Thrombosis and Haemostasis – © F. K. Schattauer Verlagsgesellschaft mbH (Stuttgart) 60 (3) 508–513 (1988)

Tissue-Type Plasminogen Activator in Patients with Intracranial Meningiomas

Hiroko Tsuda[1], Kazunari Oka[2], Youko Noutsuka[1], and Katsuo Sueishi[3]

From the Clinical Laboratory[1], Kyushu University Hospital, Department of Neurosurgery[2] and Department of Pathology[3], Faculty of Medicine, Kyushu University, Fukuoka, Japan

Key words

Intracranial meningiomas – Euglobulin fibrinolytic activity – Tissue-type plasminogen activator

Summary

Three of 13 patients with intracranial meningiomas showed the pre- and postoperative elevation of tissue-type plasminogen activator (t-PA) related fibrinolytic activity in euglobulin fractions (EFA). During operation, two of these three patients showed a significant elevation of the level of fibrin(ogen) degradation products and oozing in the operating field. However, oozing was not observed in the third patient who had been given tranexamic acid preoperatively. Fibrin autography revealed that a broad lytic band of mol wt 50–60 kDa, probably free t-PA, appeared in the plasma obtained from two of the three patients after operation when EFA elevated significantly. In all patients studied, the t-PA antigen levels were normal preoperatively but increased both during and after operation, and correlated mainly with the intensities of a lytic band of mol wt 110 kDa, probably t-PA complexed with its major inhibitor (PAI-1). These results suggest that the excessive fibrinolysis can induce the local hemorrhagic diathesis during operation and may be related to t-PA function in plasma.

Introduction

Fibrinolytic processes depend on the conversion of the inactive proenzyme plasminogen into plasmin by plasminogen activators (PAs) which are widely distributed in body fluids and tissues. The best known mammalian PAs are urokinase-type PA (u-PA), purified from urine (1), and tissue-type PA (t-PA), purified from tissue extracts (2). Both types of PA have been identified in human blood (3, 4). Physical exercise and venous occlusion have been known to increase plasma fibrinolytic activity (5), mainly due to the enhancement of t-PA release from vascular endotherial cells (4). But the mechanism of excessive plasma fibrinolysis in pathological conditions remains uncertain.

Meningiomas constitute about 15% of all intracranial tumors and most of them are histopathologically and biologically benign. They are rich in vasculature and thought to arise from the meningothelial cells lining the leptomeningial spaces. The removal of the tumors is often accompanied with unexpected bleeding from scalp, tumor tissue and leptomeninges (6). Histochemical studies of the normal human brain have shown that fibrinolytic activity is localized to meninges, choroid plexus and vascular endothelium (7). Koos et al. have reported that several mesenchymal brain tumors including meningiomas showed PA activity and suggested that the therapeutical administration of protease inhibitors could prevent the bleeding during operation (8). Cultured meningioma cells have been reported to produce PA activity, which was not inhibited by anti-u-PA IgG (9). No report is, however, available on the fibrinolytic activity of the plasma of patients with meningiomas.

Recently, we have reported on three patients with intracranial meningiomas who showed preoperative elevation of fibrinolytic activity in euglobulin fractions and oozing from the operating field (10). In this paper, we studied the fibrinolytic activity assessed by the determination of euglobulin fibrinolytic activity (EFA) and t-PA antigen (Ag) pre-, per- and postoperatively in patients with intracranial meningiomas including the reported patients. The molecular species of the PAs appearing in the plasma were studied by using fibrin autography (11), in terms of their mol wt and immunological reactivity.

Patients, Materials and Methods

Patients and Normal Subjects

Thirteen patients with intracranial meningiomas (7 males and 6 females, age range 30–72 years) were studied. The routine examinations of coagulation mechanism and platelet function were within normal ranges. None of them were under medication with any drugs known to influence fibrinolysis. Blood samples were taken on admission along with follow-up samples collected during and after tumor resection. The control group consisted of 43 apparently healthy volunteers (22 males and 21 females, age range 22–58 years).

Methods

After a 10-min period of recumbent rest, plasma samples were obtained by venipuncture into 1/10 volumes of 3.8% sodium citrate. Serum samples for FDP determination were obtained in a vacutainer glass tube containing thrombin (5 U), bathroxobin (0.4 BU) and aprotinin (250 U). From some normal subjects, blood samples were drawn both before and after 10 min of venous occlusion of the arm with a manometer cuff at 90 mmHg. Samples were immediately assayed, or devided into aliquots and stored at −80° C until use.

A specific IgG to t-PA was coupled to cyanogen bromide activated Sepharose 4 B (4 mg IgG per ml wet gel). Pooled plasma taken from normal subjects was depleted of t-PA by incubation with anti t-PA IgG Sepharose 4 B for 2 hrs at 4° C.

Euglobulin fractions were prepared with plasma dilution of 1:20 at pH 5.8. Euglobulin fibrinolytic activity (EFA) was measured on bovine fibrin plates according to the method of von Kaulla and Schultz (12), results being expressed as the mean of two perpendicular diameters of lytic area. Euglobulin lysis time (ELT) was measured by using an automated clot lysis time recorder (Riko Shoji, Japan).

t-PA Ag was measured on acidified plasma using an enzyme linked immunosorbent assay method (13) (American Diagnostica Inc., USA) in the presence of 5 mM EDTA.

