The Relationship between Fibrinolysis and Haemorrhagic Disorders

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Introduction

It is well known that the appearance of haemorrhagic disorders has close relationship with the character of blood vessels and their surrounding tissue, functions of platelet and other blood corpuscles and levels of blood coagulation factors; among them the haemorrhagic diathesis caused by the lysis of fibrinogen and/or its clotted product, fibrin, has been recognized its significant rôles in physiological and pathological phenomena inside of living body and has drawn increasing attention from various sides of biology and medicine. Actually we often observed spontaneous dissolution of experimentally manufactured thrombi or disappearance of clinically found bleeding spots and haematomas. This is the subject of the present paper, fibrinolysis.

History of fibrinolysis and its reaction mechanism

He was Dastre (1893) who recognized the phenomenon, fibrinolysis, for the first time from the scientific stand-point of view and Macfarlane (1936) made a bridge between this phenomenon and clinical symptoms, who found that a blood coagula from a cholestectomized patient was dissolved at 37° Centigrade over a night. Subsequently he also found the same type of phenomenon caused by activation of fibrinolysis system by anesthesia, strong somatic exertion and psychic excess stress. Besides them this fibrinolytic activity was found also elevated in various kinds of disease, such as anemia,

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leukemia, cancer, etc., and some of the activating substances were discovered to exist in the cultivating mixtures of a certain kinds of microorganism, for instance, β-streptococcus haemolyticus and so on. The most popular substance of this category is streptokinase (SK) and materials of the same kind or which could be activated by SK, have been found in human plasma and serum. The reaction mechanism of activation in this system can be formulated as shown in Fig. 1: the fibrin dissolving reagent, plasmin (PL), is contained in blood as an inactive precursor, plasminogen (PLg), which is activated by several activators (Act) from different sources, particularly by what is developed from proactivator (Proact) by SK or staphylokinase or by what is activated from its precursor in tissues by motives, such as fevering and other kinds of biological reactions.

Fig. 2 is a modelling formula of blood coagulation and has remarkable similarity to that of fibrinolysis in Fig. 1. As can be seen, both mechanisms have two types of reaction, one of which is in blood and of generalized nature and the other is in tissues and of local nature; although these two types of reaction can be distinguished to some extent, they keep close harmonious correlation to each other and demonstrate interestingly a certain biological reaction to stress as a whole body.

Figs. 3 and 4 show the development of fibrinolytic activity at the local site of injection and in blood in the course of experimental inflammation in mice which was induced by intramuscular injections of terpentin oil and in dogs which had injections of nicotinic acid, respectively. In tissues, Act was activated first and then PLg and PL, which were spread over to periphery in the course of time, and after the injection of nicotinic acid the fibrinolytic activity in blood was elevated very rapidly.

Here it must be emphasized as a characteristic reaction of living body that whenever fibrinolytic activity is elevated, inhibitor(s) (Inh) against the fibrinolytic agent(s) is produced simultaneously or with a little bit lag behind it. Fig. 5 demonstrates the shift of the fibrinolytic activity in rabbits before and after intramuscular injections of SK in which remarkable elevation was proved after the injections when the euglobulin fraction (eug), precipitated at pH 5.2, was checked, but not so much when plasma+SK was tested:
this might suggest the possibility that after the SK injections Inh(s) had been produced in rabbit blood against SK, PL and/or split substances induced by these reagents. The same thing could be said with human who developed the more inhibitory activity against SK in the electrophoresis fractions, particularly in albumin and α-globulin of serum, deprived after the more SK was administered, as seen in Fig. 6. The fibrinolytic enzymes and their Inhs behave just like the shape and shadow, and they build up a certain equilibrium, a sort of so-called homeostasis, sometimes cooperating and sometimes antagonizing.

**Relationship between Fibrinolysis and Haemorrhagic Diathesis**

a. The influence of fibrinolytic enzymes upon haemostasis system

In order to scrutinize the correlation between fibrinolysis system and haemorrhagic diathesis, the changes of coagulation and fibrinolysis systems were followed before and after 100,000 units of PL was injected dropwise into a patient of thrombosis of vena centralis retinae sinistra intravenously and it was found that bleeding, clotting and prothrombin times were all prolonged and the platelet count was decreased, but little changes in thromboplastin generation time, total protein and fibrinogen content in blood were proved after PL administration, as shown in Fig. 7. The fibrinolytic activity, however, was increased both in eug and in plasma+SK.

