PLASMIN AND ANTIPLASMIN
THEIR PATHOLOGIC PHYSIOLOGY

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STUDIES ON THE ANTIPLASMIN

It is known that proteolytic enzymes are found in cells and blood. Physiologically, such enzymes have been considered to be related with the protein metabolism of a living organism. Various studies have been made as to whether or not proteolytic enzymes in vivo are identical with digestive enzymes such as trypsin and pepsin. And rather recently a specific kind of proteolytic enzyme was found in the human blood and was named "plasmin" or "fibrinolysin."

Various investigations have been made as to the function of plasmin on the living organism, and many grounds have been found out for believing that plasmin in blood and/or in locus becomes active in the case of a series of pathologic states, such as surgical shock, allergy, and also of physical and mental fatigue, that the decomposition of plasma fibrinogen and/or the other proteins in vivo is caused by the above anomalous activity of the mentioned enzyme, and that the products formed by the said decomposition give troubles to the living organism.

In fact, the activation of plasmin was rapidly observed experimentally by the antigen-antibody reaction, which was brought about in vitro and in vivo (Unger & Mist), and also the toxic substance formed by the action of plasmin on the proteins in blood has the nearly same features as those of histamine, suggesting that the resultant toxic substance may play a part in those disorders in which the activation of plasmin occurred to a certain extent in blood and/or in locus.

An important suggestion was made on proteolytic enzyme, from the viewpoint of experimental medicine, by the fact that the course of inflammation was made to progress favourably in an experimental peritonitis when trypsin was given repeatedly in advance to increase the antiproteolytic power in vivo (Kay).

It has hitherto been known that certain substances such as soyabean trypsin
inhibitor have an inhibitory effect on plasmin, but such substances are not successfully used even in animal experiment because of their toxic side action. It is also known that albumin fraction of human serum has an inhibitory power to plasmin (MacFarlane and Biggs)\(^{(9)}\), and the said albumin fraction was clinically used by O. Smith and G. Smith\(^{(10,11)}\) for the case of pregnancy toxicosis, even though a mass application of such substance was difficult.

The author and his colleagues, therefore, started investigations in 1948 searching for synthetic antiplasmin substance, co-operatively working for several years with Nagasawa and his colleagues in the Laboratories of Mitsubishi Chemical Company.* One group of such substances was found out in 1952 from among those synthetic substances as results of the systematic studies made in vitro which showed inhibitory power on the fibrinolytic activity of the plasmin obtained from various serum samples. Among the compounds belonging to the said group, Ipsilon-amino-caproic acid has the inhibitory power to the fibrinolysis with plasmin system at a very low concentration of the order of \(10^{-5}\) mol\(^{(12)}\).

The screening test of anti-plasmin power in the beginning of our investigation was made as follows. A globulin fraction was obtained by diluting the plasmin of man or horse 10 times with aqua dest. and by precipitating and collecting the euglobulin fraction at \(\text{pH} 5.2\). The globulin fraction was mixed with fibrinogen solution and a certain quantity of those substances to be measured which had been diluted. Fibrin clot was made by adding thrombin to the above mixture, and the time required for the complete dissolution of fibrin clot at \(38^\circ\text{C}\) was measured and compared with the said time with that of dissolution of control fibrin clot to which the substance to be measured had not been added.

As to the effect of Ipsilon-amino-caproic acid (hereinafter abbreviated as Ipsilon) to the living organism, the following table shows that anti-anaphylaxis effect of Ipsilon in mice which occurs in protracted course.

<table>
<thead>
<tr>
<th>No. of Case examined</th>
<th>Death</th>
<th>Living</th>
<th>Death Rate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ipsilon was given</td>
<td>37 cases</td>
<td>10</td>
<td>27</td>
</tr>
<tr>
<td>Control</td>
<td>36</td>
<td>23</td>
<td>13</td>
</tr>
</tbody>
</table>

On the other hand, a remarkable inhibitory effect on the Manteaux reaction was shown by Itoga\(^{(13)}\) when local application of Ipsilon was made at the same time. It is noteworthy that such a substance having an inhibitory effect to the Manteaux reaction was scarcely known, and Ipsilon is the most powerful one.