Fibrinogen was determined by the Clauss method (14). Plasminogen and α_2-plasmin inhibitor (α_2-PI) were assayed by single radial immunodiffusion technique using antisera from Behring Institute, West Germany and Mochida Pharmaceutical Co., Japan, respectively. Fibrin(ogen) degradation products (FDP) were measured by latex agglutination test (Teikokuzoki, Japan).

Correspondence to: Dr. Hiroko Tsuda, Clinical Laboratory, Kyushu University Hospital, 3-1-1, Maidashi, Higashi-Ku, Fukuoka, 812, Japan

This document was downloaded for personal use only. Unauthorized distribution is strictly prohibited.

Table 1 Age, sex and preoperative data of fibrinolytic parameters of patients with meningioma

Patient	Age	Sex	EFA (mm)	ELT (min)	t-PA (ng/ml)	Fbg (mg/dl)	Plg (mg/dl)	α_2-PI (mg/dl)	FDP (μg/ml)
1	68	M	4.4	90	11.8	229	11.8	5.3	<2.5
2	72	F	3.8	130	17.4	247	12.8	6.9	2.5
3	61	F	3.2	140	14.1	314	11.2	6.2	2.5
4	51	M	<3.1	570	12.1	248	12.0	6.4	<2.5
5	57	F	<3.1	420	15.0	200	12.0	6.5	<2.5
6	41	F	<3.1	240	8.4	260	10.6	6.4	2.5
7	37	M	<3.1	240	15.9	211	10.6	6.6	2.5
8	54	F	<3.1	200	8.2	319	13.4	6.6	<2.5
9	59	M	<3.1	170	19.4	150	14.8	7.1	2.5
10	37	F	<3.1	500	17.1	250	15.0	8.4	2.5
11	44	M	<3.1	410	20.1	169	13.2	7.1	2.5
12	58	M	<3.1	200	7.9	188	11.0	5.9	<2.5
13	30	M	<3.1	560	14.0	241	12.6	8.6	<2.5
normal	range		<3.1	150–600	2.6–20.2	150–360	7.6–14.0	5.2–8.8	<10

Normal ranges were represented as means ± 2SD (n = 43), except EFA, ELT and FDP values.

Fibrin autography was performed by modifications of the method of Granelli-Piperno and Reich (11). Plasma samples (8 μl) were incubated with 30 μl of 0.125 M Tris (pH 6.8) containing 10% sodium dodecyl sulphate (SDS) and 33% glycerol for 30 min at 37° C. They were applied to the SDS-polyacrylamide gel, separating gel of 9% acrylamide and stacking gel of 4% acrylamide (15). After electrophoresis, the gel was soaked in 2.5% Triton X-100 to remove free SDS and placed on the fibrin agar indicator gel which was composed of 1% agarose (Agarose LGL, Nakarai Kagaku, Japan), human plasminogen (25 μg/ml), bovine thrombin (0.22 U/ml) and bovine fibrinogen (2 mg/ml, Miles, USA) in phosphate-buffered saline, pH 7.4 (PBS). The indicator gel was allowed to develop at 37° C in a moist chamber and then was photographed. Molecular weight calibration was performed using the molecular weight marker proteins, run under the same conditions. Immunological identification of the PA species was achieved by incorporating anti-t-PA or anti-u-PA IgG in the indicator gels.

Statistical analysis was performed by Student's t-test.

Reagents

Human t-PA was purified from cultured human melanoma cells as described (16). Human high molecular weight urokinase (u-PA) was obtained from Mochida Pharmaceutical Co., Japan. Antisera against t-PA and u-PA were raised in rabbits and IgGs were isolated by Protein A-Sepharose chromatography. The anti-t-PA IgG inhibited t-PA activity (ID_{50} of 100 mU/ml t-PA: 0.1 μg/ml) but did not inhibit u-PA, on the contrary the anti-u-PA IgG inhibited u-PA activity (ID_{50} of 100 mU/ml u-PA: 0.6 μg/ml) but did not inhibit t-PA. Human plasminogen was prepared from plasma by affinity chromatography on lysine-Sepharose (17). Bovine thrombin was purified from the commercially available thrombin sample (Mochida Pharmaceutical Co., Japan) as described (18).

Table 2 Effects of anti-t-PA IgG and anti-u-PA IgG on the EFA of the plasma of patient 1

		EFA (mm)
Normal IgG	(11.0 μg/ml)	5.8 ± 0.1
Anti-t-PA IgG	(1.1 μg/ml)	4.0 ± 0.0*
Anti-u-PA IgG	(11.0 μg/ml)	5.7 ± 0.1

Euglobulin fraction was precipitated from the plasma of patient 1 and resuspended in a volume of barbital buffer (0.03 M barbital, 0.12 M NaCl, pH 7.4) corresponding to half the initial plasma volume. The fraction was incubated for 15 min at 25° C with rabbit normal IgG, anti-t-PA IgG or anti-u-PA IgG at the concentration indicated in parenthesis. Subsequently EFA was measured as described under Materials and Methods. The diameters of sample wells are 3.1 mm. Data are expressed as means ± SD (n = 3).

