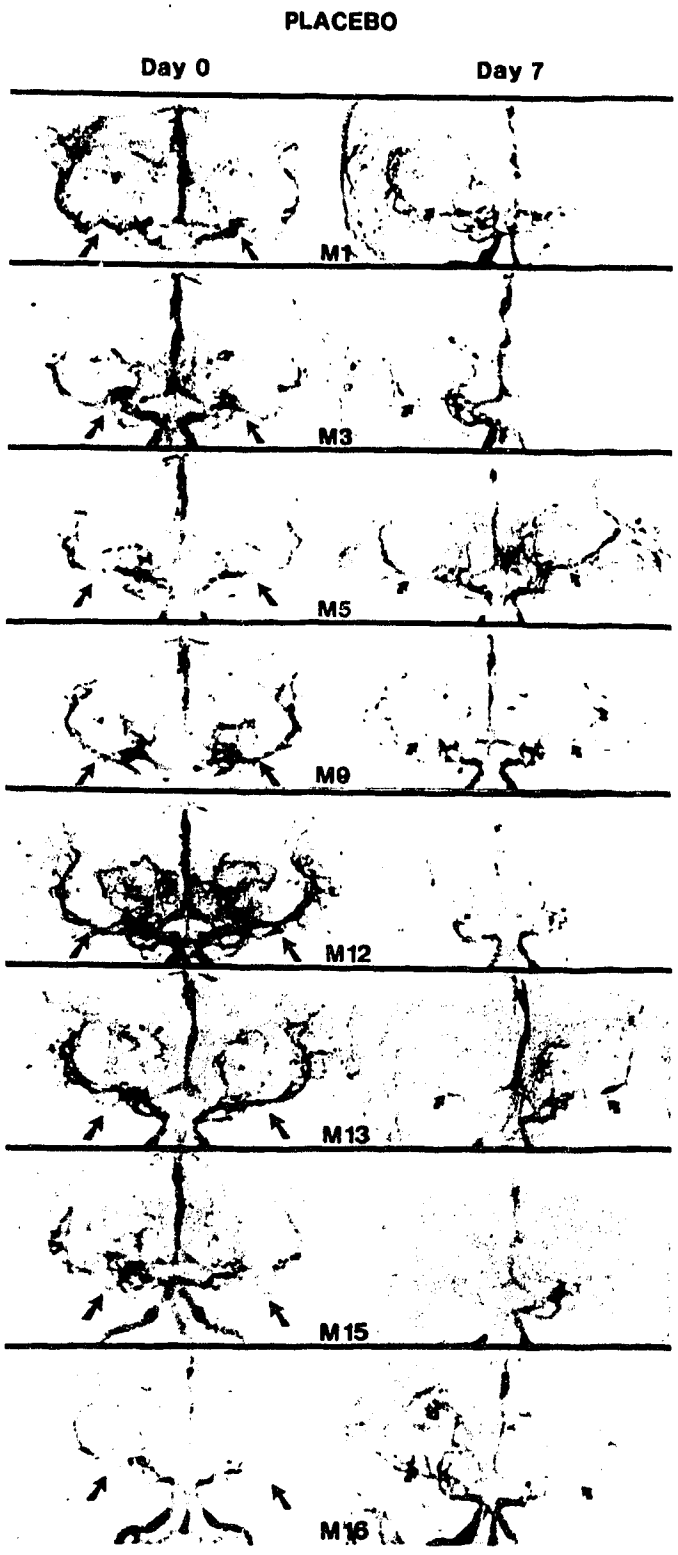

Figure IV-4
Cerebral angiograms taken on day 0 (pre-SAH), and day 7 (post-SAH), placebo group. Arrows indicate the middle cerebral arteries. Note the presence of vasospasm in the anterior cerebral vessels bilaterally at day 7. Severe vasospasm in the left carotid distributions of Monkeys 1 (M1) and 3 (M3) has markedly delayed filling of the intracranial vessels.

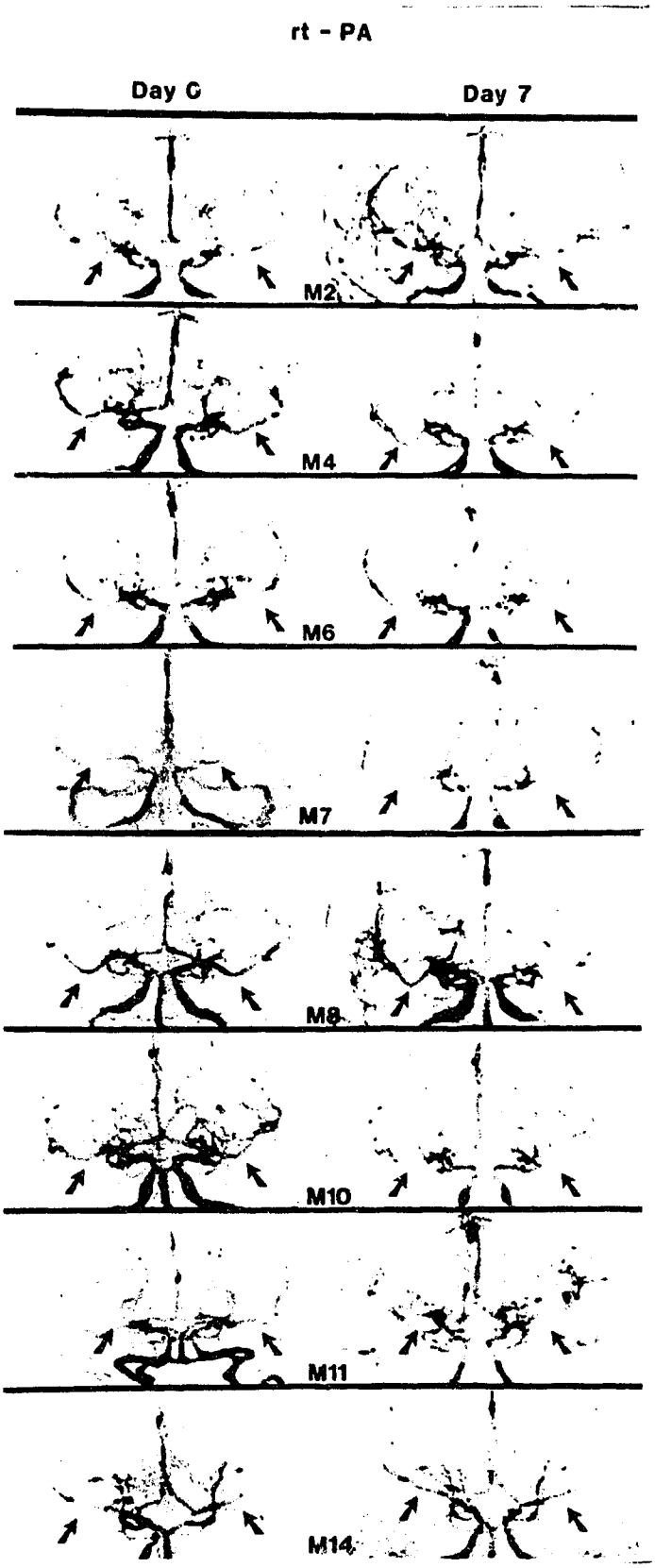

Figure IV-5
Cerebral angiograms taken on day 0 (pre-SAH) and day 7 (post-SAH), rt-PA group. Arrows indicate the middle cerebral arteries. Only mild narrowing of several middle cerebral arteries is seen on day 7 (not significant).

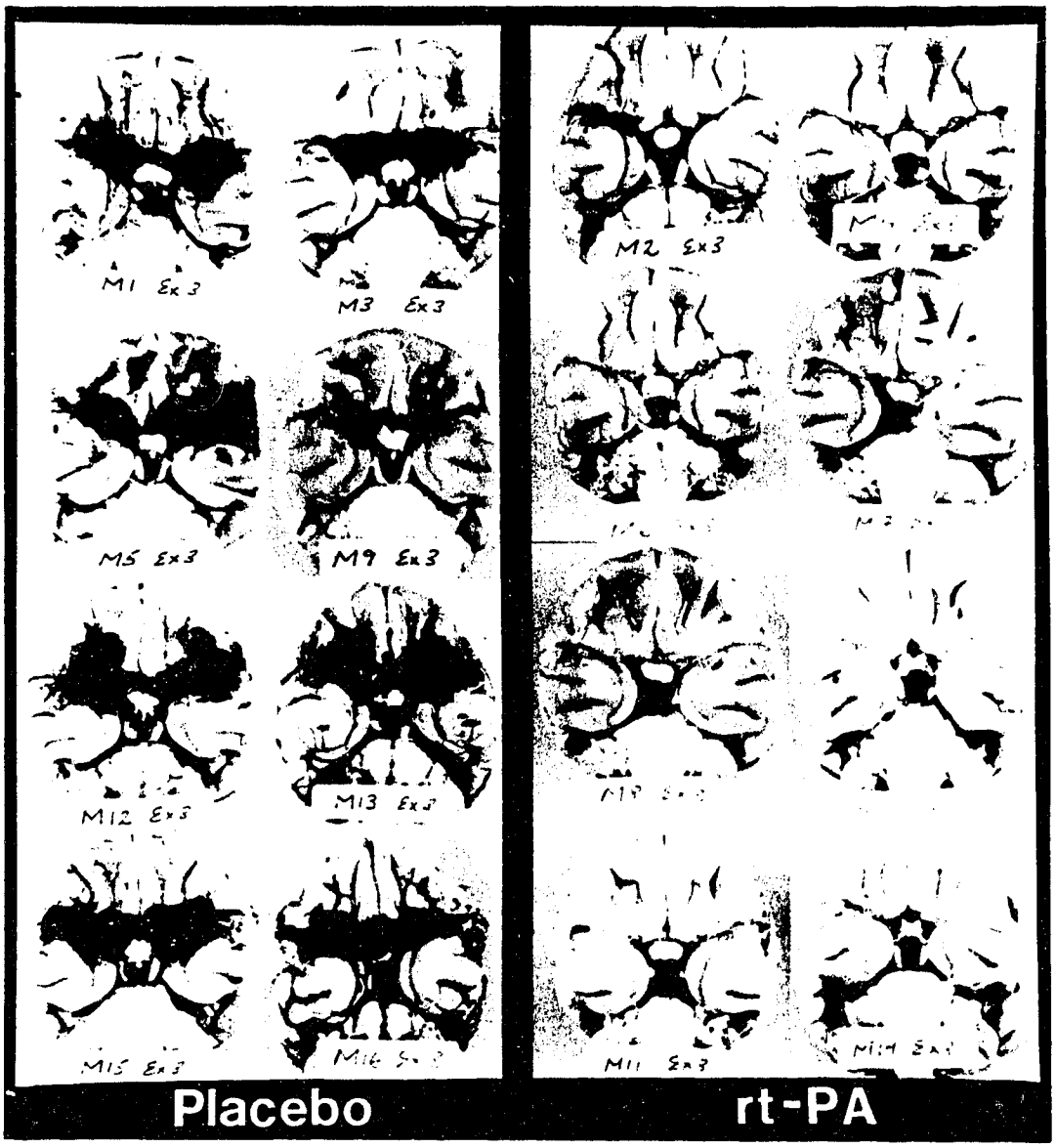

Figure IV-6
Ventral aspect of brains from monkeys in the placebo and rt-PA treated groups. Note the remaining subarachnoid blood present bilaterally on the placebo group.

