THE EFFECT OF INTRATHECAL SINGLE-BOLUS DOSE rtPA ON VASOSPASM IN EXPERIMENTAL SUBARACHNOID HEMORRHAGE
Deneysel subaraknoid kanamada vazospazm üzerine intratekal, tek doz rt PA'nın etkisi

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Summary: *The safety, prevention and treatment of the delayed cerebral vasospasm by a single administration of intrathecally applied recombinant tissue plasminogen activator (rtPA) was evaluated in a double hemorrhaged dog model of chronic cerebral vasospasm. Fourteen adult mongrel dogs were randomized into two groups of 7: clot+intrathecal normal saline, clot+intrathecal rtPA. With a single administration of 2 mg of rtPA into the cisterna magna 48 hours after the first and 6 hours after the second injection of blood in the experimental model of cerebral vasospasm, angiographic vasospasm of the basillary artery was completely prevented in all dogs so treated whereas in the control group severe vasospasm remained. All animals in the control group had a large amount of subarachnoid clot remaining at the time of sacrifice, but all animals in the rtPA group were completely free of clot. Histopathological studies of the speciemens obtained from basillary arteries showed that pathological signs of proliferative vasculopathy was present in all animals of the control group while was not demonstrable in the rtPA group. As intrathecal thrombolysis with a single rtPA injection is safe and effective in preventing cerebral vasospasm in dogs, we concluded that use of rtPA might be a promising approach for pharmacological blood clot removal.*

Özet: *Köpeklerde çift zamanlı olarak oluşturulan subaraknoid kanama sonrası meydana gelen geç serebral vazospazmın intratekal olarak verilen rekombine doku plazminojen aktivatörü (rtPA) ile engellenmesi, tedavisi ve güvenilirliği araştırıldı. Ondört yetişkin köpek herbiri 7 köpekten oluşan kontrol ve rtPA gruplarına ayrıldı. İlk subaraknoid kanamadan 48, ikinci subaraknoid kanamadan 6 saat sonra intratekal yolla verilen tek doz 2 mgr rtPA, kontrol grubunda şiddetli olarak gözlenen anjiografik vazospazmı tedavi grubunda tamamen önledi. Kontrol grubundaki hayvanlar sakrifiye edildiklerinde, subaraknoid mesafede büyük miktarda pıhtı gözlenirken, rtPA grubunda böyle bir pıhtı mevcut değildi. Basiller arterden yapılan kesitlerin histopatolojik incelenmesinde, kontrol grubundaki hayvanlarda proliferatif vaskulopatinin patolojik bulguları tesbit edildi. Köpeklerde oluşturulan deneysel subaraknoid kanamaya bağlı serebral vazospazmın önlenmesinde rtPA ile yapılan intratekal trombolizis, güvenli ve etkili bulunduğu için, farmakolojik yolla kan pıhtılarının temizlenmesi için rtPA kullanımının umut verici bir yaklaşım olabileceği sonucuna vardık.*

Although significant advances have been made in the treatment of cerebral vasospasm, the arterial vasoconstriction that commonly occurs after

subarachnoid hemorrhage (SAH) is still a leading cause of morbidity and mortality in the patients with ruptured aneurysm (1).However, the mechanism underlying cerebral vasospasm after SAH remain obscure. The importance of blood components has been substantiated by experimental and clinical studies (2-5). To prevent or arrest vasospasm, attempts have been made to

remove or neutralize subarachnoid clots, either by early surgical exploration (6-9), or by the use of various intracisternal drug administration techniques (10-13).

Recombinant tissue plasminogen activator (rtPA), a fibrinolytic agent, has been utilized both experimentally and clinically to liquefy subarachnoid clots. In an experimental study, Findlay and co-worker (10) demonstrated that almost complete lysis of subarachnoid clots could be accomplished with multiple intrathecal injection of rtPA. Since bolus application of rtPA has proven to be as efficacious for clot lysis in the circulatory system (14), it was our expectation that a single-bolus injection of this substance into the cisternal cavity would be suitable and safe to employ.

This study examines the effect of a single-bolus intracisternal administration of rtPA to prevent the development of late cerebral vasopasm in a two-hemorrhage dog model.

MATERIAL AND METHODS

A total of 14 adult mongrel dogs weighing between 20-24 kg were used for this study and divided into two groups of seven dogs each. The groups were defined by intrathechal administration of one of the followings: a treatment group receiving 2 mg rtPA in 2 ml steril water, and a control group receiving 2 ml of normal saline. The protocol was evaluated and approved by the Animal Ethics Review Commitee of Erciyes University.