As to the influence of fibrinolytic enzymes to blood corpuscles, platelet was tested as it had the most dominant relationship with coagulation and haemorrhage. Several platelet plasmas, prepared by different kinds of anticoagulant, were stored in cold room for 4 weeks and checked about their platelet count and the fibrinolytic activity of the platelet poor plasmas every week. Figs. 8 and 9 show that in the course of storage, number of platelet was more decreased in the plasmas with stronger fibrinolytic activity, for instance, in dextran sulfate plasma.

Concerning the effect of fibrinolytic enzymes on blood capillary, decrease of capillary resistance, elevation of passage and induction
of oozing haemorrhage were already reported and, as a matter of fact, there can be recognized definite causal relationship between fibrinolysis system and blood coagulation factors, platelet, capillary wall and inhibitor formation, but in actual cases the dynamic equilibria among these components are hardly disturbed. At any rate the outbreak of haemorrhagic disorders due to fibrinolysis system must be conditioned by the disturbance of homeostasis in the body.

b. The shift of fibrinolysis system at biological reactions

Prof. Tasaka classified the typical process of metabolism in living body at stresses into the following three categories: (1) increase of histamin, adrenaline and nor-adrenaline secretion, (2) mobilization of steroid hormones and (3) influence onto liberation of thyroid hormone. These mechanisms play very important rôle for the maintenance of equilibria in biological functions with good concord with the nervous control on them, as is well known.

Fig. 10 shows the change in the clotting and fibrinolytic systems which follow the intramuscular injection of adrenaline into a rabbit and demonstrates a temporary shortening of clotting time, slight decrease of total protein and fibrinogen content in plasma, prothrombin time and fibrinolytic activity of eug+SK on standard plates, but remarkable decrease of antifibrinolytic activity.

On the other hand, when acetylcholine was injected intramuscularly into a rabbit, as shown in Fig. 11, the clotting time was prolonged deeply after a momentary acceleration and finally blood came to no coagulation, and total protein and fibrinogen content diminished significantly and the fibrinolytic activity elevated profoundly. Summarizing all these data, it could be said that adrenaline evoked relatively mild and acetylcholine profound biological reactions in rabbits and invited activation of fibrinolysis system in these cases, particularly sclerotic bleeding and further shock state in the latter one.

The effect of steroid hormones on fibrinolysis system has been discussed controversially, promoting or inhibitory. Pyrogenic substances from microorganisms, such as TTG and Pyrexal, were injected into rabbits to induce fevering and activation of fibrinolysis system, as seen in Fig. 12, and prodnisolone was also injected to find their interference on the reaction of the animals. Obviously
fevering was suppressed by prednisolone and fibrinolytic activity also inhibited as shown in Fig. 13, but the level of Inh, as figured in Fig. 14, was elevated temporarily and then depressed to lower than the initial levels. Of course, the possibility must be taken into account that the inhibitory effect of prednisolone on fibrinolysis system might be secondary sequence of its suppressing activity on fevering. In order to make sure of these points, plasmas, sera and their eug were prepared at one and two hours after the prednisolone administration and their antifibrinolytic activity was checked to prove that all these samples contained 17-OHCS as shown in Fig. 15 and this steroid factor had special affinity to γ-globulin fraction of serum, exhibiting the most powerful inhibition, as seen in Fig. 16.

Among steroid substances, a pyrogenic hormone, Etiocholanolone (EC) evoked fevering and accompanying activation of fibrinolysis at 6 to 8 hours after its intramuscular injection into human subjects exclusively, as seen in Fig. 17, and the activity of Inh(s) was also strengthened subsequently, but when hydrocortisone was injected simultaneously or at a lapse of 4 hours from EC injection, fevering and elevation of fibrinolytic activity were obviously suppressed and disappeared sooner than in the control cases, as seen in Fig. 18.