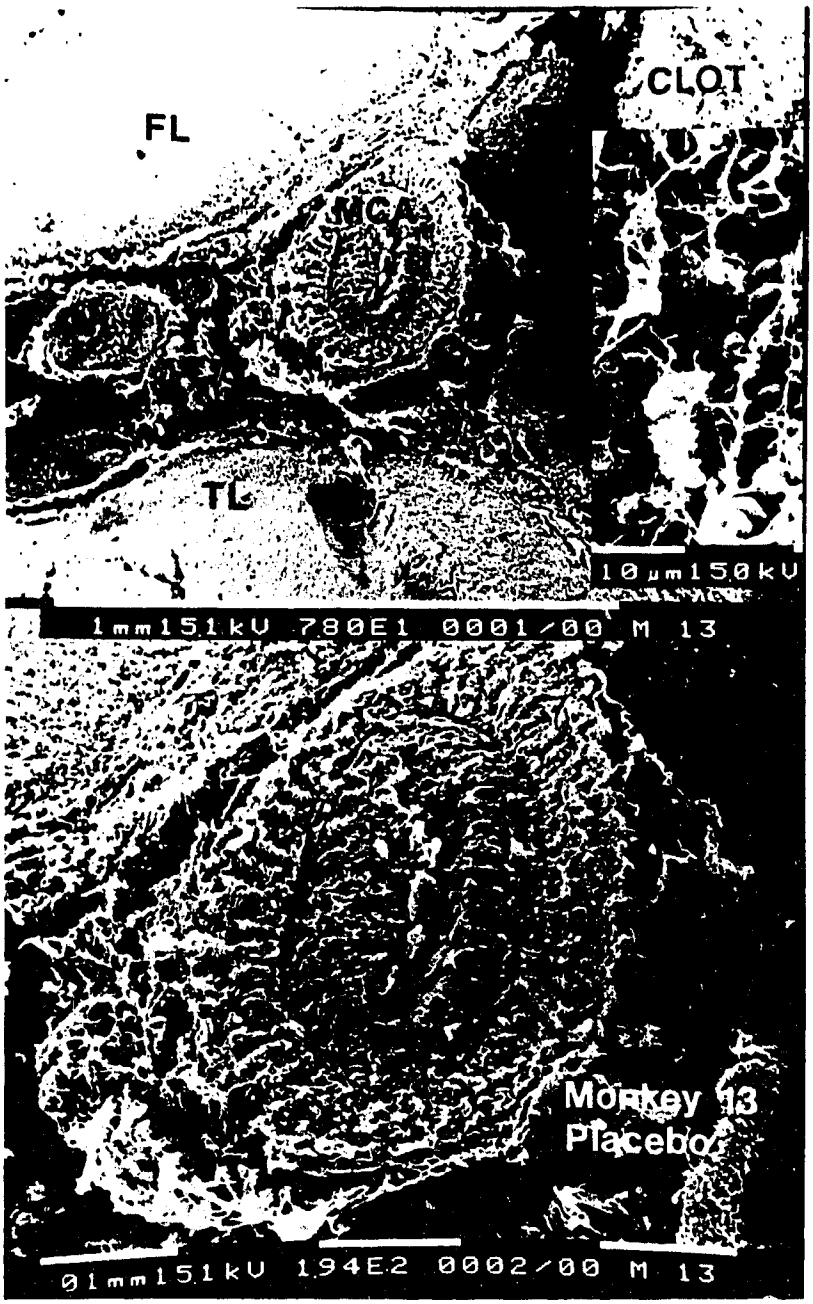

Figure IV-7
Scanning electron micrographs of the right middle cerebral artery from Monkey 13 (placebo group). Severe vasospasm with intraluminal thrombosis is present. In the upper micrograph the subarachnoid clot, which has separated from the vessel adventitia during fixation, can be seen. FL = frontal lobe, TL = temporal lobe, MCA = middle cerebral artery.

CHAPTER FIVE

INTRATHECAL FIBRINOLYTIC THERAPY AFTER SUBARACHNOID HEMORRHAGE: DOSAGE STUDY IN A PRIMATE MODEL AND REVIEW OF THE LITERATURE

SUMMARY

Because of the naturally low fibrinolytic activity of cerebrospinal fluid CSF many erythrocytes entrapped in subarachnoid blood clot undergo hemolysis *in situ*, releasing vasogenic oxyhemoglobin (OxyHb) in high concentrations around the basal cerebral arteries. In order to promote more rapid clearance of erythrocytes from the basal subarachnoid cisterns we are currently investigating intrathecal fibrinolytic therapy with human, recombinant, tissue plasminogen activator (rt-PA) in a primate model of subarachnoid hemorrhage (SAH) and cerebral vasospasm (VSP). In the present study 16 monkeys were divided into 4 groups of 4, and each group received a different dose of sustained-release gel rt-PA at the time of experimental SAH. Cerebral angiography seven days later showed that whereas no VSP occurred in the groups receiving 0.5 or 0.75 mg of rt-PA, mild to moderate VSP occurred in the groups receiving 0.125 or 0.25 mg of rt-PA. Analysis of the combined 2 smaller dosage groups revealed significant ($p<0.05$) reduction of lumen caliber in the clot-side internal carotid (C3 and C4), proximal anterior cerebral (A1) and

A version of this chapter has been published. Findlay JM, Weir BKA, Kanamaru K, Grace M, Gordon P, Baughman R, Howarth A. Can J Neurol Sci 16: 28-40, 1989.

middle cerebral (MCA) arteries. Gross subarachnoid clot remained in all of the animals in the 0.125 and 0.25 mg dose groups, in 2 of the animals in the 0.5 mg dose group, and none of the animals in the 0.75 mg dose group. It was concluded that 0.75 mg of gel rt-PA is sufficient to completely lyse a 4.25 ml SAH and prevent VSP in our primate model. The literature on fibrinolysis and erythrocyte clearance in CSF is reviewed.

INTRODUCTION

A major complication of aneurysmal subarachnoid hemorrhage (SAH) is a form of cerebral arterial narrowing most commonly referred to as vasospasm (VSP). Peaking in incidence and severity approximately one week after aneurysm rupture, VSP and the resultant reduction in cerebral blood flow (CBF) may lead to ischemia and infarction in the territories of the affected arteries. The development of VSP depends upon the presence of thick blood clot in the subarachnoid cisterns lying in sustained contact with the external cerebral arterial wall. Lysis of erythrocytes within the clot liberates oxyhemoglobin (OxyHb), which is a principal mediator of the pathological process. The primate model of SAH and VSP developed at the University of Alberta closely mimics the human condition and has proved valuable in exploring the pathogenesis [23,24,38,50,64], and testing potential treatments [25,37,55,65,66,82], of VSP. One promising treatment currently under investigation is intrathecal fibrinolytic therapy with human, recombinant, tissue plasminogen activator (rt-PA) [26,27]. The administration of this fibrinolytic enzyme into cerebrospinal fluid (CSF) has been effective in promoting rapid clearance of

subarachnoid hematoma from the basal cisterns and thereby preventing the development of VSP. Here we report the results of a dosage study using a new, sustained-release formulation of rt-PA, and review the theoretical basis of intrathecal fibrinolytic treatment after SAH.

MATERIALS AND METHODS

Details of the procedures for microsurgical exposure of the basal subarachnoid cisterns, induction of SAH, cerebral angiography and animal sacrifice used in our primate model have been published elsewhere[26], and will only be outlined here. The protocol was evaluated and approved by the University of Alberta's Animal Ethics Review Committee, and experiments were conducted with strict adherence to the standards of the Canadian Council on Animal Care.

Sixteen female Cynomolgous monkeys (Macaca fascicularis), weighing between 3.5 and 4 kg were divided into 4 groups of 4. The members of the first group received 0.125 mg, the second group 0.25 mg, the third group 0.50 mg, and the fourth group 0.75 mg of intraoperative, intrathecal sustained-release gel rt-PA. The gel is a specially formulated hyaluronidase preparation containing rt-PA, 1 mg/ml, which goes into solution and releases rt-PA over a several day period (Genentech Inc., South San Francisco, CA, USA).

After baseline cerebral angiography the animals underwent a right fronto-temporal craniectomy for opening of the basal cisterns, exposure of the cerebral vessels and placement of 4.25 ml of autogenous blood clot into the subarachnoid space (fig. V-1).

Prior to dural closure gel rt-PA was injected into the subarachnoid space in the vicinity of the Sylvian fissure, the dosage as described above.

The animals were followed for the development of any complications or delayed neurologic deficits.

Seven days after SAH and rt-PA administration the animals underwent repeat cerebral angiography and were killed by exsanguination. The brains were removed and photographed, and any remaining subarachnoid clot weighed.

Cerebral vessels were measured 4 times with a calibrated optical micrometer, and a mean value obtained. The arteries were measured bilaterally at the following points: extradural internal carotid artery (C3), intradural internal carotid artery (C4), precommunicating segment of the ACA (A1), sphenoidal segment of the MCA, azygous distal anterior cerebral artery, the first part of the PCA, basilar artery and vertebral arteries.

All data were coded, entered into a computer, and edited. Data for angiographic vessel caliber change within treatment groups between days 0 and 7 were compared by a paired t-test, and intergroup comparisons were made with t-test for unpaired variables. Analysis of other measured indices was done with either a t-test or an analysis of variance where appropriate. The level of significant for all tests of comparison was $p<0.05$ unless otherwise stated.