On day 0, the dogs were anesthetized with a combination of ketamin hydrochlorid (20/mg/kg i.m.) and xylazin (5.5 mg/kg i.m.). After the placement of a peripheral i.v line, animals received sufficient thiopental sodium to permit intubation. Manual ventilation was adjusted to maintain the arterial carbon dioxide tension ($PaCO_2$) at or near 40 mm Hg as ascertained by continuous end tidal CO_2 monitoring (Ohmeda, 5250 RGM, Germany). With the dog in the supine position, the femoral artery was dissected surgically in the groin and a No: 5 French radiopaque sigmoid-tip polyethylene catheter was introduced using the Seldinger

technique. The catheter was advanced into the left vertebral artery with fluoroscopic control. Then, a baseline cerebral angiography was performed by manuel injection of 5 ml of iopamirol corresponding to 300 mg of iodine per ml. (2, 4, 6-triiodo-5-lactamidoisoftalamid).

Thereafter, the dog was rolled into the prone position. In order to induce a "two-hemorrhage " SAH model (15), the cisterna magna was punctured with a 22-gauge needle and 0.8 ml/kg of autologous arterial blood obtained from the femoral artery was slowly injected, without removal of cerebrospinal flow (CSF). After blood injection, the operating table was tilted 300 down for 15 minutes to promote the distribution of blood within the basal cistern. After this experimental procedure, the dogs were allowed to awaken and were given free access to water and food. On day 3, the experimental injection of 0.8 ml/kg of autologous blood was repeated.

Six hour after the second SAH, the animals were resedatized and the cisterna magna was repunctured as previously mentioned.

The control group received 2 ml of physiologic saline while the treatment received 2 mg rtPA (Genentech,Inc., South San Fransisco, California) in 2 ml of normal saline intrathecally within 120 seconds. After removal of the needle, the animals were kept in a head down position at 300 for 15 minutes after injection to limit the spinal subarachnoid distribution of the drug.

On day 8, the animals were anesthetized again and ventilated manually. Arterial blood gas values were checked routinely throughout the procedure to maintain within the physiological range. The left vertebral artery was catheterized again and angiography of the basillary artery was repeated to determine the cerebral vasospasm. Following the angiographic procedure, an anterior thoracotomy was performed. The decending aorta and the vena cava were clamped. The ascending aorta was cannulated and the vena cava incised. After wash out of blood from the cerebral circulation by infusion of 2000 ml of physiological saline , intravital perfusion-fixation of cerebral vessel was

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performed by injection of 2.5% cacodylate-buffered glutaraldehyde there after the brain of the animal was removed atraumatically.

Basillary artery diamater was measured at the level of the posterior cerebral artery bifurcation by 5 ◊ magnification by an observer blind to the animal's history.

The values of radiographic measurement were averaged and the percentage of reduction in the vessel diamater was calculated by dividing the difference between the Day 0 (pre-SAH) and Day 8 (postSAH) vessel diamater by the Day 0 preSAH diamater.

Statistical evaluations of the changes in the angiographic diamater of the basillar artery were obtained using Wilcoxon test.

RESULTS

The animals in both groups showed no significant differences in heart rate, hematocrit levels and PCO2 physiological parameters at the base line and Day 8 measurement, except for body weight (Table I). There was significant alteration between Day 0 and Day 8 values in body weight in both groups

(for the control group, t value: 0, $p<0.05$; for the rtPA group, t value: 0, $p<0.05$)

Angiography of the basillar artery

Severe narrowing occured in the basillary artery in animals from the control group (mean reduction 49.8±3.8 %), whereas in the animals from rtPA group no reduction in caliber was noted.

In the control group, the mean diamater of the basillary artery on day 0 was measured as 1.26±0.05 mm (1.34 mm to 1.19 mm). All of the animals in this group showed significantly angiographic vasospasm (Fig. 1). The mean diamater of the basillar artery on day 8 was 0.63±0.07 mm (0.72 mm to 0.54 mm). The difference between preSAH and postSAH angiographic measurement was statistically significant (t value: 0, $p<0.05$) (Tab. I).

In the rtPA-treated group, none of the animals developed angiographic vasospasm (Fig. 2). While the mean diamater of the basillar artery on day 0 was measured as 1.25±0.04 mm (1.32 mm to 1.19 mm), the value obtained on day 8 was 1.25±0.05 mm (1.32 mm to 1.19 mm). The difference between 0-day and 8-day angiogram was no statistically significant (t value:10.5, $p>0.05$).