* $p < 0.01$ compared to control (Student's t-test)

Molecular weight marker proteins were obtained from BioRad Laboratories, USA, comprising myosin (200 kDa), β-galactosidase (116.25 kDa), phosphorylase b (97.4 kDa), albumin (66.2 kDa), ovalbumin (42.7 kDa), carbonic anhydrase (31 kDa).

Results

The age, sex and preoperative fibrinolytic parameters of 13 patients are listed in Table 1. Patients 1, 2 and 3 showed elevation of EFA and shortening of ELT, whereas the other 10 patients (patients 4–13) showed normal levels of EFA and ELT. The levels of the other fibrinolytic parameters including t-PA Ag, fibrinogen, plasminogen, α_2-PI and FDP were all within normal ranges, except for a slight increase of plasminogen which was observed in patients 9 and 10. None of the patients showed any signs of hemorrhagic diathesis.

The euglobulin fraction prepared from the plasma of patient 1 who showed the highest value of EFA was incubated with specific IgG to either type of PAs and then the fibrinolytic activity was examined (Table 2). The EFA was significantly inhibited by adding anti-t-PA IgG at the final concentration of 1.1 μg/ml ($p < 0.01$) but not by anti-u-PA IgG at 11.0 μg/ml, indicating that EFA was mainly ascribed to t-PA.

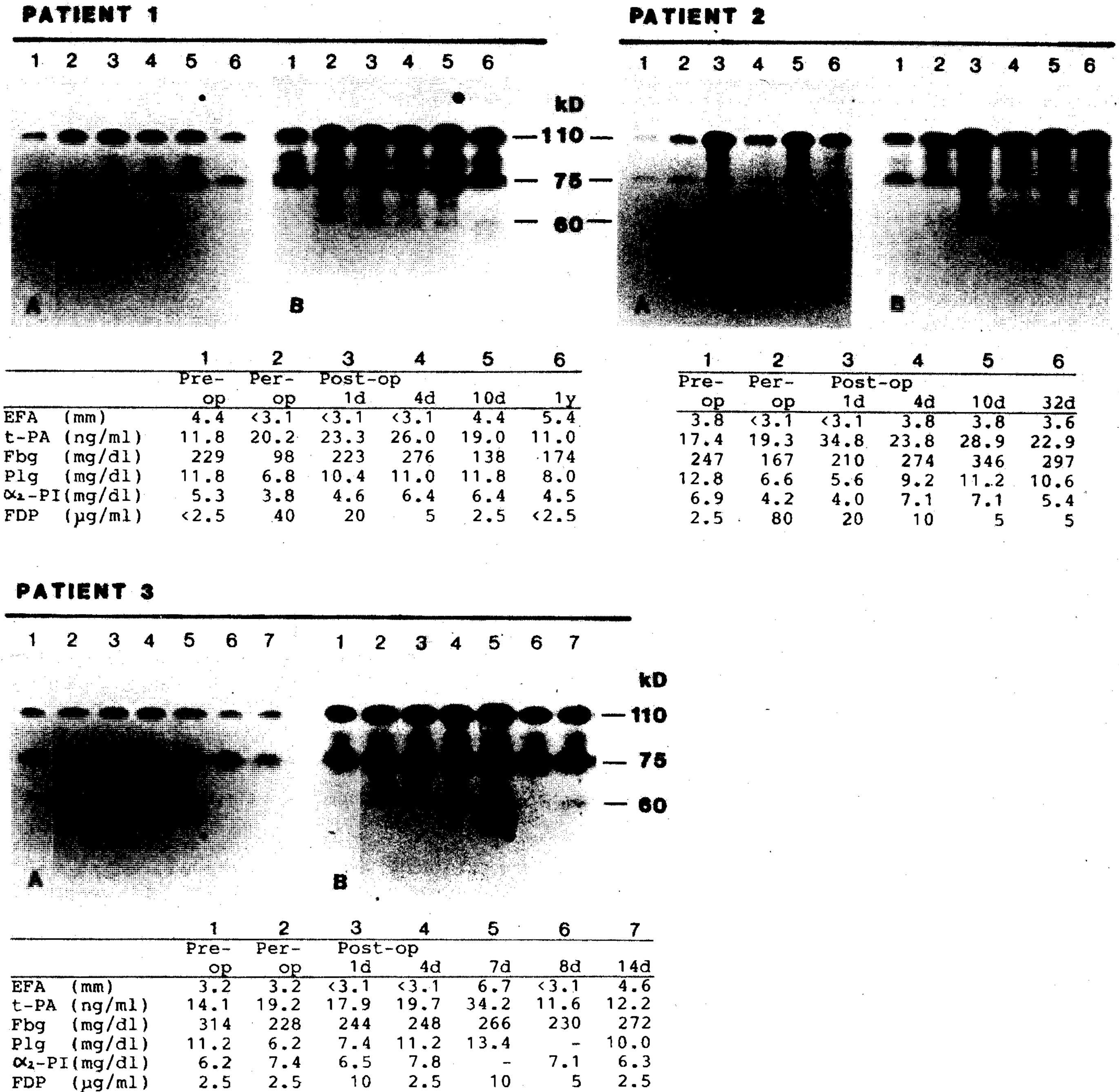
Fig. 1 shows zymograms of fibrin autography and fibrinolytic parameters of plasma samples taken from three patients (patients 1, 2 and 3) with the preoperative elevation of EFA and four patients (patients 4, 5, 6 and 7) without it, both before, during and after operation. In all patients, fibrin autography of the plasma samples obtained before operation showed mainly two lytic bands of mol wt 110 kDa and 75 kDa, as well as a weak band of 90 kDa which appeared after a longer incubation of indicator gel (Fig. 1, lane 1). These lytic bands were plasminogen dependent (data not shown) and were also observed on plasma from resting normal subject (Fig. 2, gel A, lane 1). Anti-t-PA IgG in the indicator gel abolished the 110 kDa band, whereas the 75 kDa band and the faint 90 kDa band were not completely abolished by anti-IgG to either t-PA or u-PA (Fig. 2, gel B and C, lane 1).