RESULTS

Comparisons between the 4 dosage groups showed no significant differences in day 0 or day 7 values in body weight

(3.31 ± 0.32 kg), heart rate (110 ± 15 beats per minute), or $PaCO_2$, which was always adjusted to between 36 and 42 mmHg at the time of cerebral angiography. The clinical condition of all animals remained good throughout the study period and there were no delayed neurologic deteriorations.

Mild to moderate VSP (between 10 and 50% reduction in vessel caliber compared to baseline) was seen in the C3, C4, A1 and MCA vessels in the 0.125 and 0.25 mg dose groups, and this reduction was significant in the case of C4 and the MCA in the 0.25 mg group ($p<0.05$) (Table V-1). The small number of animals in each group (4) appeared to preclude significance in the 0.125 mg group. When the 8 animals in the 2 smaller dosage groups were combined, significant narrowing ($p<0.05$) was evident in all of the clot-side anterior cerebral arteries: C3, C4, A1 and MCA. No VSP was observed in the 0.5 and 0.75 mg groups, alone or in combination (fig. V-2).

Gross clot remained in the subarachnoid space of all 8 animals in the 0.125 and 0.25 mg dose groups and in 2 of the 4 in the 0.5 mg group. The animals in the 0.75 mg group were all free of clot (fig. V-3).

DISCUSSION

Cerebral Vasospasm

In cases of aneurysmal SAH complicated by VSP the arterial narrowing is usually maximal 5 to 10 days after rupture [53,95]. The incidence and severity of VSP increases with greater volumes of blood clot present in the basal cisterns after SAH as demonstrated

by computerized tomography [29,48], but the overall incidence among survivors of aneurysm rupture has been estimated to be about 50% [28]. Vasospasm can affect arteries and arterioles of all caliber, but it is most striking in the larger arteries which course through the subarachnoid cisterns on the ventral surface of the brain. These are the same arteries upon which saccular aneurysms arise and consequently those which have greatest exposure to thick blood clot after aneurysm bleeding. Indeed, the relative rarity of VSP complicating other etiologies of SAH (eg. trauma or arteriovenous malformation rupture) is probably due to the infrequency of large cisternal blood clots in these conditions. There have been rare reports of symptomatic cerebral arterial narrowing following clipping of unruptured aneurysms [16], or presenting months after successful clipping of ruptured aneurysms [49], but such unusual cases suggest an arteriopathy fundamentally different than VSP as defined here.

From the foregoing it is evident that the pathogenesis of VSP lies in the subarachnoid clot and its effect on the cerebral arterial wall. Subarachnoid clot breakdown, which consists of lysis of both its cellular and fibrin components, releases a number of chemical substances which have been considered potential "spasmogens" [98]. The concentration of these substances is maximal near the adventitial surface of the arteries travelling through the subarachnoid cisterns, and their access to the various layers of the vessel wall may be facilitated by adventitial stomas and pathways that communicate with the subarachnoid space [23,99]. Of the many putative spasmogens liberated by the decaying subarachnoid clot, there is now considerable evidence that hemoglobin (Hb) is the most

important in the induction of VSP. The oxygenated form of the Hb molecule, oxyhemoglobin (OxyHb), is released gradually over a number of days from the red blood corpuscles enmeshed in the periarterial subarachnoid clot, and its appearance in the CSF after SAH correlates roughly with the time course of VSP[88]. As reviewed by Weir[96], numerous studies have demonstrated that red blood cell hemolysates induce prompt and sustained cerebral arterial vasoconstriction *in vitro* and when applied topically to exposed cerebral arteries *in vivo*. Intact red blood cells are not vasoactive[70], and methemoglobin (MetHb), the spontaneous auto-oxidation derivative of OxyHb that appears late and in relatively small concentrations in the CSF after SAH, is considerably less vasoactive than OxyHb[54,86]. More recently, it has been shown that erythrocytes are the sole ingredient of whole blood responsible for chronic VSP in a cat[20] and porcine[60] model of SAH. Although Boullin et al[15] failed to induce prolonged VSP in baboons with a single cisternal injection of pure OxyHb, the dosage of pigment used was small and probably substantially diluted by CSF. Given the positive correlation between VSP and the presence of a large SAH, it is likely that a high periarterial concentration of OxyHb over several days is necessary to induce VSP (as would be provided by a thick, hemolyzing, cisternal blood clot). Ohta et al[67] analyzed Hb concentrations in CSF and periarterial subarachnoid hematoma in patients with aneurysmal SAH. They considered even the maximum concentration of Hb seen in bloody CSF, approximately 5g/dl, too low to produce VSP, whereas the concentration of Hb in periarterial clot, found as high as 30 g/dl, did appear to correlate with the incidence of VSP.

It is not yet clear exactly how OxyHb induces VSP in the cerebral arterial wall, although a number of mechanisms have been proposed (fig. V-4). Although OxyHb itself induces a direct smooth muscle contractile response[33], its release into the extracellular milieu of the subarachnoid clot might also lead to the generation of various vasoactive free radicals[9], prostaglandins[69,79], and leukotrienes[72]. Hemoglobin, as well as bloody CSF obtained from patients with SAH, inhibits endothelial-dependent relaxation in canine cerebral arteries[43], suggesting another mechanism in the development of VSP.

Finally, it has recently been shown that another breakdown product of Hb found in subarachnoid clots and CSF after SAH, bilirubin, can result in pathological changes similar to VSP when applied topically to cat and baboon basilar arteries over a period of 4 hours[21].

Management strategies for VSP can be grouped under the following headings: (1) pharmacologic prevention or reversal of the arterial narrowing, (2) hemodynamic augmentation of blood flow through the narrowed and collateral cerebral circulation, (3) cerebral cytoprotection from ischemia, (4) mechanical dilatation of the narrowed arteries by transluminal angioplasty, and (5) prevention of VSP through the removal of subarachnoid clot from the subarachnoid space. It is likely that many of these approaches will have a place to play in the overall management of this condition, but thus far the only proven method to *prevent* VSP has been subarachnoid clot removal. Clinical studies have suggested[84], and experimental studies in primates have shown[37,66], that thorough

surgical clot evacuation up until about 48 hours after SAH will reduce or prevent VSP, but beyond this time the clot-vessel wall interaction has progressed to a point where some degree of VSP is inevitable. Although surgery within this time-frame for definitive aneurysm clipping to prevent rebleeding is becoming increasingly popular, vigorous clot removal is difficult and dangerous after significant subarachnoid bleeding, and it is not attempted by most neurosurgeons today. We grew interested in developing a nonmechanical way of promoting clearance of intact erythrocytes from basal subarachnoid cisterns in order to prevent VSP. The rationale of fibrinolytic therapy for SAH is best considered after reviewing the natural history of bleeding into the subarachnoid space.

Coagulation in the CSF

The blood-brain barrier (BBB) largely excludes clotting factors from the cerebrospinal fluid (CSF), resulting in mean CSF values for these proteins ranging from 1-5% to that of blood [41]. Barabas [13] observed that diluting whole blood with CSF up until approximately a 1:10 ratio of blood to CSF resulted in more rapid clotting than whole blood alone, and he attributed this to the presence of a "clotting accelerating factor" in CSF. The thromboelastographic analyses of CSF-plasma mixtures by Hindersin et al [42] verify this coagulation-promoting effect of both normal and pathological CSF, and these authors felt it is due to the presence of tissue thromboplastins in CSF. A sufficient volume of whole blood entering the subarachnoid space readily clots, the intrinsic pathway of the coagulation cascade being triggered by contact

activation with collagen in the arachnoid trabeculae[45], and the extrinsic pathway activated by thromboplastic factors released from vessel wall, leptomeninges and parenchyma. The strong activation of the coagulation system in the subarachnoid space immediately after SAH was demonstrated by Kasuya et al[45], who followed the CSF level of fibrinopeptide A (a peptide released in the formation of fibrin) by radioimmunoassay. The clotting process utilizes platelets, plasma clotting factors and fibrinogen entering the CSF as components of the hemorrhage. The volume of hemorrhage from a ruptured aneurysm varies greatly, ranging from a small leak which fails to clot to a massive bleed which not only congests the subarachnoid spaces but also spreads into brain and ventricles. The total volume of CSF in adults is approximately 150 ml, and the volume of the basal subarachnoid cisterns is roughly one-third of this. The frequent finding of free erythrocytes in CSF after SAH which fail to clot or which form a "halo" on blotting paper (as opposed to red blood cells in plasma, which readily clot) is not due to a CSF inhibitory effect, but is simply due to the dilution of coagulation proteins beyond a blood-CSF ratio which can support the coagulation cascade at sites distant from the hemorrhage.

Fibrinolysis in the CSF

The fibrinolytic system is responsible for dissolution of fibrin clots and thrombi, and is comprised of a number of serine protease enzymes. These proteases have a similar basic structure, and in particular demonstrate extensive homologies in the vicinity of the three amino acid residues directly involved in peptide bond hydrolysis (the catalytic site)[62]. The active enzymes consist of two

disulfide-linked chains[73]. The longer chains possess disulfide triple-loop bridging patterns, the so-called "kringle structures", that contain lysine-binding sites for fibrin. The smaller chain contains the catalytic site, the specificity of which is imparted by nearby amino acid sequences and secondary structures. Central to the fibrinolytic system is the proenzyme plasminogen[17]. Synthesized in the liver, it circulates in plasma in relatively high concentrations of 10 to 20 mg per 100 ml[93]. It possesses five kringle structures which impart high affinity for fibrin, and it is adsorbed onto fibrin polymers as they form, moving from the unbound "soluble-phase" to the fibrin-bound "gel-phase"[2] (fig. V-5). Plasminogen is converted to the active enzyme plasmin by the selective splitting of a single arginine-valine peptide bond, resulting in the two-chain structure of the active enzyme. The smaller chain's catalytic site is then free to hydrolyse susceptible fibrin arginine-lysine peptide bonds. Plasmin has a fairly broad range of specificity, hydrolyzing in addition to fibrin its proenzyme plasminogen, fibrinogen, coagulation factors V, VII and VIII, serum complement components and certain other plasma proteins[94]. The physiologic significance of some of these reactions are uncertain, but several mechanisms are in place to compensate for this lack of specificity: 1. plasmin is cleaved from plasminogen primarily in its gel-phase, making it inaccessible to other susceptible proteins, and 2. any plasmin that is free in plasma is rapidly inactivated by circulating plasmin inactivators, most importantly α 2-antiplasmin and α 2-macroglobulin[8]. Plasmin activated on the fibrin surface is protected from reaction with these and other circulating inhibitors.

