MINIREVIEW

The Physiology of the Fibrinolytic System

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The term hemostasis refers to the processes involved in the cessation of bleeding following injury. Hemostasis depends on a complicated interplay between serine proteases, protease inhibitors, cofactors, different types of cells and cellular receptors, extracellular matrix, and hemorheology such as blood viscosity and flow. Disturbances of the hemostatic balance may result in thrombosis, i.e. clot formation in the blood vessels, and a defect in coagulation such as hemophilia or accelerated fibrinolysis will result in bleeding tendency.

When blood vessel walls are injured by mechanical forces or due to the formation of atherosclerotic plaques, platelets are activated and adhere to these injured sites, and further form platelet aggregates. The activation of platelet results in the activation of coagulation cascade through the intrinsic and extrinsic pathways, leading to the formation of thrombin, which further activates platelet and converts fibrinogen to fibrin. The roles of thrombin in the coagulation cascades are schematically outlined in Fig. 1.

Fibrin clots in the blood vessels are normally effectively degraded and removed by the fibrinolytic enzyme system. The key enzyme is the serine protease plasmin, which degrades fibrin (rev. by Castellino [1]). Plasmin is formed from the inactive proenzyme plasminogen by the action of plasminogen activators. There are two kinds of physiological activators, one being tissue plasminogen activators (t-PA) synthesized in and released from the endothelial cells [2-4], and mainly responsible for the lysis of clots in the vessels, and the other type urinary plasminogen activator (u-PA) originally found in human urine [5, 6] and later found in plasma and many different cell types [7-10].

The regulation of fibrinolysis is effected by the production and secretion of such activators and specific inhibitors. Free circulating plasmin is rapidly inactivated by the specific inhibitor α_2 -antiplasmin (α_2 AP) [11-13], and t-PA forms a complex with plasminogen activator inhibitor-1 (PAI-1), which is also released from the endothelial cells [14, 15] (Fig. 2).

There are many publications on the biochemical aspects of fibrinolysis and therapeutic effects of thrombolytic drugs in occlusive vessel diseases such as myocardial infarction, cerebrovascular thrombosis, and venous thrombosis predisposing to pulmonary embolism. Fibrinolysis is, however, a physiological phe-

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Fig. 1. Roles of thrombin on the coagulation mechanism.

Fig. 2. Schematic representation of the fibrinolytic system. The proenzyme plasminogen is activated to the active enzyme plasmin by tissue-type plasminogen or urinary plasminogen activator. Plasmin degrades fibrin into soluble fibrin products. Inhibition of the fibrinolytic system may occur at the level of the plasminogen activators, by plasminogen activator inhibitors, or at the level of plasmin, mainly by α_2 -antiplasmin.

nomenon, which is constantly operating together with the coagulation system to secure sufficient blood flow to peripheral organs or tissues. We have examined effects of various physiological parameters on the fibrinolytic enzyme system, which will be reviewed together with results reported from other laboratories.

CHARACTERISTICS OF t-PA AND PAI-1

Before going into the physiological regulation of fibrinolysis, characteristics of two factors, t-PA and PAI-1, in fibrinolysis must be briefly reviewed [2-4, 14, 15].

Tissue plasminogen activator (t-PA)

t-PA is synthesized mainly in endothelial cells, but in other cells too. A human

melanoma cell line is often used for production of t-PA. The primary structure of t-PA has been determined by both protein and cDNA sequence analysis. The native protein is a single chain polypeptide containing 530 amino acid residues with a molecular weight of about 68,000. It is converted into a two chain molecule by a proteolytic cleavage by plasmin of the peptide bond Arg278-Ile279. Both single and two chain forms of t-PA have activator activity, although the two chain form has 7 to 8 times higher activity toward a small molecular weight substrate. The heavy chain ($M_r=38,000$) is the N-terminal portion and the light chain ($M_r=30,000$) is the C-terminal portion, containing the active center.

The heavy chain contains four domains, a finger domain homologous to similar structures in fibronectin, a growth factor domain, and two kringle domains. The finger and kringle 2 domains are involved in binding to fibrin. The activation of plasminogen by t-PA is significantly enhanced in the presence of fibrin. At physiological concentrations of plasminogen and fibrin the activation rate is increased 200–400 fold. t-PA and plasminogen forms a trimolecular complex with fibrin, facilitating the activation of plasminogen by decreasing K_m and increasing k_{cat} .