During the resection of the tumor, patients 1 and 2 showed a significant elevation of FDP level, a reduction of levels of fibrinogen, plasminogen and α_2-PI (Fig. 1), and oozing in the operating field. They were infused with 2 mg $\cdot$ kg^{-1} $\cdot$ hr^{-1} of gabexate mesilate and the oozing apparently ceased. The administration of gabexate was continued until the 5th postoperative day when the FDP level returned to a normal range. Patient 3,

This document was downloaded for personal use only. Unauthorized distribution is strictly prohibited.

showing the preoperative elevation of EFA, had an injection of 1 gram of tranexamic acid just prior to operation. She did not show the elevation of FDP level nor oozing during operation as seen in the 10 patients whose EFAs were not elevated preoperatively (patients 4–13, data of patients 8–13 were not shown).

The EFA was not detectable after the operation and elevated again several days later in patients 1, 2 and 3. In patient 3, a marked elevation of levels of EFA and t-PA Ag was observed on the 7th post operative day when the analysis of plasma using fibrin autography revealed an additional broad band of mol wt 50–60 kDa. The broad band appeared after a longer incubation of indicator gels in the plasma from patient 3 on the 14th postoperative day and from patient 1 one year after operation when EFA significantly elevated (Fig. 1, gel B). But the extents of EFA



Patient 1

	1	2	3	4	5	6
	Pre-op	Per-op	Post-op 1d	4d	10d	1y
EFA (mm)	4.4	<3.1	<3.1	<3.1	4.4	5.4
t-PA (ng/ml)	11.8	20.2	23.3	26.0	19.0	11.0
Fbg (mg/dl)	229	98	223	276	138	174
Plg (mg/dl)	11.8	6.8	10.4	11.0	11.8	8.0
α_2-PI (mg/dl)	5.3	3.8	4.6	6.4	6.4	4.5
FDP (µg/ml)	<2.5	40	20	5	2.5	<2.5

Patient 2

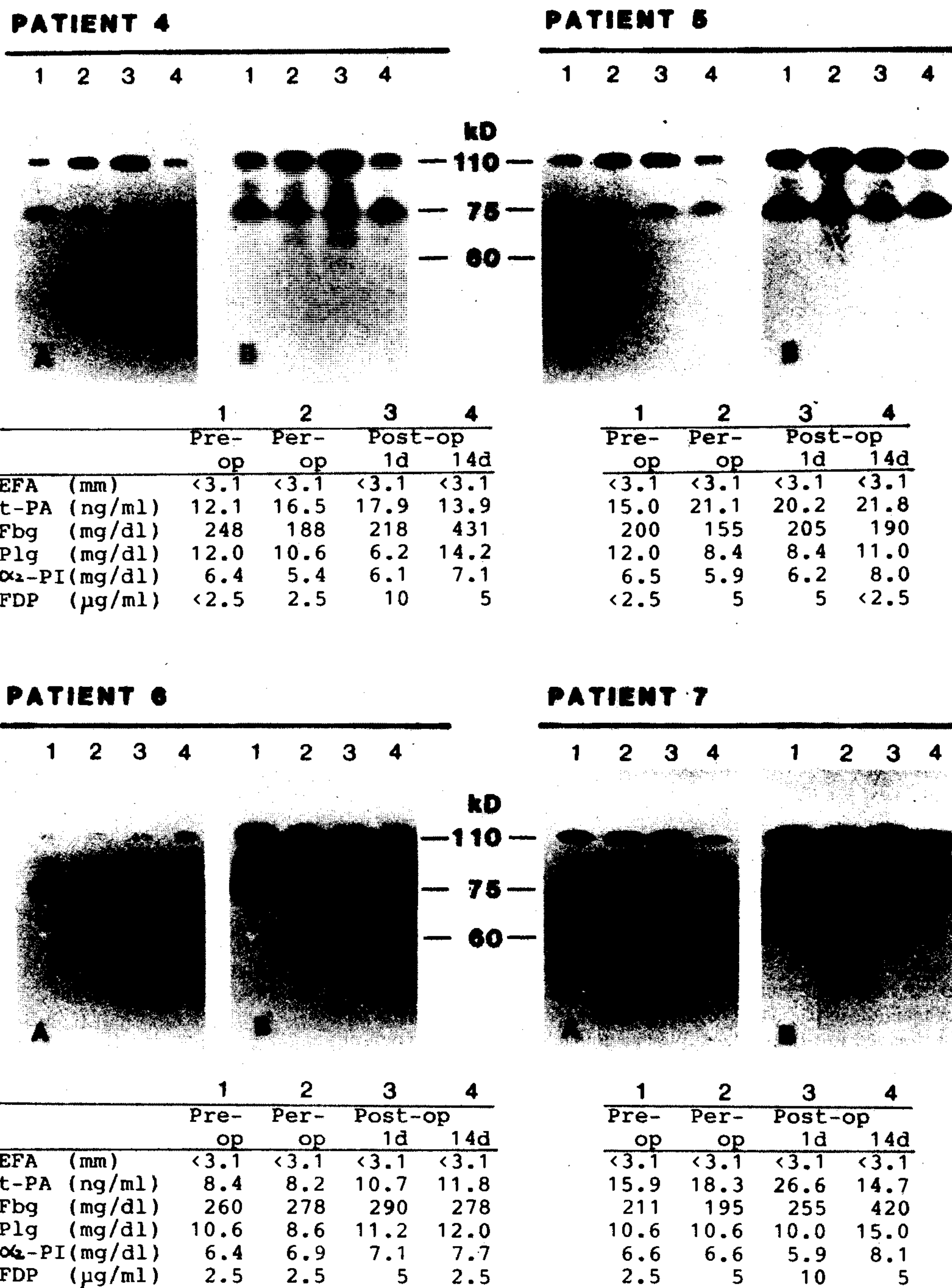
	1	2	3	4	5	6
	Pre-op	Per-op	Post-op 1d	4d	10d	32d
EFA (mm)	3.8	<3.1	<3.1	3.8	3.8	3.6
t-PA (ng/ml)	17.4	19.3	34.8	23.8	28.9	22.9
Fbg (mg/dl)	247	167	210	274	346	297
Plg (mg/dl)	12.8	6.6	5.6	9.2	11.2	10.6
α_2-PI (mg/dl)	6.9	4.2	4.0	7.1	7.1	5.4
FDP (µg/ml)	2.5	80	20	10	5	5