Since plasminogen becomes a normal clot component the regulation of fibrinolysis is exerted primarily through the availability of plasminogen activators. Locally generated and released endogenous activators include high fibrin affinity tissue plasminogen activator (t-PA) and low fibrin affinity single-chain urokinase plasminogen activator (scu-PA)[62]. Each type of activator exists in several forms, but the active enzymes all appear to once again consist of a longer polypeptide chain possessing kringle structures and a shorter chain containing the catalytic site. The most important endogenous plasminogen activator is t-PA, which is synthesized by endothelial cells of arteries, veins and capillaries[11]. Its release is triggered by a wide variety of stimuli, such as injury, ischemia, occlusion and the application of certain platelet-derived vasoactive agents[52]. Tissue plasminogen activator circulates in only nanomolar levels in the blood where it complexes with a fast acting inhibitor and is cleared primarily by the liver. Its active circulating half-life is less than 10 minutes[94]. Released on demand at the site of vessel injury and thrombus formation, t-PA's activity is protected by protein Ca, which neutralizes the plasma inhibitor of t-PA[90]. The kringle structures again serve to impart a strong affinity for fibrin, so that released t-PA diffuses into the thrombus, binds to fibrin polymers and proceeds to hydrolyse and activate the nearby fibrin-bound plasminogen into plasmin, which in turn cleaves fibrin and dissolves the clot. Minor plasminogen activator pathways employ factor XII, prekallikrein, and high-molecular weight kinogen, and descriptions of these as well as the precise molecular mechanisms of activation have recently been presented[18,62,93].

Considerably more study has been devoted to the subject of fibrinolysis than to coagulation in the CSF, owing primarily to the interest in preventing aneurysm rebleeding after SAH with antifibrinolytic agents. Early studies, such as those by Porter et al [76,77] and Takashima et al [85] relied upon biological assays for measuring fibrinolytic activity (FA) of the CSF. In such assays the presence of plasminogen activator is detected through its conversion of plasminogen to plasmin on unheated fibrin plates, resulting in zones of lysis [10]. Lysis on a heated fibrin plate indicates the presence of free plasmin in the test sample, since heating destroys the plasminogen native to the fibrin plate. In the following studies FA refers to plasminogen activator activity. These studies have shown that normal CSF lacks FA, although Takashima et al found that some FA could be detected after pneumoencephalography. Using Todd's histochemical method [87] these authors also found that the endothelial cells of the brain and meninges appeared to be the source of FA. Porter's conclusion that the CSF contained a plasminogen "proactivator", the activity of which would be completed by the addition of streptokinase or the euglobulin component of plasma, are unfounded in light of their own or subsequent data. More recent studies employing immunoassays for fibrinolytic enzymes have confirmed the impressions of these earlier studies. Kun-yu Wu et al [51] found that normal CSF contains no or only trace amounts of plasminogen, but that plasminogen could be found in the CSF of patients with breakdown of the BBB related to various disease conditions, correlating with a general increase in CSF protein levels. Similarly, Hindersin and Endler [42]

found physiologic CSF contains no plasminogen or t-PA using immunoassay.

Whereas normal CSF does not contain fibrinolytic enzymes, the CSF obtained from patients with SAH has in some instances been shown to contain FA. In an early study Tovi et al[89] examined FA by the fibrin plate method and fibrin degradation product (FDP) content of CSF samples taken at various times from aneurysmal SAH in 11 patients. Fibrinolytic activity was present in 7 of these patients at some point in time, and FDPs were seen in the CSF of all of the patients within 3 days of the hemorrhage. Hassler and Fodstad, using Todd's technique, found that tiny vessels in the adventitia of both ruptured and unruptured aneurysms produce plasminogen activator, pointing to one possible source of FA after SAH[39]. The development of simple radioimmunoassays for FDPs led to a number of reports demonstrating rapid and large increases in the concentrations of these fragments in CSF after SAH[59], although not all investigators felt that their presence reflected fibrinolysis in the CSF alone. Anderson et al[7] felt the coexistence in CSF of FDP fragments D and E with other low molecular weight proteins (plasminogen and factor IX), but not with larger proteins (fibrinogen and factor V), was more consistent with FDP leakage from the meninges or plasma into the CSF across a damaged BBB due to aseptic meningitis. Steinmetz and Grote concurred with this[83], and were unable to demonstrate any CSF plasmin or t-PA using sensitive enzyme kinetic and immunological methods in 14 patients after SAH. Vermeulen et al[91] measured CSF FDPs between days 9 and 15 after SAH in 22 patients receiving an antifibrinolytic

agent and 26 patients given placebo. Although rebleeding was significantly reduced in the former group, no difference was found in FDP levels between groups nor was any relationship evident between FDP levels and rebleeding. These authors also concluded that FDPs in the CSF reflect a damaged blood-CSF barrier rather than fibrinolysis in the subarachnoid space alone.

After experimental SAH in rabbits Fodstad et al demonstrated that the meninges were a source of plasminogen activator, although no controls were included in this study to allow comparison of meningeal FA in normal versus SAH animals [30]. Fodstad and Nilsson did study 41 patients with recently ruptured aneurysms and found that CSF plasminogen activator activity measured by fibrin plate assay increased after 1 week in the 20 not treated with the antifibrinolytic agent tranexamic acid [31].

In summary, it appears that modest FA is sometimes seen in the CSF after aneurysmal SAH as well as in other conditions that irritate the meninges [68]. This is due to the release of t-PA from endothelium of small vessels in the meninges and perhaps the adventitia of major cerebral vessels and the aneurysm sac itself. Plasma protein leakage across a damaged BBB as well as leukocytes and platelets contained within the subarachnoid thrombus are additional sources of plasminogen activators [42]. These plasminogen activators diffuse into subarachnoid clot, bind to fibrin, and activate plasminogen that was carried into the subarachnoid space as part of the hemorrhage and which became incorporated into the developing coagulum. Because of the affinity of t-PA for fibrin, t-PA activity measured in the CSF may not always detect the

presence of local fibrinolysis in subarachnoid clot. Fibrin degradation products released into the CSF are derived from both subarachnoid clot lysis and a meningitic reaction. The elevation of FDPs correlates with the size and severity of SAH, and hence also with the risk of VSP[35]. Despite the near absence of plasmin inhibitors in normal or blood-stained CSF[42], the relatively meager supply of t-PA released allows fibrinolysis to proceed slowly in the subarachnoid space, as evidenced by the longevity of blood clots deposited in the cisterns. The clearance of subarachnoid clot is not dependent upon fibrinolysis, however, and it is frequently accompanied by a degree of fibrous tissue reaction[6]. As shall be discussed, the cellular elements of the clot may be eliminated *in situ* by hemolysis or phagocytosis. In the future, application of extremely sensitive enzyme-linked immunoabsorbent assays for t-PA in large numbers of patients will more precisely define the CSF fibrinolytic response to SAH.

Clearance of Erythrocytes from the CSF

Since CSF is slightly hypertonic compared to plasma, erythrocytes extravasated into the subarachnoid space immediately become crenated[58]. Deprived of plasma glucose, their sole energy source, as well as membrane-stabilizing plasma lipids and proteins, red blood cells are unable to maintain integrity of their cell membrane and begin hemolysing in CSF. Xanthochromia describes the color of CSF imparted by liberated heme pigments, and its presence within hours of SAH was recognized by Froin at the turn of the century as a valuable means of differentiating a "bloody tap" from a spontaneous SAH[32]. It appears that erythrocytes which

escape to circulate freely in the CSF have a shorter life-span than those which become enmeshed in subarachnoid clots or clumped and trapped in the leptomeninges[36,58]. Examining necropsy material from 53 patients who had died at various intervals after aneurysmal SAH, Hammes[36] noted that blood in the subarachnoid space elicited a prompt outpouring of polymorphonuclear leukocytes, followed by the appearance of lymphocytes and large mononuclear phagocytes derived from the arachnoid. Red blood cell phagocytosis in the basal meninges was at its peak towards the end of the first week after SAH. In a similar study of 20 cases of SAH Alpers and Forster[6] found that even up to 35 days from the hemorrhage clumps of red cells remained within organizing subarachnoid connective tissue in the basal subarachnoid cisterns.

Using absorption spectrophotometry as well as biochemical analysis of the CSF, Barrows and colleagues[12] measured OxyHb, metHb and bilirubin in 31 patients after subarachnoid or ventricular hemorrhage. Oxyhemoglobin accounted for the orange xanthochromia seen within two hours of hemorrhage, and its level plateaued on the third or fourth day. At that time bilirubin could also be detected in the CSF, and its level increased over the ensuing week while the level of OxyHb gradually declined, imparting a yellow tinge to the CSF. The amount of bilirubin formed was felt to correlate with the number of erythrocytes hemolysed and the amount of OxyHb released, and bilirubin persisted in the CSF for up to 2 to 3 weeks after the hemorrhage. In this study MetHb was not detected in the CSF after SAH. In an *in vitro* experiment they found that whole blood left to incubate in

CSF released OxyHb that converted to MetHb; bilirubin never appeared, suggesting that the action of living cells is necessary for its production. In a similar study of 62 patients after SAH, Tourtellotte et al[88] confirmed the rapid appearance of OxyHb in CSF, although they found that its increasing and then declining level over days became associated with an increasing proportion of MetHb as well as bilirubin. They found that the clearance of erythrocytes and pigments was highly variable, ranging from 6 to 30 days. Slow clearing was associated with old age, diabetes, vascular disease, and greater size and severity of SAH. Patients with slow clearing of their CSF had a more delayed (typically 11 days), and far higher, plateau in OxyHb levels. Corresponding with their more severe SAH, these patients presented, and remained in poorer neurologic condition than "fast-clearers".

Matthews et al[58] found that 20 ml of whole human blood incubated in 10 ml of normal CSF releases Hb that fails to become bilirubin as it does when released in CSF *in vivo*, the factors necessary for the transformation apparently being absent in the blood clot and CSF alone.