Plasminogen activator inhibitor-1 and -2 (PAI-1 and -2)

The existence of a specific inhibitor in plasma to plasminogen activators has been shown originally in plasma, then found in the conditioned media of cultured endothelial cells. PAI-1 is a single chain glycoprotein of a molecular weight of about 50,000 with an isoelectric point of 4.5–5.0. The molecule has 379 amino acid residues without cysteine residues, thus no disulfide bonds. PAI-1 belongs to a serpin family with the reactive center of Arg-Met. The regulation of PAI-1 activity is not clear yet. A variety of compounds are known to stimulate PAI-1 release in cell culture system: endotoxin, thrombin, TGF- β , interleukin-1, TNF, glucocorticoids, and basic FGF. Physical stimuli are, however, not identified.

PAI-2 is not usually present in the plasma except at the late stage of pregnancy. PAI-2 has been purified from human placenta, but is now considered to be produced from monocyte/macrophage. The importance of PAI-2 has been recognized with respect to the regulation of tumor growth which will be reviewed later. PAI-2 has a molecular weight of 70,000, and belongs to a serpin family with the reactive center of Arg-Thr.

AGE

It is well known that the incidence of thrombotic diseases increases in older age. Fibrinolytic activity appears to be present in the blood early in fetal life [16]. Plasminogen has been detected in the blood of 10.5 week embryo, levels rising through fetal life [17]. Plasminogen concentration is, however, low in the newborn, usually around 50% of adult values [18], and reaches the adult levels rapidly [19]. There are also many reports suggesting increases in various coagula-

tion factors in the aged. We have measured t-PA antigen, PA activity, and PA inhibitor activity in various age groups [20]. Antigen levels of t-PA increased with age. Since t-PA exists not only in the free form but in the complex with various inhibitors such as PAI-1 or α_2 AP, there is a possibility that an increase in t-PA antigen is due to an increase in t-PA-PAI-1 complex, thus active t-PA levels decrease. We then measured plasma levels of PA inhibitor activity and PA activity, the latter representing the concentration of free t-PA levels [21]. PA inhibitor activity increased and PA activity decreased with age, which correlates well with the higher incidence of thrombotic disease in the aged. Plasma plasminogen levels did not change between 2 years old to 90 years old [22]. We have examined the contents of serotonin (5-hydroxytryptamine: 5HT) in platelets of males of various ages. Serotonin contents in platelets decreased with increase in age [23], which may mean impaired uptake into and enhanced release of serotonin from platelets [24].

SEX

It has been repeatedly shown that the incidence of thrombotic disease is less in females than in males. A possible explanation lies in the different endocrine make-up of men and women. In fact, the cardiovascular disease incidence was lower in premenopausal than postmenopausal women [25]. We have shown [26] that plasma levels of t-PA antigen were higher in males than females, and those in females gradually increased with increase in age, reaching the same levels as those of males at their 60s. Plasma levels of t-PA-PAI-1 complex were also higher in males than females, indicating that t-PA was mainly in the form of a complex with PAI-1 in males. Plasma levels of free PAI-1 were higher in males than in females up to their 60s, suggesting that male blood was less fibrinolytic than female blood. In fact euglobulin clot lysis time was faster in females than in males [27].

CIRCADIAN FLUCTUATION

There is a circadian variation in the incidence of stroke, myocardial infarction, and sudden death. The analysis of data revealed the high incidence of onset of pain of myocardial infarction between 6 a.m. and 12 noon [28]. The higher vascular tone in the morning was implicated in the high incidence of myocardial infarction [29]. The possibility exists, however, that there is high coagulability and low fibrinolytic activity in the morning. Fearnley and his co-workers first noted the circadian variation of fibrinolytic activity [30], showing that fibrinolysis was considerably higher in the afternoon. Since then many groups of workers have confirmed the results. Analysis of the blood fibrinolytic components indicated that t-PA activity increased from 9 a.m. to 3 p.m., and that both t-PA and PAI-1 antigen levels decreased in the afternoon [31]. We have shown [27] that euglobulin clot lysis time (ELT) was longer in the morning and gradually shortened in the

afternoon in both males and females, ELT of females being shorter than that of males. Plasma levels of t-PA, free PAI-1, and t-PA-PAI-1 complex were high in the morning, decreasing towards evening. Those values were lower in females than in males. Similar circadian variation of fibrinolytic parameters was recognized in patients with angina pectoris [32]. It is now clear that higher fibrinolytic activity in the afternoon is mainly due to the decreased release of PAI-1 from endothelial cells, not due to increase in t-PA release.