Table I. Measurement of physiological parameters and vessels diameters between Day 0 and Day 8 in two animals group●

Groups	Number	Weight	Heart rate	Hematocrit	PCO2	Vessel caliber
Control Day 0	7	20.85±2.11	126.7±2.92	41.14±1.34	38.42±0.97	1.26±0.05
Day 8	7	20.14±2.26	126.57±5.85	41.14±0.90	38.57±0.97	0.63±0.07
p value		$p<0.05$*	$p>0.05$	$p>0.05$	$p>0.05$	$p<0.05$*
rtPA Day 0	7	21.00±1.91	127.57±3.25	41.00±1.29	38.57±0.97	1.25±0.04
Day 8	7	20.00±1.93	127.28±3.94	40.85±0.90	38.57±1.51	1.25±0.05
p value		$p<0.05$*	$p>0.05$	$p>0.05$	$p>0.05$	$p>0.05$

●*Values are mean±standart deviation; * Significantly different from Day 0*

Morphologic examination

At the time of sacrifice, all animals in the control group had thick blood clot filling the cisterna magna. In contrast, animals in the rtPA group had no subarachnoid clot within the cisterna magna.

Histological examination in the control group displayed changes in the basillary artery corresponding with proliferative vasculopathy. However, in the rtPA group, histological examination of the specimens of the basillary artery showed no morphological evidence of posthemorrhagic vasculopathy. There was marked contrast between the control and rtPA group with regard to the thickness of the vessel wall, the folding of the internal elastic lamina, and the shape of the smooth muscle cell (Fig. 3)
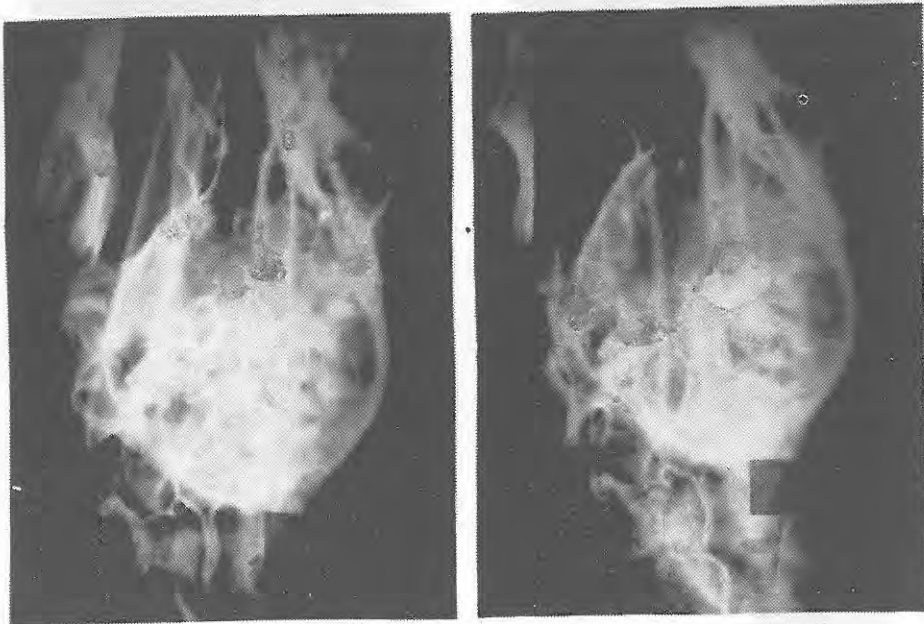
Figure 1. Cerebral angiogram taken on Day 0 (left) and Day 8 (right), control group. Note the presence of vasospasm in the basillar and posterior cerebral artery at Day 8