Patient 3

	1	2	3	4	5	6	7
	Pre-op	Per-op	Post-op 1d	4d	7d	8d	14d
EFA (mm)	3.2	3.2	<3.1	<3.1	6.7	<3.1	4.6
t-PA (ng/ml)	14.1	19.2	17.9	19.7	34.2	11.6	12.2
Fbg (mg/dl)	314	228	244	248	266	230	272
Plg (mg/dl)	11.2	6.2	7.4	11.2	13.4	-	10.0
α_2-PI (mg/dl)	6.2	7.4	6.5	7.8	-	7.1	6.3
FDP (µg/ml)	2.5	2.5	10	2.5	10	5	2.5

Fig. 1 a

This document was downloaded for personal use only. Unauthorized distribution is strictly prohibited.



Patient 4

	1 Pre-op	2 Per-op	3 Post-op 1d	4 Post-op 14d
EFA (mm)	<3.1	<3.1	<3.1	<3.1
t-PA (ng/ml)	12.1	16.5	17.9	13.9
Fbg (mg/dl)	248	188	218	431
Plg (mg/dl)	12.0	10.6	6.2	14.2
α_2-PI (mg/dl)	6.4	5.4	6.1	7.1
FDP (μg/ml)	<2.5	2.5	10	5

Patient 5

	1 Pre-op	2 Per-op	3 Post-op 1d	4 Post-op 14d
EFA (mm)	<3.1	<3.1	<3.1	<3.1
t-PA (ng/ml)	15.0	21.1	20.2	21.8
Fbg (mg/dl)	200	155	205	190
Plg (mg/dl)	12.0	8.4	8.4	11.0
α_2-PI (mg/dl)	6.5	5.9	6.2	8.0
FDP (μg/ml)	<2.5	5	5	<2.5

Patient 6

	1 Pre-op	2 Per-op	3 Post-op 1d	4 Post-op 14d
EFA (mm)	<3.1	<3.1	<3.1	<3.1
t-PA (ng/ml)	8.4	8.2	10.7	11.8
Fbg (mg/dl)	260	278	290	278
Plg (mg/dl)	10.6	8.6	11.2	12.0
α_2-PI (mg/dl)	6.4	6.9	7.1	7.7
FDP (μg/ml)	2.5	2.5	5	2.5

Patient 7

	1 Pre-op	2 Per-op	3 Post-op 1d	4 Post-op 14d
EFA (mm)	<3.1	<3.1	<3.1	<3.1
t-PA (ng/ml)	15.9	18.3	26.6	14.7
Fbg (mg/dl)	211	195	255	420
Plg (mg/dl)	10.6	10.6	10.0	15.0
α_2-PI (mg/dl)	6.6	6.6	5.9	8.1
FDP (μg/ml)	2.5	5	10	5

Fig. 1 Zymograms of fibrin autography and fibrinolytic parameters on plasma samples taken from 7 patients before, during and after resection of tumors. The indicator gel was allowed to develop at 37° C for 13 hr (A) or 20 hr (B) and then was photographed. The number of the lane of zymogram is corresponding to that of the column of table and represents the each plasma sample examined

Fig. 1 b

elevation were less than that observed in patient 3 and the t-PA Ag did not increase. The intensity of the 110 kDa band increased in all patients during operation or on the 1st postoperative day and the elevation continued for several days, although the extent and duration of elevation varied widely among individuals (Fig. 1, gel A). The level of t-PA Ag also increased in the same period and correlated well with the intensity of the 110 kDa band. Several new bands between 60 kDa and 110 kDa appeared in patient 2 postoperatively when the intensities of the 110 kDa band and the t-PA Ag levels increased significantly. After a prolonged incubation of gels, similar new bands also appeared in the other patients, except patient 6, during or after operation when the intensities of 110 kDa band and the t-PA Ag levels were high (Fig. 1, gel B).