Higher coagulability in the morning may also be related to the higher activity of platelet. Serotonin contents of platelets are higher in the morning and decrease in the afternoon [23]. Serotonin released from platelets causes the contraction of smooth muscle of blood vessel, which may be one of the reasons for higher vascular tone in the morning.

ALCOHOL, SMOKING, AND MENTAL STRESS

There has been strong interest among epidemiologists in the relationships between risk factors and atherosclerosis and occlusive cardiovascular disease. As to effects of alcohol ingestion on fibrinolysis, Morgagni [33] reported hyperfibrinolysis in a heavily drunken man. Meade *et al.* [34] showed that fibrinolytic activity was raised by habitual consumption of moderate amounts of alcohol. Several epidemiologic studies have demonstrated a negative correlation between alcohol consumption and fatal ischemic heart disease. This preventive role of ethanol has been attributed to increase in the concentration of high-density lipoprotein (HDL) in the plasma [35] as well as to the decreased aggregability of platelets [36, 37]. We have examined the acute effects of alcohol ingestion in plasma fibrinolytic activity [38] and platelet serotonin metabolism [39]. Ingestion of 250 ml of whisky (43% alcohol, v/v) did not result in any change in fibrinolytic parameters such as t-PA or PAI-1 in the plasma compared with controls who took tea. Euglobulin clot lysis time prolonged on the next morning due not to decrease in the release of t-PA but to the increase in the release of PAI-1. On the other hand, platelet activity including serotonin uptake was inhibited immediately after alcohol ingestion [39]. One half of alcohol's protection action against coronary heart disease appears to be caused not by hyperfibrinolysis but increased HDL levels [40], and the rest will be mediated by significant alcohol-induced inhibition of platelet function as its aggregation, adhesion, and thromboxane A₂ release.

In comparing groups of heavy smokers, Billimoria *et al.* [41] reported on decrease in fibrinolysis which concords with many other researches. There were no reports on the effects of short-term smoking, although no change in plasma fibrinogen has been noted following one-cigarette smoking [42]. Our results on the effects of short-term smoking indicated that both PA and PAI-activities increased after cigarette smoking. Especially plasma t-PA antigen levels increased after smoking. Plasma epinephrine levels also increased, which may be related to the increased release of t-PA after cigarette smoking.

Physical and mental stress were reported to influence the activity of coagulation (reviewed by Marsh [43]). Macfarlane and Biggs [44] noted that nearly 50% of patients showed increase in fibrinolytic activity when given premedication prior to an operation. The fear of the impending operation was considered to be the major factor in influencing fibrinolysis. The impending venopuncture and medical examination were used to test fibrinolysis, confirming that fear was the factor causing enhanced fibrinolysis, possibly through change in sympathetic nervous activity. We took advantage of the bleeding time test in which students cut the ear of their colleagues and measured time for the cessation of bleeding [45]. The procedure was fearful because students did not know the extent of pain that would be incurred by the incision, and the precision of the technique used by their non-skilled friends. We intentionally stressed the fact that the incision must be large enough to secure a reasonable extent of bleeding. The results indicate that students showed dramatic increase in fibrinolysis as measured by shortening of ELT even after the verbal explanation of the procedure. The test of the bleeding time itself also shortened ELT, but to less extent compared with the effects of verbal explanation. The analysis of change in fibrinolytic parameters revealed decrease in the release of PAI-1 from endothelial cells. When platelet responses were analyzed, increase in the response to serotonin, and ADP was noted. Plasma levels of serotonin, epinephrine, and norepinephrine increased after bleeding time procedure [46]. It is possible that mental stress enhanced fibrinolytic activity by lowering plasma levels of PAI-1, which may be caused by changes in sympathetic nerve activity.

