The Role of Platelets in Blood Coagulation

Manabu YAMANAKA

Department of Central Laboratory, Tokushima University Hospital, Tokushima University School of Medicine

The clotting activity of platelet, called platelet factor 3 which may be liberated from platelet during the process of its physical changes, referred to as viscous metamorphosis, would seem to be most important, and its intrinsic thromboplastin generation activity is probably related to its phospholipid contents. It has been shown that phospholipid derived from sources other than platelets can act as fairly efficient substitutes for platelet in the thromboplastin generation. Recent investigations have been directed to study phospholipid components of human platelets and their effects in blood clotting. However, the results have shown a good deal of disagreement. While, the morphological changes of platelets as corpuscles in clotting process, have been much investigated but little is known about the nature of the surface of platelet, which is likely concerned with the aggregation or adhesion of platelets and absorption of protein. In this paper, attempt was made to analyse the chemical constituents of platelet phospholipid and compare their clotting activities and clarify the nature of the surface of platelet.

Methods

Platelet suspension: Platelets were separated from citrated blood by differential centrifugation and then washed thrice and resuspended in physiological saline to one third of original platelet rich plasma volume. The number of platelets in the suspension was not taken into consideration.

Thromboplastin generating activity of platelets: The test was carried out according to Hicks and Pitney.

Estimation of ATP: Firefly luminescence (Luciferin-Luciferase) method was employed.

Determination of electrophoretic mobilities of platelets was performed using Northrop-Kunitz observation cell. Details of method were reported elsewhere.

Results

A. Platelet Factor 3
1. Its chemical constituents and clotting activity

Using chloroform extract of brain, Trostin and Soya-Lecithin substituted for
platelet suspension, maximal thromboplastic activities were shown at their optimal concentrations. According to Marinetti's method, paper chromatographical analyses of human platelets, crude cephalin and Soya-Lecithin were carried out. As a result, the main components of each material were phosphatidyl ethanolamine (PE), phosphatidyl serin (PS) and lecithin in common. Of these, PE and lecithin were most effective in low concentration and not in high concentration. PS on the contrary assumed an attitude of the reverse direction.

Although particle size of the phospholipid in solution also needed to be considered, the clotting activity of platelet would be affected when qualitative and quantitative equilibria among these fractions of phospholipid in platelet were broken under certain conditions.

2. Clinical aspects on platelet factor 3 activity

Recently, hemorrhagic tendency in a case of the reduction of platelet factor 3 activity due to the defect in its liberation from platelet has been reported. Comparative studies of thromboplastic activity of normal platelet suspension and disintegrated platelet by freeze-thawing, which would allow the release of factors concerning with thromboplastin formation, showed that highly concentrated platelet, especially disintegrated platelets behave in anticoagulant fashion. The platelet factor 3 activity of patients with hypoplastic anemia and leukemia was markedly reduced, unexpectedly that of essential thrombocytopenia revealed normal. In diabetes mellitus, nephritis and liver disease associated with deficiency of fat metabolism, the activity of disintegrated platelets was reduced inspite of normal activity in platelet suspension. It was thought that the antithromboplastic activity of phospholipid fraction which would hardly be released from platelet, was liberated as a result of distintegration. The thromboplastic activity in the disintegrated platelet was reduced in proportion to the reduction in the number of the platelet of peripheral blood, but unchanged in the platelet suspension. This would explain that functional clotting activity may not be limited to the number of platelets and the normal activity was observed in the platelet suspension of patients with thrombocytopenia.

B. Changes in platelet ATP during blood clotting and the mode of action of ADP

During blood clotting, platelets undergo viscous metamorphosis, then liberate their own ATP and platelet factor 3 to adhere themselves or to accelerate clotting process. The changes in platelet ATP during clotting were investigated and the fact that ATP content of platelet was decreased in course of clotting process was found. This should indicate the liberation of ATP from platelet. A poor correlation between the content of ATP in platelet and platelet adhesiveness rate or the rate of clot retraction and no difference in content of ATP in so-called non-adhesive platelets passed through glass filter and whole platelets before filtration which contained adhesive and non-adhesive platelets, were observed. When ADP was added to non-adhesive platelet, the platelet adhesiveness increased and the rate and extent of the increase were greater in cases with lower adhesiveness of whole platelets.
V. Platelets as corpuscles

1. Possible activation of contact factor by the surface of platelet

Platelet fixed with formalin or heated at 100°C for ten minutes, was added to normal non-contact plasma in siliconed tubes and recalcification time of each tube was determined. As the time of incubation increased, the substrate plasma clotting time was reduced but normal intact platelet has no ability to shorten the clotting time. No reduction of clotting time could be detected in Hageman factor deficient plasma used as a substrate plasma. It shows that the surface of platelet which would be altered under certain conditions, could activate the contact factor which was adsorbed on to the surface of platelet or present in the surroundings of platelets.

2. Microelectrophoretic studies of platelets

The mobility of unwashed or washed normal platelet was $-1.55 \pm 0.09 \mu\text{sec}^{-1}\text{volt}^{-1}\text{cm}^{-1}$ and $-1.81 \pm 0.10 \mu\text{sec}^{-1}\text{volt}^{-1}\text{cm}^{-1}$ respectively. After washing the platelet, the mobility had been increased but was ceased to change by more than three times washing, however, the washed platelet exposed to plasma, restored the mobility to that of original unwashed platelet. These results suggest that the mobility of platelet circulating in plasma may be limited due to the protein coated on the surface of platelet. Platelets exhibited true isoelectric point at pH 4.0 for unwashed and pH 3.5 for washed platelet. It seems likely that the surface of platelet may be near phospholipid rather than acidic protein in nature.

**Summary and Conclusion**

It may be said that the platelet carried within and immediately about itself all
the substances necessary either to promote or interfere with coagulation and hemo-
stasis. Regarding platelet activity, it seems to be of great importance how to release
these components from platelet besides these qualitative and quantitative changes.
A further studies on the nature of the surface of platelet membrane are needed to
clarify the mode of the action of platelets as corpuscles, bearing a strong negative
charge.

References

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