The PA activities in the plasma from a normal subject after venous occlusion and those from patients 2 and 3 after operation were characterized using anti-t-PA or anti-u-PA IgG (Fig. 2). The post-venous occlusion plasma produced two additional bands, a broad band of 50–60 kDa and a faint one of 42 kDa. The 110 kDa band was intensified in comparison with that of resting plasma. The broad 50–60 kDa band in the plasma from patient 3 comigrated with that in post-venous occlusion plasma, and both bands were inhibited by anti-t-PA IgG but not by anti-u-PA IgG. Whereas, the 42 kDa band, which was located slightly cathodal in

This document was downloaded for personal use only. Unauthorized distribution is strictly prohibited.

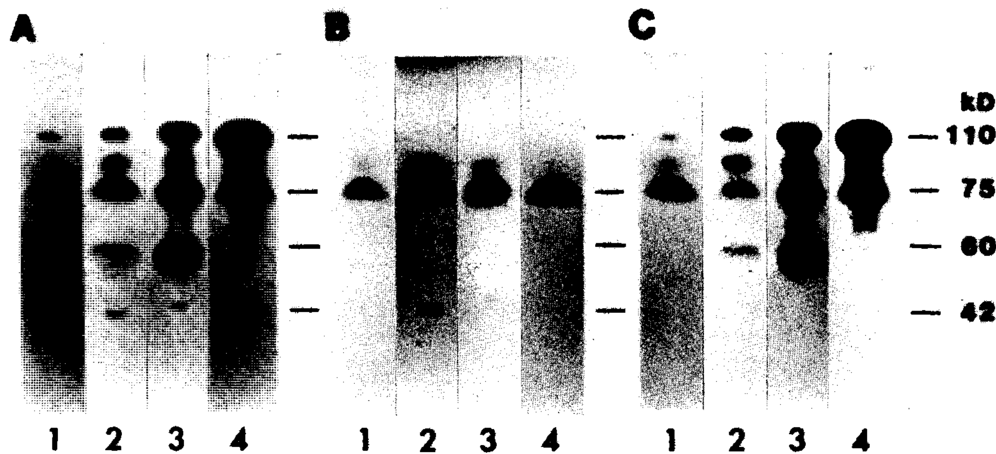


Fig. 2 Analysis of plasma samples by fibrin autography with rabbit normal IgG (A), with anti-t-PA IgG (B) and with anti-u-PA IgG (C) in the indicator gel. Final concentrations of IgGs were 10 μg/ml. Plasma obtained from a normal subject at rest (lane 1), after venous occlusion (lane 2), from patient 3 on the 7th postoperative day (lane 3) and from patient 2 on the 1st postoperative day (lane 4) were studied

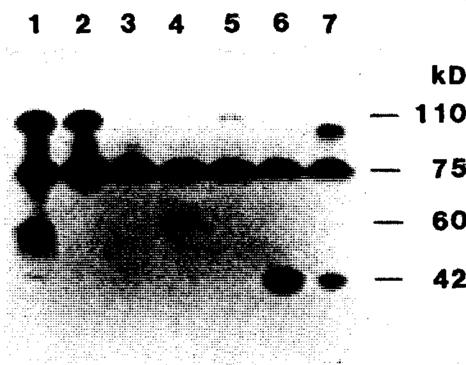


Fig. 3 Zymographic study of complex formation of free PA with its major inhibitor in plasma. Free t-PA (final 6 ng/ml) or u-PA (final 3 ng/ml) was added to t-PA depleted plasma. Immediately after addition or after incubation for 15 min at 37° C, the mixture was subjected to SDS-PAGE and analysed by fibrin autography. Lane 1: plasma from patient 3 on the 7th postoperative day; lane 2: pooled plasma not depleted of t-PA; lane 3: t-PA depleted plasma; lanes 4 and 5: t-PA depleted plasma incubated with t-PA for 0 and 15 min, respectively; lanes 6 and 7: t-PA depleted plasma incubated with u-PA for 0 and 15 min, respectively

the plasma of patient 3 probably due to the decreased level of albumin (3.5 mg/dl) (19), was inhibited by anti u-PA-IgG and not by anti t-PA-IgG. Thus, the 50–60 kDa band and the 42 kDa band may ascribe to t-PA and u-PA, respectively. Almost all the new bands of PA activity appearing in the plasma from patient 2 were inhibited by anti-t-PA IgG.