Furthermore the influence of sexual hormones having similar structures to the above mentioned hormones onto fibrinolysis system was also very interesting and instructive. Fig. 19 shows the clinical course of a patient of prostate hypertrophy who was proved elevation of fibrinolytic activity and haemorrhagic diathesis. In this patient, a female gland stimulating hormone suppressed fibrinolysis system whereas a protein anabolic hormone, which had secretion stimulating effect on male glands, restored this depressed fibrinolysis to the initial level. As you could understand, some steroid hormones with similar structure showed contrary actions on fibrinolysis system and their basic mechanism must be studied further on.

The next substance in biological reactions is thyroid hormone. In Fig. 20 thyroxin was injected to a rabbit intramuscularly solely or with adrenocorticotropic hormone (ACTH) and it was found that fibrinolysis was not changed at plasma+SK, but remarkably increased
at eug+SK by a thyroxin injection which was minimized by simultaneous administration of ACTH, and a same type of shift was observed with Inh for fibrinolysis system as seen in Fig. 21. In order to scrutinize the reaction mechanism of thyroxin, an aliquot of a dilution series of thyroxin was placed over an equal aliquot of a standard PL solution on fibrin plates and it was proved that the lysis area on standard plates was decreased at higher concentration of thyroxin, but no difference was recognized between the samples with different concentration of this on heated plates, as shown in Fig. 22; in other words, thyroxin was imagined to have inhibiting effect on Act.

The relationship of fibrinolysis system with reticuloendothelial system, which has significant influence on biological reaction, seems to be also interesting from this point of view. Rabbits were injected Indian ink or nitrogen mustard N-oxide (NM) intravenously as preliminary treatments, then immunized with dysentery vaccine for a fortnight and their fibrinolytic activity was measured throughout these period. As seen in Fig. 23, the antifibrinolytic activity in serum was elevated by Indian ink treatment but unchanged or decreased by NM and then depressed by the immunological treatment sooner or later in both cases; in other words, fibrinolysis system in rabbit sera showed a tendency of elevation finally in all cases. Among the protein fractions of these sera, β-globulin had the most intimate concord with the shift of antifibrinolytic activity as seen in Fig. 24. We have so far observed that all the serum fractions, albumin, α-, β- and γ-globulins could show antifibrinolytic influence and realized that further studies were needed on the line of Inh.

Herewith, ε-aminocaproic acid (ε-ACA) must be also mentioned as an Inh for fibrinolysis system which has special reactivity with PL and its Act system, showing no inhibitory activity on other proteolytic enzymes such as trypsin and so on, as seen in Fig. 25. When it was injected into human intravenously at the dose of one to four grams, it exhibited immediate antifibrinolytic influence and maintained activity for about 4 hours, being excreted through kidney. When this substance could prove therapeutic effect on haemorrhagic disorders in patients, as described in the following
chapter, who had higher fibrinolytic activity and bleeding symptoms, this haemorrhage might be able to be attributed to the higher level of fibrinolysis system.

**Fibrinolysis system in haemorrhagic patients**

The first case was a 19 years old male patient of chronic myelogenous leukemia (Fig. 27). Together with severe epistaxis and purpura, remarkable elevation of fibrinolysis system was proved and the administration of ε-ACA with various kinds of antileukemic agents and steroid hormones improved his bleeding tendency significantly, depressing the fibrinolytic activity. In the course of this therapy, however, haemorrhage came back to him inspite of the sufficiently depressed fibrinolysis system, but the increase of ε-ACA dose saved this bleeding again. Of course, the bleeding tendency in the cases of leukemia could not be explained exclusively by the elevation of fibrinolysis system, but it must tell some real circumstances that this substance stopped the bleeding in the case directly by suppression of fibrinolytic activity or indirectly through this effect.

The next case was a 53 years old male patient of essential kidney bleeding (Fig. 28). No special organic defect was unveiled throughout several prudent urological examinations and haematuria was not influenced by many haemostatics, so that ε-ACA was administered and proved its effect on this haematuria, particularly no bleeding was revealed since a daily dose of 8 grams was given through mouth at intervals of 6 hours, and this non-haematuria continued until today.