* Shosuke Okamoto, Fujio Nagasawa, Eichi Takagi, Yuzo Tsukada, Mikio Yokoi, M. Sato and others were engaged in this cooperative work.
which has been discovered.

Another experiments were made by giving Ipsilon per se to experimental animals, and it was made clear that the repeated administration of a large amount of Ipsilon was practically not toxic. In the case of injecting intravenously 1 g per 1 kg of the weight of a rabbit continuously for ten days once a day, the rabbits were sacrificed after the lapse of one week, and the abnormality of organs was examined histologically and proved to be little.

Therefore, clinical examinations on the effect of the intravenous administration of Ipsilon in a appropriate solution were searchingly conducted by Sato and his colleagues and by Itoga and his colleagues. Thus, Sato and others revealed the effectiveness of Ipsilon on some patients where plasmin was active in the venous blood. Itoga and others denoted that the administration of Ipsilon could be effective on some skin diseases.

Since then, Ipsilon became to be clinically applicable wider and wider, and the recent achievements made by the members of the committee of our research project are detailed in the several papers of this issue.

METHOD AND DISCUSSION

Very marked progress in the chemistry of blood proteins and coagulation factors in recent years has resulted in the rapid development of knowledge and method on the fibrinolysis with plasmin, the more precise investigations being able to be facilitated than those described by us in the form of a patent specification 1953.

In particular, highly purified preparation of bacterial streptokinase has become so easily available to the laboratory work that some studies have been carried out by several workers on the mechanism of Ipsilon on plasma activated with streptokinase (T. Abe, et al., T. Igawa, et al., and S. Okamoto, et al.).

Either clinical or experimental investigations have been variously conducted by numbers of workers and the physiological concept of the fibrinolysis has been developing and changing rapidly. Moreover, the promising development of fibrinolytic therapy in such diseases as a thromboembolic disease and some infection diseases has cast a new light on the physiology of plasmin system in the living organism.

An urgent task, however, was facing us; that is, the most adequate and practical method for revealing some significant aspects of plasmin system in the healthy and patients should be established. This was the reason why it was intended by our committee on Research Project to make a comparison of those
different methods for determining plasmin activity in blood, which have been described and applied by different workers and in different laboratories.

More than 1,200 blood samples furnished from different divisions of the Hospital of Keio University, School of Medicine, were examined in several different methods at the same time in our technical center and the results obtained are briefly summarised in the present paper and will be discussed in the several other papers following.

The improvement of the methods described by MacFarlane et al. (3), Loomis (23), Unger and Mist (6), Astrup and others (24) has been noticed by us and those representative methods were adopted in our technical team, although the excellent fibrin plate method by Astrup is now on the way to be compared with the other methods.

So far as our results obtained until today, the whole clot lysis test by Ratnoff and the euglobulin lysis test by Loomis were both constantly reproducible, although those different tests seem to represent each different aspect of plasmin system. Results indicated that the whole clot lysis test was the most positive in most cases of the skin diseases suspected to be allergic, and that the euglobulin lysis test was very often positive in some haemorrhagic diseases.

It may be reasonably assumed that results of euglobulin lysis test by Loomis are less influenced by the amount of anti-plasmin than those of whole clot lysis test, and this presents one of the reasons why results from these two methods are different each other. This problem will be later discussed in the paper written by Asahi, Takamura and Okamoto.

Details of the results obtained with streptokinase activation test are now ready to be published by U. Okamoto et al. (25). These results have been highly reproducible, but their significance seems to be clearly different from those obtained by the methods mentioned.