EXERCISE

Since Biggs *et al.* [2] reported on enhanced fibrinolysis upon exercise, many groups demonstrated increase in blood fibrinolysis using a wide variety of exercise forms. Recently the quantitative procedures of treadmill and bicycle ergometer tests were employed. Increase in fibrinolysis was shown to be due to a rise in plasminogen activator level [47]. The mechanism of exercise-induced fibrinolysis is poorly understood. Cash and Allan [48] originally proposed adrenergic stimulation to be responsible for the release of plasminogen activator. If changes in plasminogen activator, pulse rate, and catecholamine level are examined at intervals after exercise, plasminogen activator and pulse rate rise in parallel with increase in workload, whereas catecholamines increase only after exhaustive exercise [49]. Furthermore, when β -blockers are used at the time of catecholamine infusion, plasminogen activator response can only be partially blocked whereas the release of von Willebrand factor from endothelial cells has been totally abolished [50]. Similar results have been observed in exercise, thus it is now suggested that fibrinolysis induced by exercise is not mediated via β -adrenergic receptors [51].

Except for changes in plasminogen activator after exercise, little changes have been reported in plasma levels of fibrinogen and plasminogen [52]. Antiplasmin

levels hardly change [53]. As to plasma levels of fibrinogen degradation products (FDP), there is still controversy. Some workers have been unable to show any change in serum FDP concentration, but others have shown two- to fourfold increase.

We have examined changes in fibrinolytic parameters with myocardial infarction. Plasma levels of t-PA increased and PAI-1 decreased after exercise in normal persons [54]. Basic levels of PAI-1 were higher in patients with myocardial infarction. In fact one of our patients showed a high level of PAI-1 just before the onset of myocardial infarction [55]. When comparison was made between patients with single vs. multivessel disease, PAI-1 levels were high in patients with multivessel disease. The extent of increase in t-PA after exercise was less in patients with multivessel disease [56]. PAI activity as well as plasma levels of PAI-1 decreased more after exercise in patients with multivessel disease, which may mean that exercise is of protective value in patients with myocardial infarction.

EMOTION AND ITS DISORDERS

Some lines of evidence suggest that disturbances in the regulation of circadian rhythms may be of prime importance in the pathophysiology of depression [57, 58]. It has been also reported that patients with affective disorders have a higher than expected rate of mortality from cardiovascular disease [59, 60]. Further evidence suggests that major depression has a direct negative impact on the outcome of cardiovascular disease [61]. We have measured change in fibrinolytic parameters in patients with neurosis or depression [62]. Patients with depression and neurosis had lower levels of t-PA in plasma. Neurotic patients seemed to have lower levels of t-PA than depressive patients. Plasma levels of free PAI-1 and t-PA-PAI-1 complex were lower in depression and neurotic patients than in normal controls. ELT was shorter in depressive and neurotic patients, mainly due to the lower release of PAI-1 from endothelial cells. These results show that fibrinolytic activity of depressive and neurotic patients was enhanced, which findings cannot be consonant with epidemiological evidence identifying thrombogenesis in such patients as a cause of cardiovascular disease.

We then measured circadian fluctuations of plasma fibrinolytic activity as well as substances related to serotonin [63]. Clinical studies have provided evidence that serotonin is implicated in the state of depression as its precursors, and selective inhibitors of its uptake are very effective in its treatment [64, 65]. As human blood platelets may be regarded as a reliable model for serotonergic synaptosomes because of their specific biochemical mechanisms for uptake, storage, metabolism, and release of the amine, the whole blood measurement of serotonin is considered to provide a peripheral model for the study of serotonergic procedures in the brain [66, 67]. Since there is some evidence that disturbances in the regulation of circadian rhythms may be of prime importance in the pathophysiology of affective disorders, we examined whole blood serotonin at various times of the day in

Fig. 3. Diurnal variations in whole blood serotonin concentrations. Each points represents the mean $\pm$ SE.

psychiatric patients in hospital and control volunteers. Whole blood serotonin levels, which nearly represent platelet serotonin, were high in the morning in normal persons and gradually decreased in the afternoon. On the other hand, blood serotonin levels were low in the morning in depressive and neurotic patients, and gradually increased, which indicates differences in diurnal pattern of blood serotonin between patients with affective disorders and normal persons [68] (Fig. 3). Blood concentration of 5-hydroxyindole acetic acid (5-HIAA), a major metabolite of serotonin, also showed a circadian fluctuation. In depressed patients group, the highest value was observed at 8:30 a.m. and decreases in the afternoon. In the control group, the lowest value was at 8:30 a.m. and increased progressively in the afternoon. These patterns were exactly in a minor image in comparison to the patterns of blood serotonin content. Since plasma 5-HIAA was not only produced and released from platelets but also directly from the brain [69], the higher levels of plasma 5-HIAA in depressive patients do not necessarily mean higher monoamine oxidase activity of platelets.