Hemoglobin consists of two pairs of unlike polypeptide chains, each chain carrying an iron-containing heme moiety. The iron atom in Hb is in the ferrous form, which can bind oxygen reversibly and thus bestows Hb with its oxygen carrying capacity. Oxyhemoglobin, or oxygen bound Hb, becomes MetHb through loss of electrons (oxidation) from the ferrous ion, converting it to the ferric state. This is a spontaneous reaction in the presence of water, and occurs extracellularly in the CSF or within subarachnoid

clot. Either OxyHb or MetHb can be catabolized within certain cells into protein (globin), iron, and biliverdin (the remainder of the heme molecule)[14]. This cleavage is accomplished by a microsomal heme oxygenase system. Biliverdin is rapidly reduced by biliverdin reductase into bilirubin. In an experimental model of SAH in rats Roost et al[78] found that the enzymes necessary for bilirubin production after SAH in the central nervous system are found in arachnoid and choroid plexus. It is probable that macrophages migrating into the subarachnoid hematoma are also responsible for bilirubin formation in the CSF[75].

Not all erythrocytes that escape entrapment in subarachnoid clot for freedom in the CSF undergo hemolysis. It has been shown that free erythrocytes in the subarachnoid space, which frequently reach levels of over one million cells per cubic mm, travel with CSF flow to the arachnoid granulations where they engorge the villi[22]. It remains unclear whether or not there exists in man any open channels through the villi into dural sinuses large enough to allow passage of whole erythrocytes[47]. Ultrastructural examination of the arachnoid villi from several different animal species indicate that there is restricted passage of red cells into the circulation, many of the cells becoming entrapped in the central core of the villus to be digested by macrophages[3,34]. These findings are in agreement with tracer studies using labelled red blood cells injected into the subarachnoid space, which show most erythrocytes remain fixed in the arachnoid[1].

In summary, clearance of erythrocytes from the CSF is accomplished by 3 basic mechanisms. The first is hemolysis, which

an overall significant difference was found then a Sheffe's test was applied to examine for pair-wise differences. Analysis of other measured indices was done with either a t-test or an analysis of variance where appropriate. The level of significance for all tests of comparison was $p<0.05$ unless otherwise stated.

RESULTS

Comparisons in the 5 treatment groups showed no significant differences in the baseline and day 7 values for body weight, mean arterial blood pressure, heart rate, or $PaCO_2$ (Table VI-1).

Clinical Status

All animals awoke from surgery without a neurological deficit. On day 7 one animal in the 72-hour group (monkey 29) was discovered left-hemiplegic and obtunded on the cage bottom, and this was interpreted as a delayed ischemic deficit.

Extent of Cerebral Vasospasm

At day 7 in the control group there was a 28, 33 and 70% reduction in vessel caliber compared to baseline in the right (clot)-side C4, A1 and MCA vessels, respectively, which were all significant changes ($p<0.05$) (Table VI-2). No significant change in vessel calibers occurred in the 0-, 24- and 48-hours groups. In the 48-hour group there was a 23% reduction in lumen caliber for the right A1 segment, but this change was not significant. In the 72-hour group significant VSP developed in the right C3 (15% reduction), C4 (24% reduction), and MCA (39% reduction) ($p<0.05$) (fig. VI-2). Severity of VSP paralleled the length of delay before intrathecal fibrinolytic therapy was administered (fig. VI-3), and intergroup comparisons revealed a significant difference between the

72-hour group and controls for A1 and the MCA. Monkey 29, which developed a delayed right hemisphere infarct, had a 57% reduction on the right MCA caliber with a left shift of the azygous distal A2 segment.

Pathology

All control animals had a large right-sided subarachnoid clot remaining at sacrifice, with a mean weight of 1.18 gm (SD, 0.12). A single animal each in the 0- and 24-hour groups had a small clot remaining, as did 3 animals in the 48-hour group and 5 in the 72-hour group (fig. VI-4). All treatment groups differed significantly in remaining clot weight from controls, but it appeared that older clots were more resistant to fibrinolysis (fig. VI-4).

DISCUSSION

Of the many management strategies tested or employed for VSP several have proved effective. Augmentation of cerebral blood flow (CBF) by way of induced hypertension, volume augmentation and hemodilution is effective in reversing deficits and is in wide usage [3,9]. The calcium antagonist nimodipine, although apparently ineffective in dilating vasospastic arteries, does confer a degree of protection from ischemic damage [2,8,11,12,13], either by dilating sma teral arterioles [15] or preventing calcium influx to the ische. euron [14]. Until now the only way of preventing VSP itself rather than its consequences has been subarachnoid clot removal. Clinical trials suggested that surgical clot evacuation at the time of aneurysm clipping was effective in reducing the incidence and severity of ensuing VSP, providing it was done within the first several days after SAH and was thorough [16]. This was confirmed in

our primate model of VSP, where it was shown that clot evacuation up until 48 hours after experimental SAH prevented significant VSP, whereas clot removal at 72 and 96 hours failed to prevent progressively worse VSP[7,10]. Although surgery within 48 hours of aneurysm rupture for aneurysm clipping to prevent rebleeding is popular, vigorous clot removal is difficult and dangerous after significant aneurysm bleeding.

We have previously shown that 0.5 mg of rt-PA injected into the subarachnoid space 3 times over 24 hours beginning 24 hours after SAH uniformly lysed a mean clot volume of 4.38 ml and virtually eliminated VSP measured at 7 days[4]. Neither this dose, nor a much higher dose of 10 mg administered intrathecally to 6 separate monkeys[5], had any adverse effect on the coagulation systems or pathologic appearance of the brain or meninges. Subsequently it was found that a combination of rt-PA suspension (0.5 mg) plus sustained-release gel rt-PA (1.25 mg), administered intraoperatively at the time of experimental SAH, was effective in clearing a bilateral 6 ml subarachnoid clot and preventing VSP[5]. As in the first study, 1 placebo animal developed a delayed cerebral infarct. A dosage study using gel rt-PA alone[6] demonstrated that 0.75 mg was sufficient to completely lyse a 4.25 ml SAH and prevent VSP in the primate model.

In this study we determined that clot removal with gel rt-PA (0.75 mg) within 72 hours of experimental SAH is effective in preventing VSP, which is the same result we obtained with surgical removal of clot in monkeys[7]. And as with surgical clot removal, the severity of VSP appeared to parallel the duration of contact

between the blood clot and the cerebral vessels. These findings are also in agreement with a recent report from Alksne et al[1]. They found that delaying injection of the enzyme plasmin into the cisterna magna of pigs 2, 4 and 6 days after experimental SAH resulted in a progressive increase in the extent and severity of subendothelial smooth muscle cell proliferation, a marker of VSP in their model.

We are hopeful that intrathecal fibrinolysis with rt-PA may prove to be effective preventative therapy for VSP after aneurysmal SAH. The results of this study suggest that it will be necessary to employ it in conjunction with aneurysm clipping within 72 hours of the initial hemorrhage.

BIBLIOGRAPHY

1. Alksne JF, Branson PJ, Bailey N: Modification of experimental post-subarachnoid hemorrhage vasculopathy with intracisternal plasmin. Neurosurgery 23: 335-337, 1988.
2. Allen GS, Ahn HS, Prezosi TJ, et al: Cerebral arterial spasm - A controlled trial of nimodipine in patients with subarachnoid hemorrhage. N Eng J Med 308: 619-624, 1983.
3. Awad IA, Carter P, Spetzler RF, Medina M, Williams FW Jr: Clinical vasospasm after subarachnoid hemorrhage: Response to hypervolemic hemodilution and arterial hypertension. Stroke 18: 365-372, 1987.
4. Findlay JM, Weir BK, Steinke D, Tanabe T, Gordon P, Grace M: Effect of intrathecal thrombolytic therapy on subarachnoid clot and chronic vasospasm in a primate model of SAH. J Neurosurg 69: 723-735, 1988.
5. Findlay JM, Weir BKA, Gordon P, Grace M, Baughman R: Safety and efficacy of intrathecal thrombolytic therapy in a primate model of cerebral vasospasm. Neurosurgery 24: 491-498, 1989.
6. Findlay JM, Weir BKA, Kanamaru K, Grace M, Gordon P, Baughman R: Intrathecal fibrinolytic therapy after subarachnoid hemorrhage: Dosage study in a primate model and review of the literature. Can J Neurol Sci 16: 28-40, 1989.
7. Handa Y, Weir BKA, Nosko M, Mosewich R, Tsuji T, Grace M: The effect of timing of clot removal on chronic vasospasm in a primate model. J Neurosurg 67: 558-564, 1987.

8. Jan M, Buchheit F, Tremoulet M: Therapeutic trial of intravenous nimodipine in patients with established cerebral vasospasm after rupture of intracranial aneurysms. Neurosurgery 23: 154-157, 1988.

9. Kassell NF, Peerless SJ, Durward QJ, Beck DW, Drake CG, Adams HP: Treatment of ischemic deficits from vasospasm with intravascular volume expansion and induced arterial hypertension. Neurosurgery 11: 337-343, 1982.

10. Nosko M, Weir BKA, Lundt A, Grace M, Allen P, Mielke B: Effect of clot removal at 24 hours on chronic vasospasm after SAH in the primate model. J Neurosurg 66: 416-422, 1987.

11. Ohman J, Heiskanen O: Effect of nimodipine on the outcome of patients after aneurysmal subarachnoid hemorrhage and surgery. J Neurosurg 69: 683-686, 1988.

12. Petruk KC, West M, Mohr G, et al: Nimodipine treatment in poor-grade aneurysm patients. Results of a multicenter double-blind placebo-controlled trial. J Neurosurg 68: 505-517, 1988.

13. Philippon J, Grob R, Dagreou F, Guggiari M, Rivierez M, Viars P: Prevention of vasospasm in subarachnoid hemorrhage. A controlled study with nimodipine. Acta Neurochir (Wien) 82: 110-114, 1986.

14. Seisjo BK: Cerebral circulation and metabolism. J Neurosurg 60: 883-908, 1984.

15. Takayasu M, Bassett JE, Dacey RG: Effects of calcium antagonists on intracerebral penetrating arterioles in rats. J Neurosurg 69: 104-109, 1988.

16. Taneda M: Effect of early operation for ruptured aneurysms on prevention of delayed ischemic symptoms. J Neurosurg 57: 622-628, 1982.