Results obtained by MacFarlane's test and Kuroyanagi's modification (26) of the said test are delicate, and the results seemed to be largely influenced by the amount and state of anti-plasmin in the sample.

Ultraviolet absorbance test by Unger is sensitive and very ideal when the purified plasmin fractions are used, but it does not seem to be available widely in such complicated sample as blood obtained from patients. The paper by Sasaki and others indicated that the variation of ultraviolet absorbance does not go parallel with the increasing digestion of fibrinogen with plasmin activity. And, the other numerous results obtained in various pathologic states are now under investigation in relating with pathological pattern of the dynamic aspect of plasmin in each disorders.
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I should note here that, in most of the cases examined in our technical center such methods as fibrinogen determination by Gramm(27), whole clot lysis test by Ratnoff, euglobulin lysis test by Loomis, streptokinase activation test, Unger's Ultraviolet test, MacFarlane test and its Kuroyanagi's modification were adopted as the routine methods for determining the plasmin activity in the blood sample obtained from the healthy person and the patients ailing various diseases.

PLASMIC AND ANTIPLASMIC STATES

Either clinical or experimental investigation has been so variously conducted that only a few achievements will be somewhat tentatively cited in the present paper relating to our investigation.

In the first place, the author should like to note the activation of plasmin was rapidly observed experimentally by the antigen-antibody reaction in vivo and in vitro as well. MacFarlane and Biggs(9), Rocha e Silva(28), Unger and Mist(6), Okamoto and Tsukada(7) respectively noticed and revealed the mechanism of the activation of plasmin with such reactions.

The second point is that the strong activation of plasmin in blood with some pathologic stages of man proved to cause an increased tendency of bleeding: Ratnoff described in 1952 a very typical case of the activation of plasmin in a patient who died from a severe haemorrhage after an operation and in whom no other direct cause of haemorrhage but the activation of plasmin was observed with various kinds of blood examination.

A- or hypo-fibrinogenemia in the course of child delivery has come to be noticed in recent years, and it is now regarded as closely associated with the strong activation of plasmin. A series of such evidences clearly indicated that the strong activation of plasmin can produce, more or less, such a haemorrhage as oozing in a surgical operation.

On the other hand, the successful application of streptokinase to patients ailing thromboembolic diseases or some infection seems to bring about a confusion in the classical concept of the fibrinolysis in vivo. The author wishes to point out the presence of those two types of pathologic states, where the balance of plasmin system is shifted; the former is a plasmic state, the latter an antiplasmic state. Clinical investigations made by Miller and others indicate streptokinase should be available to the latter.

On the contrary, plasmic states in some disorders can be successful combated with the administration of a synthesized antiplasmic substance, i.e. L-α-amino-α-caproic acid, as described in those papers written by Okamoto et al., Mikata et al.(17,18), Sato et al.(16) respectively. It may be a somewhat schematic way
to divide the pathologic states into such two types, yet the author regards it as a useful and convenient way for determining whether some new methods such as an antiplasmin treatment, as it were, should be applied or not.

It was clearly demonstrated in our animal experiments that the unfavourable side actions caused by the intravenous administration of streptokinase (of large amount!) were suppressed by the administration of \( \varepsilon \). This fact supports the possibility of the effective usage of \( \varepsilon \) for removing the unfavourable side action caused by the overdosing of streptokinase.

While it is possible to remove the antiplasmic action of \( \varepsilon \) by giving the streptokinase to patients. The words of Dr. J. Miller (Private communication) may be cited here: That is, “streptokinase in right hand, \( \varepsilon \) in left hand.”

The following papers in this issue will approach to the pathologic physiology of plasmin system in man and animal, suggesting more precise concepts of the fibrinolysis and a rather unique way of treating those disorders belonging to a hyper-plasmic group.

Acknowledgement

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13. Itoga, G.: (Private communication)
14. Sato, S.: (Private communication)
15. Itoga, G.: (Private communication)