Blood tryptophan levels were significantly lower in depressive patients. The patterns of the circadian fluctuation of tryptophan were similar between depressive patients and normal controls, the plasma levels being higher in the morning and lowering in the afternoon. Plasma levels in both t-PA and PAI-1 were lower in depressive patients, their levels in both depressive patients and normal controls being high in the morning and lowering in the afternoon.

These results clearly show that platelet serotonin metabolism and plasma fibrinolytic activity were influenced in states of affective disorders.

NUTRITION AND BODY WEIGHT

In general, many data suggest that lipemia inhibits fibrinolysis. However, the mechanism of fat-induced inhibition is not elucidated. In lipemia, there is chylomicron in plasma, which is known to have antifibrinolytic activity *in vitro* and *in vivo*. Beta-lipoprotein, which rises after fat ingestion, also has antifibrinolytic activity. Hamsten [70] shows that increase in plasma levels of triglyceride results in increase in plasma levels of PAI-1 concentration. In the case of high triglyceride levels, VLDL of larger particle sizes caused the release of PAI-1 from cultured endothelial cells [71]. We have had volunteers take butter (50 or 100 g) at 8:30 and measured fibrinolytic parameters and platelet aggregability. It was shown that ELT of volunteers loaded with 100 g of butter significantly prolonged at 14:30 and 16:30 compared with controls or 50 g butter loaded volunteers. PAI-1 also significantly increased at 10:30, 2 h after fat intake, which means that triglyceride induced the release of PAI-1 [72]. Platelet response to ADP and collagen was inhibited. These results suggest that lipemia itself is thrombogenic with respect to fibrinolysis, but antithrombogenic in platelet activity. Probably, the prolonged presence of lipemia results in atherosclerosis, which causes thrombosis.

There is a general agreement on decrease in fibrinolytic activity in obesity. There was an inverse relationship between body weight and fibrinolytic activity in normal persons [34, 73]. The reduction of fibrinolysis in obese people seems to be mainly due to decrease in plasma levels of t-PA [74, 75]. Fibrinolysis was not only reduced in a resting condition, but the response to exercise or venous occlusion was also impaired in obesity. Ogston and McAndrew [76] showed that dietary restriction resulted in increase in fibrinolytic activity in both obese and normal subjects.

We measured various fibrinolytic parameters in normal controls and diabetic patients. Body weight positively correlated with t-PA, PAI-1, and ELT in normal subjects [77]. The prolongation of ELT is considered to be caused by increase in plasma levels of PAI-1 [78]. Juhan-Vague and Alessi [79] indicated that PAI-1 was correlated with insulin levels in both normal subjects and diabetic patients. Apparent correlations between PAI-1 and body weight or triglyceride levels may reflect the fact that insulin levels were correlated with both body weight and triglyceride levels.

In diabetic patients, correlations between body weight and fibrinolytic parameters were obscure in contrast to good correlations in normal subjects [80]. It is suggested that the regulatory mechanisms which underlie the relation of body weight to fibrinolytic parameters are impaired in diabetes mellitus.

LIPOPROTEIN(a)

Coronary heart disease is caused by clot formation on the atherosclerotic

plaques of the artery. There are several risk factors attributed to the incidence of coronary heart disease: high cholesterol, high diastolic pressure, and cigarette smoking. It has been known that a genetic make-up plays a role. In 1963, Berg discovered a variant of LDL called lipoprotein(a) (Lp(a)) [81]. The amounts of Lp(a) in the plasma varies from undetectable to 100 mg/dl. High levels of Lp(a) are strongly associated with atherosclerosis. cDNA cloning and the studies on the structure of Lp(a) revealed that Lp(a) consists of LDL-like core joined to B-100 by a disulfide linkage. Another protein, called apolipoprotein(a), is linked to B-100 by a covalent bond. Apolipoprotein(a) was found to be very similar in structure to plasminogen. Apolipoprotein(a) consisted of kringle structure (K4) repeated more than 30 times, one kringle 5 and protease domain, which lost protease activity by mutation. Because of the structural similarity between plasminogen and apolipoprotein(a), it has been suggested that Lp(a) induces atheromatous plaques by competitive inhibition of fibrinolysis or binding of Lp(a) to fibrin formed on injured vessel walls.