Table VI-1

Values of measured indices by group*

Parameter	Control		0 Hr		24 Hrs		48 Hrs		72 Hrs	
	day 0	day 7	day 0	day 7	day 0	day 7	day 0	day 7	day 0	day 7
no. of monkeys	6	6	6	6	6	6	6	6	6	6
body weight (kg)	3.59±0.30	3.55±0.29	3.44±0.27	3.42±0.26	3.73±0.60	3.72±0.58	3.90±0.50	3.90±0.06	3.32±0.32	3.23±0.32
MABP (mmHg)	113±5	112±4	114±2	112±3	110±5	112±4	105±10	106±4	108±6	110±5
heart rate (min^{-1})	110±4	110±4	110±0	111±2	110±5	110±5	111±2	112±3	110±5	109±5
$PaCO_2$ (mmHg)	40±1	40±1	40±1	39±1	40±1	40±1	40±1	41±2	40±1	40±1
Vessel Caliber (mm)										
C3-R	1.73±0.08	1.55±0.21	1.68±0.09	1.68±0.07	1.64±0.14	1.65±0.13	1.63±0.08	1.64±0.07	1.69±0.09	1.43±0.19
C3-L	1.73±0.11	1.54±0.33	1.65±0.10	1.69±0.07	1.67±0.16	1.67±0.14	1.68±0.06	1.66±0.08	1.72±0.07	1.60±0.15
C4-R	1.44±0.18	1.03±0.35	1.47±0.13	1.40±0.08	1.43±0.23	1.42±0.20	1.28±0.15	1.10±0.16	1.39±0.17	1.05±0.14
C4-L	1.44±0.22	1.24±0.31	1.48±0.10	1.46±0.07	1.46±0.20	1.44±0.18	1.29±0.13	1.30±0.15	1.43±0.19	1.29±0.14
A1-R	0.49±0.21	0.33±0.08	0.50±0.29	0.41±0.16	0.56±0.14	0.49±0.14	0.56±0.18	0.43±0.26	0.67±0.08	0.41±0.26
A1-L	0.63±0.15	0.70±0.14	0.72±0.16	0.66±0.11	0.59±0.11	0.60±0.13	0.52±0.27	0.51±0.28	0.64±0.08	0.59±0.07
A2	0.89±0.11	0.81±0.08	0.81±0.09	0.80±0.08	0.75±0.14	0.78±0.14	0.75±0.06	0.75±0.04	0.77±0.03	0.75±0.03
MCA-R	0.83±0.12	0.25±0.08	0.76±0.09	0.75±0.09	0.80±0.10	0.72±0.14	0.76±0.04	0.73±0.06	0.89±0.07	0.53±0.21
MCA-L	0.82±0.10	0.84±0.11	0.79±0.05	0.80±0.04	0.77±0.10	0.78±0.10	0.78±0.04	0.79±0.01	0.84±0.04	0.79±0.02
BA	0.96±0.23	0.98±0.21	0.90±0.09	0.92±0.04	0.74±0.09	0.74±0.08	0.79±0.12	0.87±0.14	0.93±0.16	0.91±0.13
weight of remaining clot (gm)		1.18±0.12		0.005±0.013		0.002±0.004		0.007±0.007		0.033±0.013

*Values are means ± standard deviations. MABP = mean arterial blood pressure; C3 = extradural internal carotid artery; C4 = intradural internal carotid artery; A1 = precommunicating segment of the anterior cerebral artery; A2 = distal azygous anterior cerebral artery; MCA = sphenoidal segment of the middle cerebral artery; BA = basilar artery

Table VI-2

Change from baseline in right-sided cerebral vessel diameters by treatment timing group (%)*

Vessel	Control	0 Hr	24 Hrs	48 Hrs	72 Hrs
C3	-10 ± 3	0 ± 2	+1 ± 3	+1 ± 2	-15 ± 3 †
C4	-28 ± 7 †	-5 ± 3	-1 ± 5	-7 ± 5	-24 ± 4 †
A1	-33 ± 13 †	-18 ± 18	-12 ± 10	-23 ± 15	-8 ± 5
MCA	-70 ± 10 †	-2 ± 5	-10 ± 6	-4 ± 3	-39 ± 6 †
A2	-9 ± 4	0 ± 4	+4 ± 6	0 ± 3	-3 ± 2
BA	+2 ± 13	+1 ± 3	0 ± 5	+10 ± 8	-2 ± 6

*Values are percentage change in means ± standard error of the means. C3 = extradural internal carotid artery; C4 = intradural internal carotid artery; A1 = precommunicating segment of the anterior cerebral artery; MCA = sphenoidal segment of the middle cerebral artery; A2 = distal azygous anterior cerebral artery; BA = basilar artery

† $p<0.05$

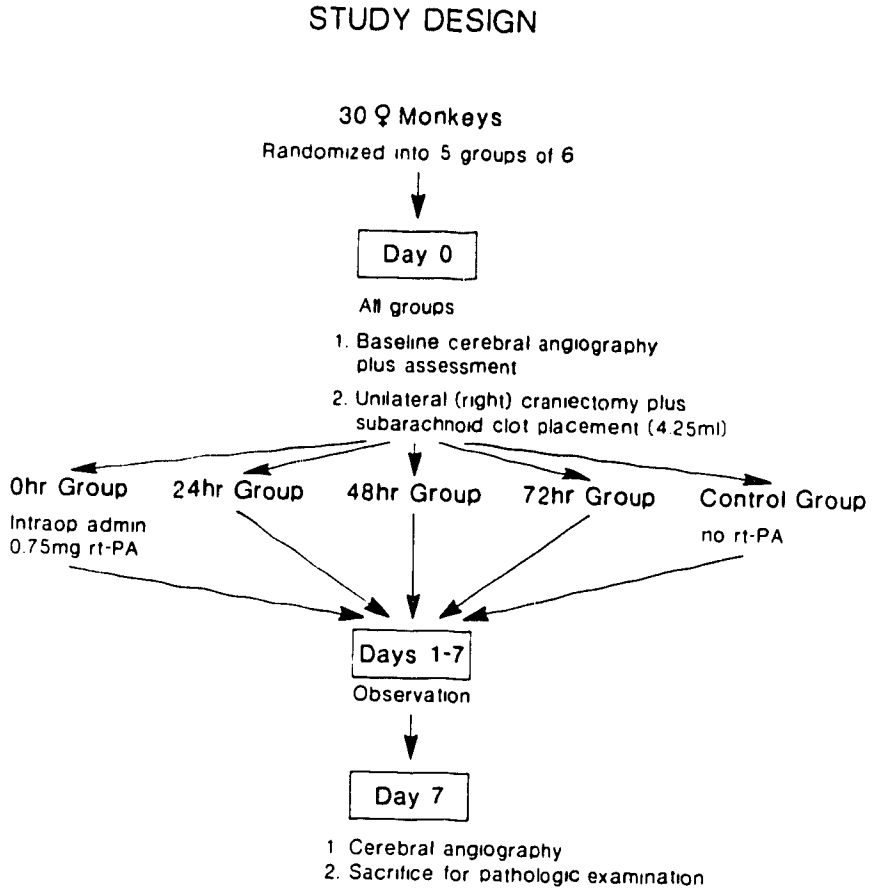

Figure VI-1
Study design of Timing Study for intrathecal rt-PA therapy.

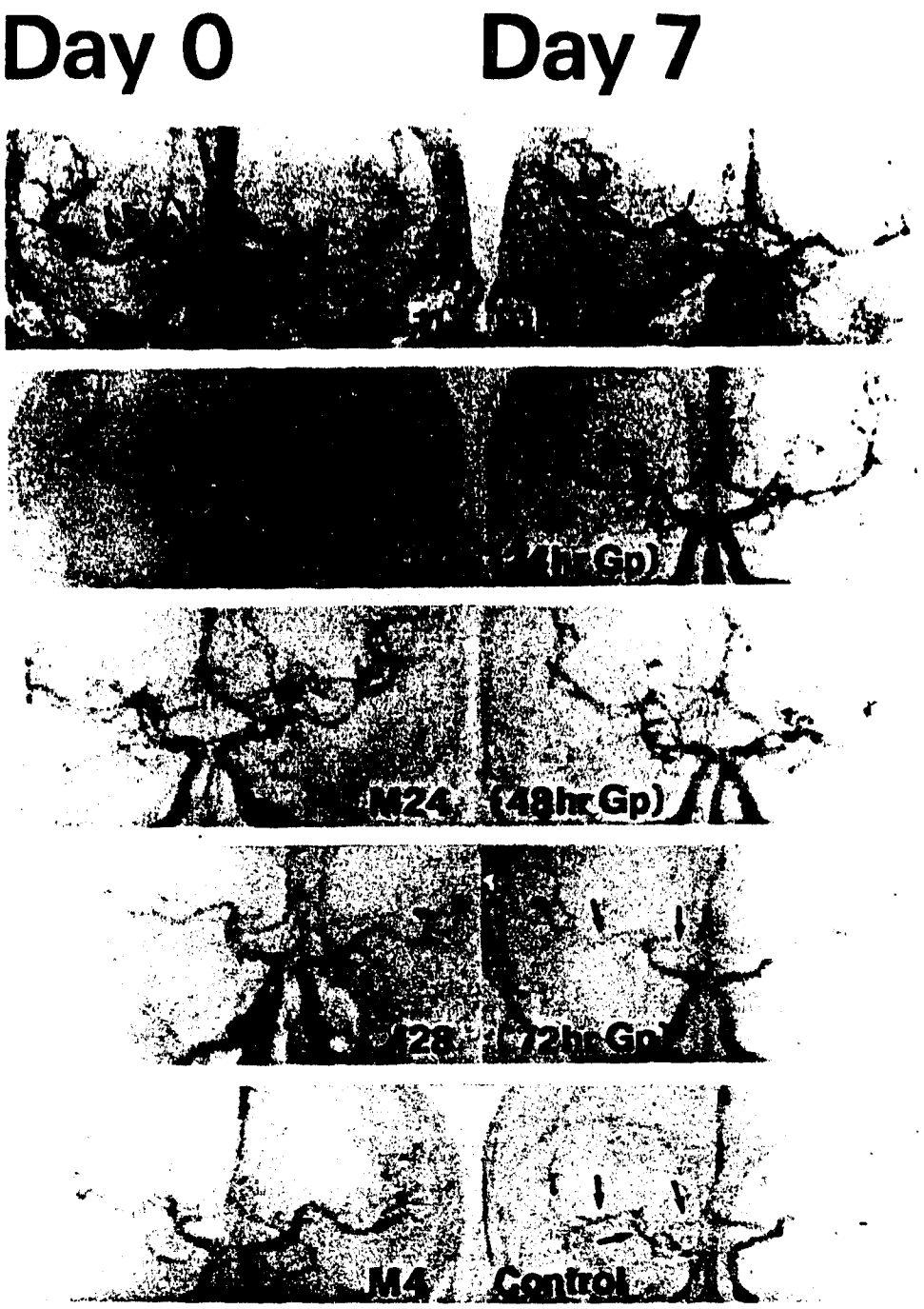

Figure VI-2
Examples of baseline (day 0, left column) and presacrifice (day 7, right column) cerebral angiograms from a monkey in each timing group and the control group. On the day 0 angiogram of Monkey 11 (M11) the right (clot-side) middle cerebral artery (MCA) is indicated with parallel arrows, the proximal anterior cerebral (A1) with a single arrow, and the intradural internal carotid (C4) with opposing arrows. The day 7 angiograms of M11 (0-hour group), M16 (24-hour group) and M24 (48-hour group) are without significant vasospasm, whereas vasospasm is apparent on day 7 in M28 (72-hour group) and M4 (control group) (arrows).