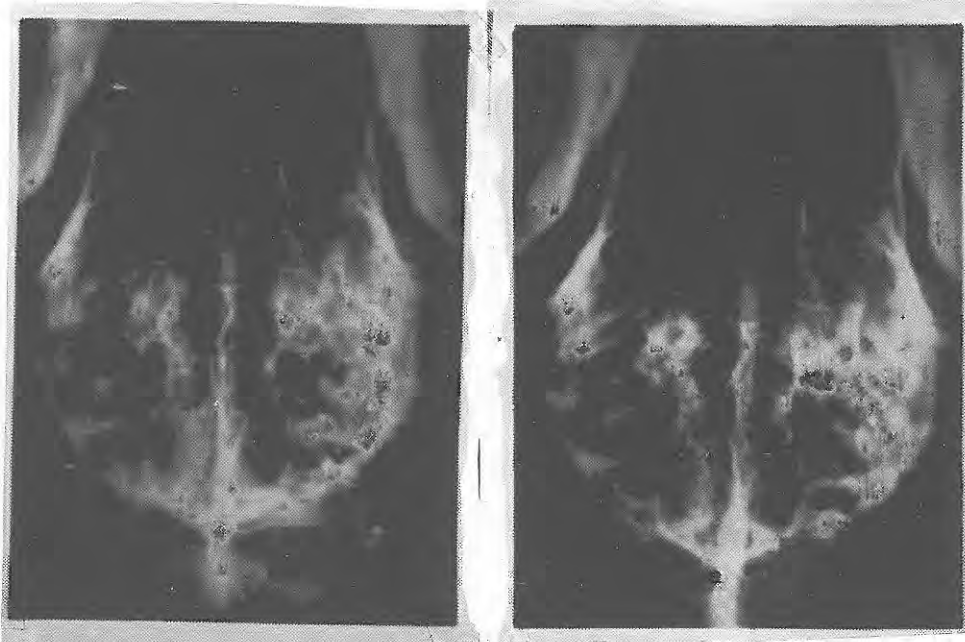
Figure 2. Cerebral angiogram taken on Day 0 (left) and Day 8 (right), rtPA group. There was no narrowing of the basillar and posterior cerebral artery on the postSAH angiogram. Compare with the degree of spasm in Figure 1
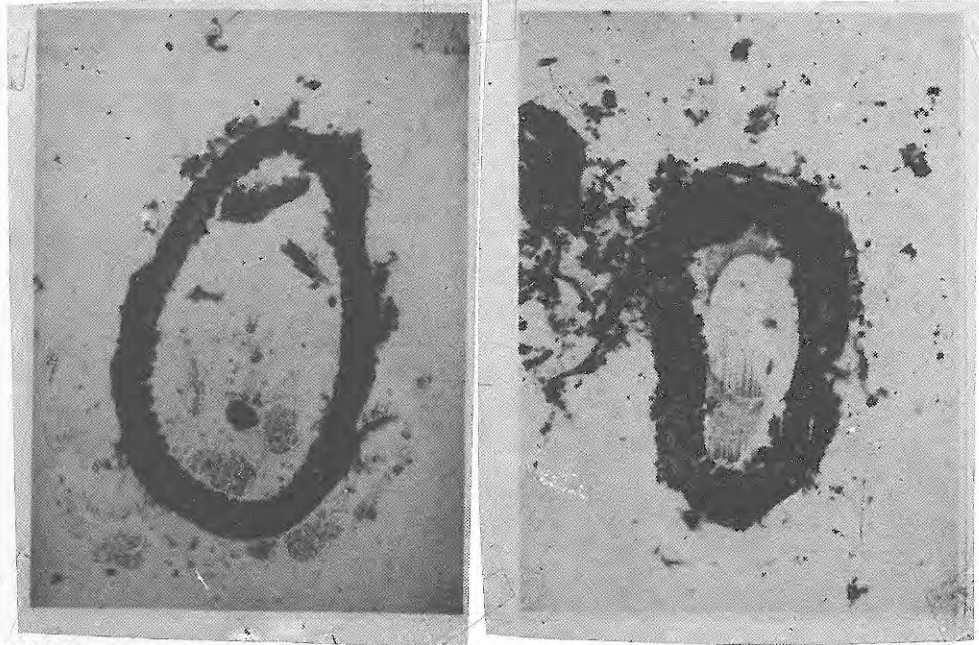
Figure 3. Light microscopic appearence of the section of the basillar artery obtained from autopsy. In the control group (left), severe narrowing of the vessel diameter with tortuous changes and corrugation of the intimal and internal elastic lamina. Normal diameter and appearence of the basillar artery in the rtPA group (right)

DISCUSSION

A remarkable feature of the cerebral vasospasm that is associated with SAH is its delayed onset. Vasospasm is rarely seen on angiogram immediately after the rupture of an aneurysm. However, cerebral vasospasm is a common occurence between 3rd and 17th days after hemorrhage (16, 17). The incidence of severe vasospasm in SAH is related to the volume of blood present intracisternaly (2). On the other hand, the manifestation of brain ischemia is related to the magnitude of vasospasm (4). Over a period of several days, the erytrocytes of the blood clot lyse and release vasospastic and vasotoxic substances which permeate the walls of cerebral arteries and induce muscular spasm and vessel wall damage (18-21)

The faculty behind the administration of fibrinolytic substances is to achieve a premature lysis of the subarachnoid clots, thereby facilitating the normal clearing process of the bloody cistern from spasmogenic metabolites by the circulating CSF.