The bands of PA activity in plasma samples were further identified using free PA and t-PA depleted plasma (Fig. 3). When the t-PA was depleted from plasma by incubation with anti-t-PA IgG coupled Sepharose 4B, the 110 kDa band disappeared (lane 3). Free t-PA added to the t-PA depleted plasma produced a 60 kDa band (lane 4) which seems likely to be identical to the tailing edge of the t-PA related broad 50–60 kDa band in the plasma from patient 3 (lane 1). The 110 kDa band reappeared and the 60 kDa band disappeared after incubation for 15 min at 37° C (lane 5). The 42 kDa band appeared immediately after the addition of free u-PA to t-PA depleted plasma (lane 6). The 95 kDa band was produced and the intensity of the 42 kDa band reduced after incubation for 15 min at 37° C (lane 7). Thus, free t-PA or u-PA added to t-PA depleted plasma rapidly formed a 110 kDa complex of t-PA or 95 kDa complex of u-PA with its major circulating inhibitor, PAI-1, in line with a previous report (20).

Discussion

We have demonstrated that three (patients 1, 2 and 3) of 13 patients with intracranial meningiomas showed the elevation of t-PA related EFA before operation. Significant changes of fibrinolytic parameters and oozing in the operating field were observed in patients 1 and 2 during the resection of tumors but not in patient 3 who had been injected with tranexamic acid before the operation. In these three patients, the elevation of EFA disappeared within a few days after the operation and reappeared afterwards. Fibrin autography revealed that a broad lytic band of mol wt 50–60 kDa appeared in the plasma showing a significant rise of EFA. The plasma from normal subjects after venous occlusion produced a similar broad lytic band of mol wt 50–60 kDa. By the immunological technique using specific IgGs to PAs and the reconstitution experiments using free t-PA and t-PA depleted plasma, it was suggested that the broad lytic band of mol wt 50–60 kDa was primarily produced by free t-PA. A similar broad 50–60 kDa band has been observed in the euglobulin fraction after venous occlusion (21) or exercise (22) and in the plasma after exercise (22) as well as in the plasma from patients with alcoholic cirrhosis showing shortened ELT (23). It has been supposed that the appearance of free t-PA in plasma was due to the enhancement of t-PA release from vascular endothelial cells in the cases of venous occlusion and exercise (4) or to the impairment of hepatic clearance of t-PA in the case of cirrhosis (23). Meninges, choroid plexus and vascular endothelium in normal brain (7), meningioma tissue (8) and meningioma cell line (9) have been shown to produce PA. The free t-PA observed in plasma from meningioma patients may be derived from meningioma tissues including endothelial cells in tumor stroma or meningioma cells themselves. However, the finding that the broad 50–60 kDa band is observed even after the major part of

This document was downloaded for personal use only. Unauthorized distribution is strictly prohibited.

tumor has been removed suggests that free t-PA may originate from tissue other than that of the tumor, e.g. from meninges, choroid plexus or systemic vascular endothelial cells.

The oozing which occurred during the operation was well controlled by the fibrinolytic inhibitor, tranexamic acid, or the synthetic serine protease inhibitor, gabexate mesilate (24), and the participation of excessive fibrinolysis can be postulated. The release of PA from the surgical site into the circulation may cause the oozing as suggested regarding the bleeding complications following the surgery of the urinary tract or prostate (25). It is at present unknown whether the pre- or postoperative elevation of EFA and appearance of a broad lytic band of 50–60 kDa in plasma and the excessive fibrinolysis during operation are due to the PA originating from the same tissue. Although these findings were obtained from only three patients, it seems likely that preoperative EFA levels may have a predictive value for identification of patients requiring prophylaxis of hemorrhagic diathesis during operation.

In all patients, the levels of t-PA Ag were normal preoperatively but increased both during and after operation, and correlated mainly to the intensities of a lytic band of mol wt of 110 kDa. The 110 kDa band was related to t-PA and appeared in t-PA depleted plasma by incubating with free t-PA, suggesting that it is due to a complex of t-PA with its major circulating inhibitor of mol wt 50 kDa, PAI-1, in agreement with previous reports (20, 22, 26). When the intensities of 110 kDa band and the t-PA Ag levels increased significantly, several new bands between 60 kDa and 110 kDa appeared, and those activities were inhibited by anti-t-PA IgG. The t-PA Ag assay using polyclonal antibodies does not distinguish between free t-PA and t-PA-inhibitor complexes (27). Therefore, the rise of t-PA Ag after operation seems likely to result mainly from that of t-PA-PAI-1 complex. A significant increase of t-PA Ag and t-PA inhibition has been observed after major abdominal surgery (28, 29), or total hip replacement (30), and it is suggested that PAI-1 behaves like an acute phase plasma protein (28).

Acknowledgements

We wish to thank Dr. K. Okochi for critical review of the manuscript, Dr. Y. Sakata (Jichi Medical School) for helpful suggestions on the fibrin autography method and Drs K. Kamikaseda and R. Nakamura for allowing us to study their patients. This study was supported in part by grant from the Ministry of Education, Science and Culture of the Government of Japan.