We have measured Lp(a) in normal subjects and diabetic patients. Lp(a) was negatively correlated with body weight, but not correlated with plasma levels of PAI-1 or ELT. In diabetic patients, plasma levels of Lp(a) were higher than in normal subject. Lp(a) was not correlated with ELT in either normal or diabetic subjects. It is our suggestion that Lp(a) may not function through the inhibition of fibrinolysis to accelerate atherosclerosis.

ROLES OF FIBRINOLYSIS IN CELL GROWTH AND MIGRATION

It was shown that activated macrophages and transformed tumor cells produced u-PA [82, 83]. Since then numerous reports have been published concerning the roles of plasminogen activator in cell growth and migration, especially metastasis of tumor cells. The presence of u-PA, PAI-1, and PAI-2 in tumor cells has been shown by the quantitative determination of these factors and immunohistochemical stainings. We have shown increases in u-PA, PAI-1, and PAI-2, but no change or rather a decrease in t-PA in tumor tissue in stomach cancer [84], ovarian and uterine tumor [85], breast cancer [86, 87], and colon cancer [88]. The mechanisms by which these parameters function with respect to tumor progression are poorly understood. There are three hypotheses: (1) proteolytic degradations of the matrix by plasmin converted from plasminogen by u-PA, (2) the activation by plasmin of procollagenase to collagenase, which digest collagen in the matrix and the basement membrane of the blood vessels, (3) the stimulation of tumor cell growth by the binding of u-PA or u-PA-PAI-1 complex to the receptor of u-PA. Recently roles of u-PA receptor (u-PAR) have attracted much attention. u-PAR provides not only a mechanism to confine and localize u-PA at the cell surface, but enables the activation of pro-u-PA and plasminogen, thereby rendering the cell surface favorable for the generation and activity of plasmin. In a variety of human tumors, u-PA and u-PAR are consistently at invasive foci, either expressed in the

same or adjacent cells.

If roles of u-PA are to activate plasminogen, the inhibition of u-PA by PAI-1 should result in the inhibition of proliferation and metastasis of tumors. The concentrations of u-PA and PAI-1 in tumor tissues are higher in proportion to the tumor size, and tumors with metastasis have large amounts of u-PA and PAI-1. On the other hand, tumors with high levels of PAI-2 stay in smaller sizes and are less metastatic [87, 88]. These results may indicate that roles of PAI-1 and PAI-2 in tumor growth and metastasis are different, PAI-1 being facilitative, and PAI-2 being inhibitory. There are various hypotheses to explain differences of functions of PAI-1 and PAI-2, but further studies are definitely needed.

PREGNANCY AND PUERPERIUM

There is a general consensus that fibrinolysis decreases during pregnancy [89, 90]. The decrease starts at about the twelfth week of pregnancy and continues up to around the 26th week, thus reaching a plateau. Euglobulin clot lysis time prolongs at these periods. It is claimed that plasma levels of plasminogen activator inhibitors remain constant, and that there are marked increases in the levels of α_2 -macroglobulin and α_2 -antiplasmin [91]. Because of the diminished response to venous occlusion, it is suggested [92] that impairment of the synthesis and release of t-PA may be the major cause of the decrease of fibrinolysis during pregnancy.

During labor, in spite of strenuous physical activity and mental stress, fibrinolytic activity remains low; however, fibrinolysis abruptly increases after delivery. Such rise takes place before the umbilical cord is clamped and while the placenta is still *in situ* [89]. Placental separation seems to be much responsible, however, for the further rise in fibrinolysis at parturition. We have measured plasma levels of t-PA and u-PA in pregnant women, and found either t-PA or u-PA levels did not increase significantly in the first and second trimesters, but both activators increased after the third trimester of pregnancy. The high levels of PAs continued throughout the first stage of labor. The levels of t-PA further increased for the first few hours after delivery, whereas the levels of u-PA returned to normal immediately following childbirth. In order to know the roles of the uterus or placenta in the elevated levels of PAs, we took samples from the uterine venous blood. There was no significant difference in the levels of t-PA antigens in between peripheral and venous blood in the third trimester of pregnancy. The levels of t-PA were higher, however, after than before delivery. The results indicate that the uterus is involved in the high levels of t-PA in the circulation. Further increase in t-PA in the post partum may be explained by the possibility that the involuting uterus produces large amounts of t-PA. As stated above, the separation of the placenta may partly be responsible for the high levels of t-PA in the circulation due to the damage to the endothelium in the placental bed.