The major contemporary theory concerning the pathogenesis of vasospasm is that the periarterial blood clot, during its gradual degredation, release certain vasoactive substance that cause vascular spasm.

Although a number of subtances in whole blood produce vasoconstriction in isolated cerebral arteries (22), Mayberg et al. (23) have suggested that arterial narrowing after SAH is mediated by mechanism releated to prolonged exposure of the vessel wall to hemoglobin or its catabolites from lysing subarachnoid erythrocytes. There is a great deal of information involving oxyhemoglobin (24). It is speculated that free radicals produced during the oxidative conversion of oxyHb to methemoglobin can produce vasoconstrictive and vasotoxic lipid peroxides and prostaglandins. This is based upon the premise that the beginning of vasospasm can be hindered by early evacuation of the clot prior to its degregation to vasoconstrictive and vasotoxic subtances.

It has been reported in clinical and experimental trials that timely and through surgical evacuation of the cisternal blood clot will prevent vasospasm if it is employed within 48 hours after SAH (6-9). However, even in these optimistic early report, this approach such as total evacuation of clot from the basal cistern is both technically difficult and potentially dangerous (11).

A number of studies have shown that early operation and removal of subarachnoid blood clot after SAH could diminish the incidence of vasospasm and improve outcome (6-8). Early surgery for aneurysm combined with aggressive surgical evacuation of subarachnoid and cisternal clot is theoretically attractive in the prevention of cerebral vasospasm but is technically difficult and potentially dangerous in patients with significant and diffuse subarachnoid hemorrhage (10).

Since more intensive premature lysis of the residual blood clots is inaccessible during the surgery of aneurysm, intrathecal administration of thrombolytic agents as a method of lysing and preventing vasospasm have stimulated interest. (13).

Fibrinolytic system refers to a group of enzymes when activated cause fibrin breakdown of fibrinolysis and later dissolution of the blood clot. The active fibrinolytic enzyme is plasmin, the activated product of the enzyme plasminogen.

Conventional activators of the fibrinolytic system streptokinase and urokinase, induce a systemic lytic state that may result in bleeding (12, 25). Conversely, rtPA exhibits considerable fibrin specifity when compared to these conventional thrombolytic agents. It converts plasminogen to plasmin in the presence of fibrin with relatively little activity against freely circulating plasminogen, effectively localizing the fibrinolytic process to the surface of the fibrin clot (26). This agent has been shown to be effective clinically in the treatment of the coronary artery thrombosis and is not associated with systemic fibrinolytic activation which plagues other therapeutic agents Both urokinase and rtPA are human enzymes that produce fibrinolysis by directly converting plasminogen to plasmin. One difference between rtPA and urokinase is that the former requires

fibrin for plasminogen activation and is therefore more clot-selective fibrinolysis (26). Therefore, rtPA is less likely to consume circulating plasminogen and clotting factor and causes less frequently generalized lytic state when administrated by i.v., iatrogenicaly (27). Additionally, kinetics of conversion of plasminogen to plasmin are considerably accelerated in the presence of fibrin. Although efficacy of washout of blood clots by urokinase have been showed in experimental and clinical investigation (13), the possibility of inducing systemic fibrinogenolysis by intracisternal administration of urokinase was prevented widespread use of this drug (12, 25). All these observations and experiences make rtPA the ideal fibrinolytic substance to liquefy the subarachnoid clot in the basal cisterns.

Findlay (10) was first to demonstrate the efficacy of multiple injections of rtPA on preventing vasospasm in primate model of SAH. We have not preferred a continuous catheterization for multiple injection of this agent because in clinical setting this form of application may cause inherent complication such as infection, CSF leakage, and damage to vital structures.

In our experimental study using the double-hemorrhage model in dogs, a single injection of rtPA into the cisterna magna was given 48 hours after the first and six hours after the second injection of blood. This experimental model was designed to pretend a similar clinical situation.

None of the animals in the rtPA group developed arterial narrowing in the postSAH angiogram on day 8, whereas in all animals of the control group severe angiographic vasospasm occured.

In this experimental study, intracisternal single injection of rtPA in a dog model of SAH has prevented the delayed onset of cerebral vasospasm. Our results indicate that rtPA is effective in lysing clots within subarachnoid cisterns and preventing cerebral vasospasm.