References

1 White W F, Barlow G H, Mozen M M. The isolation and characterization of plasminogen activator (urokinase) from human urine. Biochemistry 1966; 5: 2160–9.
2 Rijken D C, Wijngaards G, Zaal-de-Jong M, Welbergen J. Purification and partial characterization of plasminogen activator from human uterine tissue. Biochim Biophys Acta 1979; 580: 140–53.
3 Wun T-C, Schleuning W-D, Reich E. Isolation and characterization of urokinase from human plasma. J Biol Chem 1982; 257: 3276–83.
4 Rijken D C, Wijngaards G, Welbergen J. Relationship between uterine tissue plasminogen activater and the activaters in blood and vascular wall. Thromb Res 1980; 18: 815–30.
5 Prowse C V, Cash J D, Path M R C. Physiologic and pharmacologic enhancement of fibrinolysis. Semin Thromb Hemostas 1984; 10: 51–60.
6 Northfield D W C. The Surgery of the Central Nervous System. Blackwell Scientific Publications, Oxford 1973.
7 Takashima S, Koga M, Tanaka M. Fibrinolytic activity of human brain and cerebrospinal fluid. Br J Exp Pathol 1969; 50: 533–9.
8 Koos W, Valencak E, Kraus H, Blumel G, Bock F. L'activité fibrinolytique relevée dans les tumeurs intra-craniennes. Neuro-Chirurgie 1971; 17: 549–58.
9 Tucker W S, Kirsch W M, Martinez-Hernandez A, Fink L M. In vitro plasminogen activator activity in human brain tumors. Cancer Res 1978; 38: 297–302.
10 Oka K, Tsuda H, Kamikaseda K, Nakamura R, Fukui M, Noutsuka Y, Sueishi K. Meningiomas and hemorrhagic diathesis. J Neurosurgery (in press).
11 Granelli-Piperno A, Reich E. A study of proteases and protease-inhibitor complexes in biological fluids. J Exp Med 1978; 148: 223–34.
12 von Kaulla K N, Schultz R L. Methods for the evaluation of human fibrinolysis. Studies with two combined technics. Am J Clin Pathol 1958; 29: 104–9.
13 Bergsdorf N, Nilsson T, Wallén P. An enzyme linked immunosorbent assay for determination of tissue plasminogen activator applied to patients with thromboembolic disease. Thromb Haemostas 1983; 50: 740–4.
14 Clauss A. Gerinnungsphysiologische Schnellmethode zur Bestimmung des Fibrinogens. Acta Haematol 1957; 17: 237–40.
15 Laemmli U K. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature 1970; 227: 680–5.
16 Rijken D C, Collen D. Purification and characterization of the plasminogen activator secreted by human melanoma cells in culture. J Biol Chem 1981; 256: 7035–41.
17 Deutsch D G, Mertz E T. Plasminogen: Purification from human plasma by affinity chromatography. Science 1970; 170: 1095–6.
18 Lundbald R L, Kingdon H S, Mann K G. Thrombin. In: Methods in Enzymology. Lorand L (ed), Vol 45. Academic Press, New York 1976; pp 156–76.
19 Tissot J-D, Schneider P, Hauert J, Ruegg M, Kruithof E K O, Bachmann F. Isolation from human plasma of a plasminogen activator identical to urinary high molecular weight urokinase. J Clin Invest 1982; 70: 1320–3.
20 Kruithof E K O, Tran-Thang C, Ransijn A, Bachmann F. Demonstration of a fast-acting inhibitor of plasminogen activators in human plasma. Blood 1984; 64: 907–13.
21 Stalder M, Hauert J, Kruithof E K O, Bachmann F. Release of vascular plasminogen activator (v-PA) after venous stasis: electrophoretic-zymographic analysis of free and complexed v-PA. Br J Haematol 1985; 61: 169–76.
22 Booth N A, Walker E, Maughan R, Bennett B. Plasminogen activator in normal subjects after exercise and venous occlusion: t-PA circulates as complexes with C1-inhibitor and PAI-1. Blood 1987; 69: 1600–4.
23 Booth N A, Anderson J A, Bennett B. Plasminogen activators in alcoholic cirrhosis: demonstration of increased tissue type and urokinase type activator. J Clin Pathol 1984; 37: 772–7.
24 Muramatu M, Fujii S. Inhibitory effects of ω-guanidino acid esters on trypsin, plasmin, thrombin and plasma kallikrein. J Biochem 1968; 64: 807–14.
25 Kwaan H C. The role of fibrinolysis in desease processes. Semin Thromb Hemostas 1984; 10: 71–9.
26 Wun T-C, Capuano A. Initiation and regulation of fibrinolysis in human plasma at the plasminogen activator level. Blood 1987; 69: 1354–62.
27 Rijken D C, van Hinsberg V W M, Sens E H C. Quantitation of tissue-type plasminogen activator in human endothelial cell cultures by use of an enzyme immunoassay. Thromb Res 1984; 33: 145–53.
28 Kluft C, Verheijen J H, Jie A F H, Rijken D C, Preston F E, Sue-Ling H M, Jespersen J, Aasen A O. The postoperative fibrinolytic shutdown: a rapidly reverting acute phase pattern for the fast-acting inhibitor of tissue-type plasminogen activator after trauma. Scand J Clin Lab Invest 1985; 45: 605–10.
29 Mellbring G, Nilsson T, Bergsdorf N, Wallén P. Tissue plasminogen activator concentrations in major abdominal surgery. Relationship to postoperative deep vein thrombosis. Thromb Res 1984; 36: 331–4.
30 Páramo J A, Alfaro M J, Rocha E. Postoperative changes in the plasmatic levels of tissue-type plasminogen activator and its fast-acting inhibitor – Relationship to deep vein thrombosis and influence of prophylaxis. Thromb Haemostas 1985; 54: 713–6.

Received May 17, 1988 Accepted after revision August 18, 1988

This document was downloaded for personal use only. Unauthorized distribution is strictly prohibited.