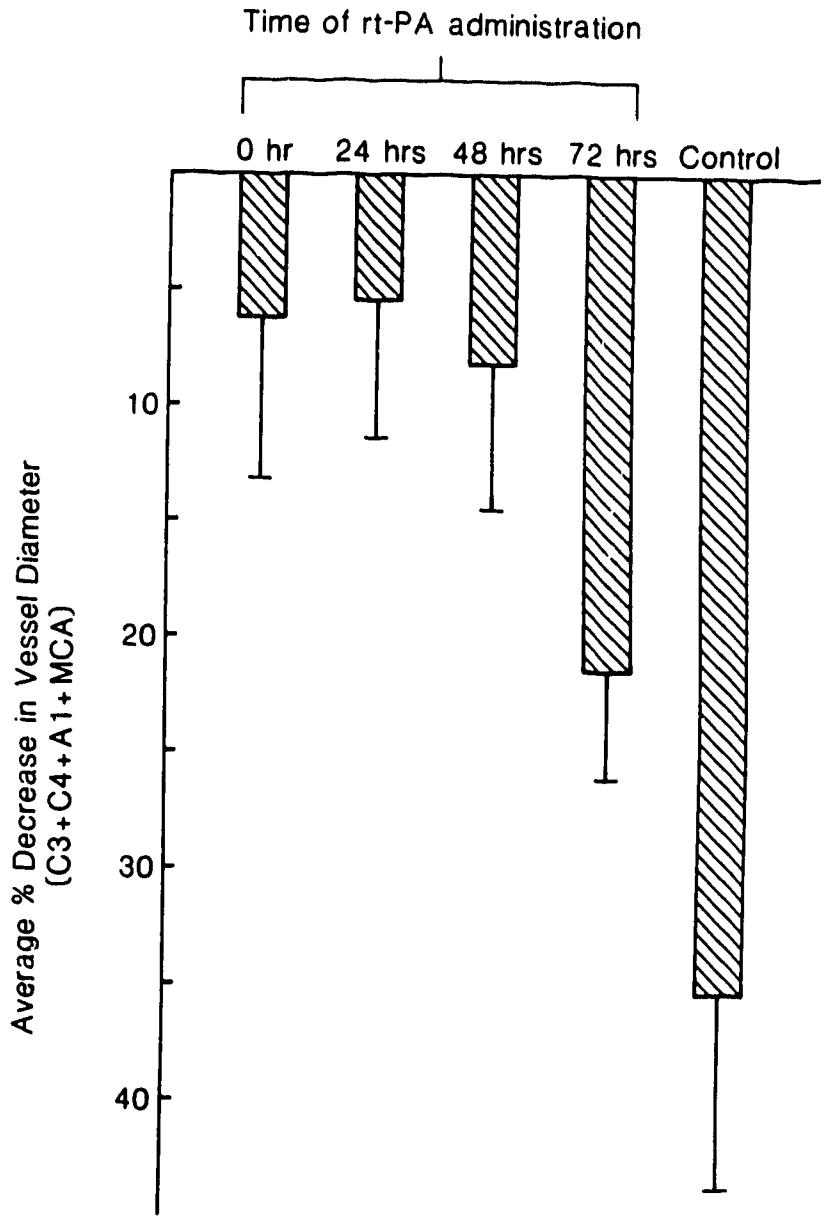

Figure VI-3
Bar graph showing the average cumulative decrease in vessel caliber (compared to baseline) for the right-sided internal carotid (C3 & C4), proximal anterior cerebral (A1), and middle cerebral arteries (MCA), for the different timing groups and controls. The amount of vasospasm correlated with the duration of time the clot was in contact with the vessel wall ($p<0.05$).

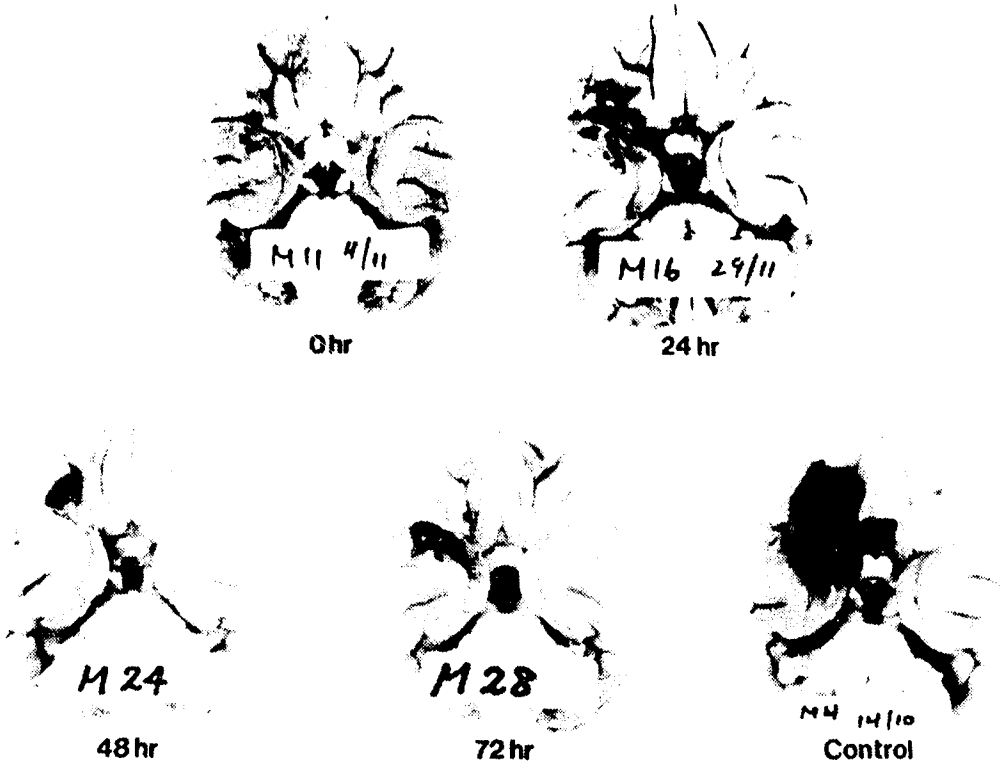

Figure VI-4
Ventral surfaces of the brains from a monkey in each timing group and the control group. The 0- and 24-hour group examples are free of gross clots, and small clots are present in the 48- and 72-hour group examples in the vicinity of the right Sylvian fissure. The control group example demonstrates a large, right cisternal clot.

CHAPTER SEVEN

CONCLUSIONS AND RECOMMENDATIONS

This work demonstrated that intrathecal administration of fibrinolytic agent human recombinant tissue plasminogen activator (rt-PA) within 72 hours of experimental subarachnoid hemorrhage (SAH) was effective in clearing large, diffuse subarachnoid clots in primates, thereby preventing the onset of cerebral vasospasm (VSP). Even an appreciably larger dose of rt-PA than required for clot lysis did not result in enough transfer of enzyme from the cerebrospinal fluid (CSF) into the circulation to cause systemic fibrinolysis. Tissue plasminogen activator, a relatively selective protease, did not inflame or injure the intact brain. Not surprisingly, however, epidural leakage of the enzyme resulted in temporary wound bleeding.

How do we progress from here? These encouraging results have stimulated interest in a human clinical trial. Our experimental work suggests that it would be appropriate to study patients with a proven aneurysmal SAH in whom surgery is undertaken within 72 hours of rupture for aneurysm clipping. The computerized tomographic (CT) scan of the patient should indicate a SAH, placing the patient at risk for VSP. Clinical grading will be less important in patient selection, the patient having already been considered a surgical candidate. After cerebral angiography patients with multiple aneurysms should be excluded because of the danger of repairing an incorrect (unruptured) aneurysm and then inducing lysis of a hemostatic clot over the previously ruptured

aneurysm. If one assumes that a typical large volume SAH deposits approximately 50 to 75 ml of blood into the basal cisterns, then extrapolating from the monkey data an appropriate dose of rt-PA in humans would be 7.5 to 10 mg (0.5 and 0.75 mg of rt-PA were effective in preventing VSP in the primate dosage study which involved a 4.25 ml clot). This extrapolation seems reasonable given the close similarity in fibrinolytic systems of man and the cynomolgous monkey.

It is uncertain whether the sustained-release gel formulation of rt-PA is necessary. The hyaluronidase gel has been prepared to go into solution and release its content of rt-PA over an approximately 24 hour period. This gradual release was initially felt to be advantageous because it would allow for the elimination of rt-PA from the subarachnoid space by inactivation and absorption into the circulation. We found that CSF is devoid of plasmin or rt-PA inactivators, and the doses of gel rt-PA mentioned above in the clot lysis studies resulted in peak concentrations of rt-PA at 24 hours followed by declining but still high levels of rt-PA until sacrifice 7 days later (fig. VII-1). After administration of 0.75 mg of gel rt-PA in a group of 4 monkeys, which went into solution within 24 hours and resulted in peak CSF rt-PA concentrations one day later, the CSF level of rt-PA measured at seven days (9 ng/ml) was almost 1000 times that of normal serum (enzyme-linked immunosorbant assays for rt-PA concentrations done by Genentech Inc., USA) (fig. VII-1).

These results indicate that elimination of rt-PA is slow in the subarachnoid space, and considering that rt-PA is rapidly adsorbed

onto fibrin polymers after administration, it is likely that a single intraoperative injection of rt-PA suspension into the exposed basal cisterns is all that is necessary to effect clot lysis.