In appraising the results of our experimental study, it is proposed that pharmacological lysis of subarachnoid clot by a single intracisternal injection of rtPA might be promising method for the hindering of cerebral vasospasm. Further studies are needed to verify the safety of this method of administration.

Acknowledgement

REFERENCES

1. *Kassell NF, Sasaki T, Colohan ART, Nazar G. Cerebral vasospasm following aneurysmal subarachnoid hemorrhage. Stroke 1985;16:562-572.*
2. *Fisher CM, Mistler JP, Davis JM. Relation of cerebral vasospasm to subarachnoid hemorrhage visualized by computerized tomographic scanning. Neurosurgery 1980;6:1-9.*
3. *Kistler JP, Crowell RM, Davis KR et al. The relation of vasospasm to the extent and location of subarachnoid blood visualized by CT scan: A prospective study. Neurology 1983;33:424-436.*
4. *Graham DI, MacPherson P, Pitts LH. Correlation between angiographic vasospasm, hematoma, and ischemic brain damage following SAH. J Neurosurg 1983;59:223-230.*
5. *White RP, Robertson JT. Role of plasmin, thrombin, and anti-trombin III as etiological factors in delayed cerebral vasospasm. Neurosurgery 1985;16:27-35.*
6. *Handa Y, Weir BKA, Nosko M, et al. The effect of timing of clot removal on chronic vasospasm in a primate model. J Neurosurg 1987;75:558-564.*
7. *Nosko M, Weir B, Lunt A, et al. Effect of clot*

removal at 24 hours on chronic vasospasm after SAH in the primate model. *J Neurosurg* 1987;66:416-422.

8. Taneda M. Effect of early operation for ruptured aneurysm on prevention of delayed ischemic symptoms. *J Neurosurg* 1982;57:622-628.

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

10. Findlay JM, Weir BKA, Kanamaru K, et al. Intrathecal fibrinolytic therapy after subarachnoid hemorrhage: Dosage study in a primate model and review of the literature. *Can J Neurol Sci* 1989;16:28-40.

11. Findlay JM, Weir BKA, Kanamaru K, et al. The effect of timing of intrathecal fibrinolytic therapy on cerebral vasospasm in a primate model of subarachnoid hemorrhage. *Neurosurgery* 1990;26:201-206.

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

13. Yoshida Y, Ueki S, Takahashi A. Intratechal irrigation with urokinase in ruptured cerebral aneurysm cases. Basic studies and clinical application *Neurol Med Chir* 1985;24:987-997.

14. Eisert WG, Callisen H, M.ller TH. Multiple bolus injection of rtPA lyse in the rabbit jugular vein model as efficient as continuous infusion. *J Cell Biochem* 1987;11:184.

15. Varsos VG, Liszczak TM, Han DH, et al. Delayed cerebral vasospasm is not reversible by aminophylline, nifedipine. or papaverine in two-hemorrhage canine model. *J Neurosurg* 1983;58:11-17.

16. Kwak R, Niizuma H, Ohi T, Suzuki J. Angiographic study of cerebral vasospasm following rupture of intracranial aneurysm: Part 1. Time of the appearence. *Surg Neurol* 1979;11:257-262.

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

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

19. Macdonald RL, Weir BKA, Runzer TD, et al. Etiology of cerebral vasospasm in primates. *J Neurosurg* 1991;75:415-424.

20. Macdonald RL, Weir BKA. A review of hemoglobin and pathogenesis of cerebral vasospasm. *Stroke* 1991;22:971-982.

21. Towart R. The pathophysiology of cerebral vasospasm, and pharmacological approach to its management. *Acta Neurochir (Wien)* 1982;63:253-258.

22. White RP. Pharmacodynamic features of delayed cerebral vasospasm. In:Wilkins RH (ed), *Cerebral Vasospasm*. Raven Press, New York 1988, pp 373-382.

23. Mayberg M, Okata T, Bark D. Cerebral arteriopathy and contractile changes after SAH are related to erythrocyte component of whole blood. *Stroke* 1988;19:130-135.

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

25. Del Zoppo GJ, Zeumer H, Harker LA. Thrombolytic therapy in stroke: Possibilities and hazards. *Stroke* 1986;17:595-607.

26. Sobel BE, Gross RW, Robinson AK. Thrombolysis, clot selctivity, and kinetics. *Circulation* 1984;70:160-164.

27. Fears B. Biochemical pharmacology and therapeutic aspects of thrombolytic agents. *Pharmacol Rev* 1990;42:201-???