Plasminogen activators are implicated also in ovulation. Roles of plasminogen activator in the follicle rupture has been proposed, based upon the observations that

plasmin is capable of weakening follicle wall strips *in vitro* [93], and that increasing amounts of plasminogen activators are produced by rat granulosa cells toward the time of ovulation [94]. We have induced the ovulation in rat by the injection of human chorionic gonadotropin (HCG), and measured the concentration of PA in the ovarian extract [95]. The concentration of PA dramatically increased up to just before the ovulation and then dropped to low levels after the ovulation. These processes were shown to be independent of prostaglandin systems [96]. These results also raise the possibility that PA is involved for the degradation of the supporting structure such as collagen or elastin, and leading to the rupture of ovarian follicles.

FIBRINOLYSIS IN PHYSIOLOGY

Fibrinolytic system has evolved to remove fibrin clots formed at injury, and to facilitate blood flow to secure recanalization of a blood vessel. Blood clots are not only formed at the site of vascular injury, but on the atheromatous plaques, leading to thrombosis. Research interest has focused on the therapeutic effects of thrombolytic agents such as streptokinase (SK), u-PA, or t-PA. Recent progress in biotechnology has enabled production of recombinant t-PA and scu-PA (pro-UK). Great hope developed in the use of t-PA for the treatment of thrombosis of coronary artery or cerebrovascular disease, because t-PA was known to be very fibrin specific in the expression of its activity. Former agents such as SK and u-PA have been said to be non-discriminatory towards fibrin and fibrinogen. The degradation of fibrinogen due to the activation of plasminogen by SK or u-PA was considered to cause bleeding side effects. Activation of plasminogen in the plasma also results in the depletion of inhibitors such as α_2 -antiplasmin, which, in turn is considered responsible for the bleeding due to the lack of the regulation of plasmin activity.

Clinical trials, however, have revealed unexpected results with the use of t-PA in the treatment of myocardial infarction [97]. Two randomized trials of SK and two kinds of recombinant t-PA showed no significant difference in mortality between SK and t-PA. Furthermore there was a significant excess of cerebrovascular bleeding in t-PA usage. Four extra strokes per 1,000 patients were observed in t-PA groups. In order to overcome the shortcomings of t-PA, many mutants were

Fig. 4. Protein family tree. This tree shows a hypothetical reconstruction of the evolutionary relations within an extended group of molecules that includes apolipoprotein (a) and plasminogen. All the proteins are derived from an ancestral protease. Molecules with new properties arose through the addition, deletion, or duplication of functional domains in the proteins and also through the gradual accumulation of amino acid substitutions through mutations. P, protease; K, kringle; Pr, propeptide; C, calcium binding domain; E, EGF domain; F', fibronectin' domain; F'', fibronectin'' domain.

developed. Insertion and deletion of various domains of t-PA, hybrid molecules of t-PA with scu-PA, and molecules without one or more carbohydrate moieties have been tried [98]. Thus far the results are not satisfactory yet. The major reason for the failure of the modification of the molecule to a more effective one is the lack of the autonomy of each domain of t-PA. Change in one domain influences the other domain of t-PA, resulting in a molecular form with unexpected effects.

These experiences obtained from therapeutic trials called our attention to the fact that many mechanisms are unknown yet as to how fibrinolysis works. Without knowing the mechanisms of physiological fibrinolysis, clinical trials may fail due to unexpected outcomes.

Molecules involved in coagulation and fibrinolysis were considered to have evolved from ancestral protease similar to trypsin [99]. The addition of kringle domains to the protease domain gave rise to plasminogen and apolipoprotein(a), and the addition of finger, EGF (epidermal growth factor), and two kringle domains resulted in the formation of t-PA (Fig. 4). Out of these molecules (t-PA) and its inhibitor (PAI-1) are synthesized and released from endothelial cells of the blood vessels, and many stimuli bring about the release of these factors. Physiological stimuli to control the synthesis and release of t-PA and PAI-1 are still far from clear. Renewed efforts to clarify the physiological regulation of fibrinolysis are expected in the future.

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