It was surprising to witness the disappearance of such a large subarachnoid clot (4 to 6 ml) in small primates treated with rt-PA. The premise of this therapy is to prevent high periarterial concentrations of oxyhemoglobin (OxyHb) generated by slowly hemolysing erythrocytes entrapped in subarachnoid clots. However, early dissolution of the fibrin mesh and liberation of intact erythrocytes into the CSF is not tantamount to removing erythrocytes from the subarachnoid space, nor does it prevent hemolysis of freed red blood cells. It appeared, however, that treated animals were able to cope with the increased load of free erythrocytes in the CSF (for example there were no instances of obstructive hydrocephalus), and levels of OxyHb, presumably diluted by the total volume of CSF, must have been insufficient to induce VSP.

Despite this, it may be advantageous in human patients with much larger subarachnoid clots to provide some form of CSF drainage, as has been done in the past to encourage red blood cell clearance from the CSF without any form of intrathecal fibrinolysis[1]. This could take the form of cisternal, lumbar or ventricular drainage. This intervention would facilitate the daily monitoring of CSF erythrocyte, hemoglobin and rt-PA levels, for comparison to controls. The risk of incisional hemorrhages associated with epidural rt-PA leakage requires that dural closure be secure, and may mitigate against a cisternal drain passing through the operative site.

Patients should be monitored with regular postoperative CT scans and transcranial Doppler examinations to assess effect of treatment on subarachnoid clot and delayed arterial narrowing. The greatest danger of fibrinolytic therapy is induction of intracranial hemorrhages, be they epidural, subdural, subarachnoid or intracerebral. These will be monitored by clinical examination and CT scans.

The initial patients could be entered as part of a phase 2 safety trial, ie 15 to 25 patients in an open investigation with intense scrutiny for untoward effects, such as hemorrhage. If the treatment is safe and appears to have some effect on postoperative clot and/or VSP then further trials should follow, instituting blinding and comparison to controls. Both treatment and placebo control groups should be given any therapy already proved effective in reducing the risk of delayed ischemia, such as nimodipine (where available), and hypervolemic, hypertensive therapy.

This test of intrathecal fibrinolytic therapy will only be applicable to a certain percentage of the overall population of patients afflicted with aneurysmal SAH. They will have to be seen early, with a large but nondestructive SAH, and they will require almost immediate operation with minimal, atraumatic dissection for aneurysm clipping.

In the past 10 years several effective therapies have emerged which appear to significantly reduce brain ischemia and hence morbidity and mortality attributable to VSP alone. These are hypervolemic, hypertensive therapy and the calcium antagonist

nimodipine. It is our hope that intrathecal fibrinolytic therapy may also prove to have a measurable effect on those statistics, and thereby become a useful addition to the armamentarium of surgeons caring for patients suffering from rupture of intracranial aneurysms.

BIBLIOGRAPHY

1. Sakaki S, Ohta SH, Kuwabara H, Shiraishi M: The role of ventricular and cisternal drainage in the early operation for ruptured intracranial aneurysms. Acta Neurochir (Wien) 88: 87-94, 1987.

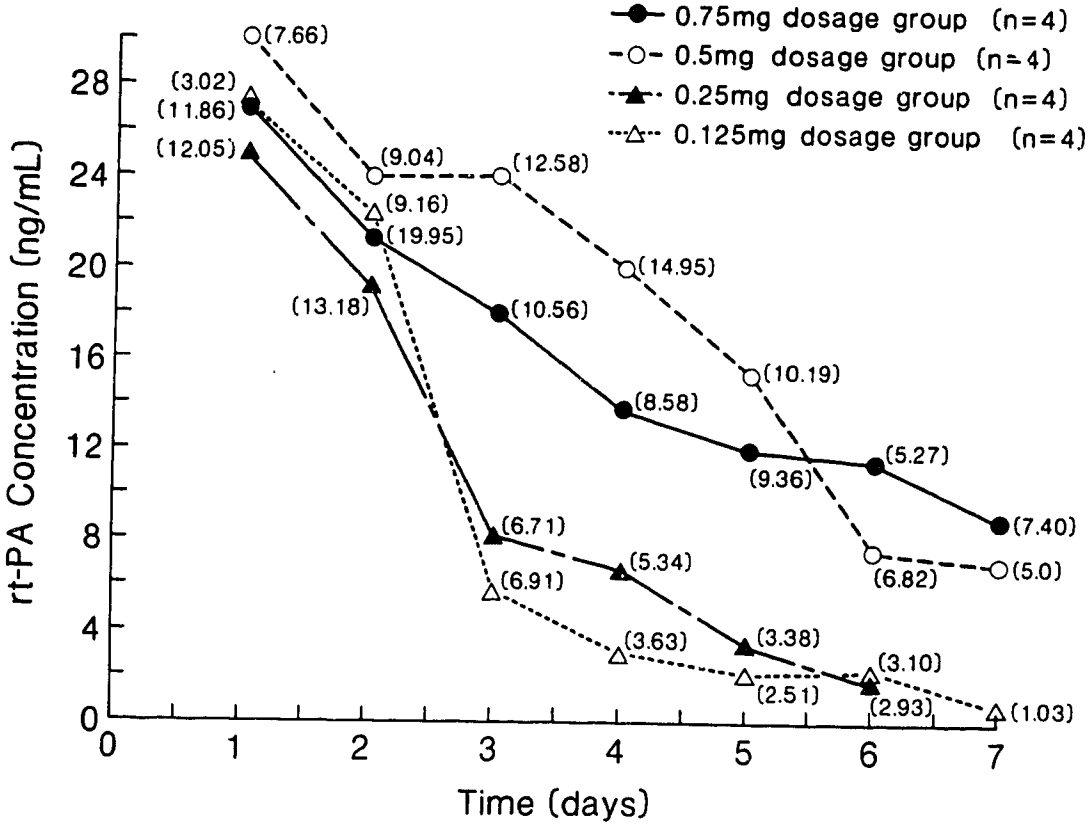

Figure VII-1
Sixteen monkeys were divided into 4 equal groups, each group receiving either 0.125, 0.25, 0.50 or 0.75 mg of gel rt-PA after subarachnoid hemorrhage (SAH). CSF taken prior to hemorrhage was devoid of rt-PA in all animals. Daily CSF samples were taken after SAH and rt-PA administration and the level of rt-PA measured. In each dosage group peak levels of rt-PA were seen 24 hours after administration, and declined thereafter. Doses effective in lysing the subarachnoid clot, 0.50 and 0.75 mg, were associated with relatively slow elimination of rt-PA from CSF, and the mean level on day 7, (9 ng/ml) is over 1000 times greater than the level of rt-PA in normal serum. *Values in parentheses are standard deviations*.

PUBLICATIONS AND AWARDS

1. Findlay JM, DeMajo W: Cerebral Fat Embolism, Can Med Assoc J 131: 755-757, 1984.
2. Findlay JM, Lougheed WM, Gentili F, Walker PM, Glynn MFX, Houle S: Effect of perioperative platelet inhibition on postcarotid endarterectomy mural thrombus formation, J Neurosurg 63: 693-698, 1985.
3. Findlay JM, Chui M, Muller PJ: Fenestration of the supraclinoid internal carotid artery, Can J Neurol Sci 14: 159-151, 1987.
4. Findlay JM, Chaisson D, Hudson ARH, Chui M.: Giant-cell tumor of the middle cranial fossa. Case Report, J Neurosurg 66: 924-928, 1987.
5. Findlay JM, Bernstein M, Vanderlinden RG, Resch L: Microsurgical removal of intramedullary spinal cord metastases, Neurosurgery 21: 911-915, 1987.
6. Macdonald RL, Findlay JM, Tator CH: Post-traumatic microcystic spinal cord degeneration, a cause of post-traumatic progressive myelopathy, J Neurosurg 68: 466-471, 1988.
7. Findlay JM, Weir BK, Steinke D, Tanabe T, Gordon P, Grace M: Effect of intrathecal thrombolytic therapy on subarachnoid clot and chronic vasospasm in a primate model of SAH J Neurosurg, 69: 723-735, 1988.
8. Macdonald RL, Findlay JM, Tator CH: Sphenoethmoidal sinusitis complicated by cavernous sinus thrombosis and pontocerebellar infarction. Can J Neurol Sci 15: 310-313, 1988.

9. Findlay JM, Weir BK: Subarachnoid and Intracerebral Hemorrhage. Ch in Weinstein PR and Faden AI (eds), *Protection of the Brain from Ischemia*, 1989 (in press).

10. Findlay JM, Weir BK, Gordon P, Grace M, Baughman R: Safety and efficacy of intrathecal thrombolytic therapy in a primate model of cerebral vasospasm. Neurosurgery 24: 491-498, 1989.

11. Findlay JM, Weir BKA, Kanamaru K, Gordon P, Baughman R, Howarth A: Intrathecal fibrinolytic therapy after subarachnoid hemorrhage: Dosage study in a primate model and review of the literature. Can J Neurol Sci 16: 28-40, 1989.

12. Steinke DE, Weir BKA, Findlay JM, Tanabe T, Grace M, Krushelnycky BW: A trial of the 21-aminosteroid U74006F in a primate model of chronic cerebral vasospasm. Neurosurgery 24: 179-186, 1989.

13. Findlay JM, Weir BKA, Kanamaru K, Espinosa F: Arterial wall changes in cerebral vasospasm. Neurosurgery (accepted for publication, 1989)

14. Findlay JM, Weir BKA, Kanamaru K, Grace M, Baughman R: The effect of timing of intrathecal fibrinolytic therapy on cerebral vasospasm in a primate model of subarachnoid hemorrhage. Neurosurgery (submitted for publication, 1989)

15. Findlay JM, Weir BKA: Intrathecal thrombolytic therapy in the prevention of vasospasm following subarachnoid hemorrhage in: Sawaya R (ed), *Fibrinolysis in the Neurosciences*. Hanley & Belfus Inc., (in press).

1988 Kenneth G. McKenzie Memorial Award for Neurosurgical Research, Annual Meeting of the Canadian Neurosurgical Society, Quebec City, June 1988.

1988 Galbraith Award in Cerebrovascular Research, American Association of Neurological Surgeons and Congress of Neurological Surgeons, Seattle, Washington, October 1988.

1988 Publication Prize Award, University of Alberta Hospital

1989 Robert G. Siekert Young Investigator Award in Stroke, 14th International Joint Conference on Stroke and Cerebral Circulation, American Heart Association, San Antonio, Texas, February 1989.