The third female patient was 19 years old and complained of epigastrial pain at hunger and tenderness of breast, back and abdomen. Meanwhile she noticed red coloration of urine and was admitted to our hospital on account of severe haemorrhage and anaemia. A blood examination revealed remarkable decrease of fibrinogen content as well as anemia and elevation of fibrinolytic activity in blood; blood transfusion and many haemostatic could not give her so much therapeutic effect that ε-ACA was employed to decrease purpura, particularly significantly at the combination with steroid hormones.
In the course of sickness, however, ascites was recognized and some tumour cells were proved in it, suggesting that her original suffering might be some sort of malignant tumour which was certified at autopsy to be stomach cancer (Fig. 29).

The fourth case was a 37 years old multipara who was attacked by ablatio placentae on her pregnant day of 40 weeks and 4 days, when haemorrhagic discharge appeared suddenly, and transmitted to our hospital immediately, but on her way to hospital next bleeding came out to induce non-consciousness and shock. As shown in Fig. 30, she received Caesarian operation after recovery from this urgent condition through adequate treatments and found to have several bleeding foci, such as a sugillation on anterior surface of uterus, haematomas at plica uteri dextra, etc., as seen in Fig. 31. After the operation, however, she had still positive bleeding through uterus mouth, mixing occasional big blood discharge and was proved to have a up-most fibrinolytic activity in blood as described in Fig. 32, so that energetic therapy with ε-ACA was applied to secure immediate stoppage of bleeding and favorable clinical course thereafter (Fig. 30).

The fifth male patient had prostate cancer and was admitted to our hospital because of dysuria and haematuria. The appearance of haematuria and shift of fibrinolysis system were illustrated in Fig. 33 and castration decreased the lysis area on fibrin plates developed by plasma+SK and eug+SK, but haematuria and high level of fibrinolytic activity of eug itself were still demonstrated, so that ε-ACG was administered to diminish haematuria and suppress the activity of eug and urine Act.

For the last case, the author would like to mention the relationship between haemophilia and fibrinolysis. In 1960 Boudreau and Frampton of the United States of America published from their own experience as haemophiliacs that mouthful intake of peanut could inhibit bleeding, and many discussions were opened on its reaction mechanism, etc. Dr. Astrup, my teacher, insisted that peanut had some sort of Inh activity on fibrinolysis system, and if it is the case, ε-ACA must work on haemophilic, too. On this assumption daily doses of 5 to 10 grams of ε-ACA were given orally to Factor VIII (AHG) or IX (PTC) deficient patients and their
therapeutic response was checked. Fig. 34 shows episodes of bleeding and blood transfusion in a haemophilia A patient, a 4 years old boy who has had purpura or larger scaled haemorrhage since he began to crawl on floor at 9 months and was admitted to hospital sometimes for treatments, for instance, blood transfusion. Since he started the ε-ACA therapy recently, the frequency and grade of bleeding were distinguishedly reduced and has never received blood transfusion since January of this year even in the winter season when he used to have blood transfusion every year before. Furthermore he had not so many incidences of bleeding at slight contusions as before and obtained self-confidence in usual movement and daily life. Some blood examinations before and after ε-ACA administration revealed definite depression in fibrinolysis system, but he did not show any abnormal elevation of fibrinolytic activity before the treatment, and could prove no apparent improvement in thromboplastin generation test. Thirteen cases of haemophilia including three of control, were treated, as shown in Fig. 35 and showed more or less effect, minimizing the frequency, amount and duration of bleeding. However, ε-ACA could not replace the missing blood clotting factors, nor work on all haemophiliacs and let haemorrhage subside perfectly even though it worked to some extent, and particularly gave us not so much expectation when bleeding was once started. Nevertheless, it proved obvious prophylactic effect and seemed to give us a gleam of hope in the poor treatment of haemophiliac. Although its reaction modus must be studied furthermore, of course, it is quite obvious that this substance had some influence on bleeding in haemophiliac.

Conclusion

So far the relationship between fibrinolysis system and haemorrhagic disorders was studied experimentally and clinically and a certain causality in the living body was proved, but it dealt with only one type of haemorrhagic disorders. The passive adaptation and active self-control of the living body can conquer, get rid of the change of milieu externe et interne and prohibit the occurrence of bleeding. It is our sincere hope and task to study the original
motives which can maintain the dynamic equilibria inside of body, using more sensitive and multivalent techniques for fibrinolysis measurement.

Because of the limitation of space all figures and literatures in English, listed in the Japanese issue, were dropped: Editor.