Prothrombin Utilization Following Addition of Platelet Cofactor I Concentrates to Hemophilic Plasma

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Platelet cofactor I is a procoagulant found in plasma but not in serum. Concentrates have been prepared by adsorbing the activity on kaolin (1). These concentrates usually have three components when examined with an ultracentrifuge. The main component comprises 85% of the total and upon electrophoresis has a mobility nearly like that of the β-globulins. The activity in the concentrates is readily destroyed by heating at 40—45° C. When added to hemophilia A blood or plasma the clotting time shortens but not to the same extent as with normal blood or plasma (2).**) The question then arises whether this shortening of clotting time is accompanied by prothrombin utilization and if so whether there is any difference in this respect between hemophilia A and hemophilia B (PTC of Aggeler).

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**) I went to Philadelphia with concentrates of platelet cofactor I and platelet factor 3 to carry out these experiments with Leandro M. Tocantins and Ruth R. Holburn. Subsequently; namely in April 1958, I went there a second time and we worked with hemophilia B patients. Although the results varied amongst the four patients studied the outstanding observation was a shortening of the clotting time of whole blood with platelet cofactor I. I was then wondering about the potency of the concentrates on account of the loss in activity during the drying from the frozen state. In my laboratory we also had plans to concentrate the activity further. As soon as we found out that much platelet cofactor I activity is lost upon drying and when we obtained better concentrates attention was again directed to hemophilia B and we employed the 2-stage prothrombin method for determining prothrombin utilization, instead of studying whole blood and plasma recalcification clotting times. While our attention to this work has removed the basis for many uncertainties inherent in the first surveys our conclusion is of the same general nature as found in Philadelphia, and I give all appropriate credit to Leandro M. Tocantins and Ruth R. Holburn and express my appreciation for their contribution to the initial developments. Walter H. Seegers

In our endeavour to find some information concerning what happens when platelet cofactor I concentrates are added to hemophilic blood we found that it shortens the clotting time in both hemophilia A and B. Then we used the technic of Brinkhous to study the prothrombin utilization (3). Already in 1939 he observed that prothrombin is not consumed in hemophilia. With the addition of platelet cofactor I we found that the utilization of prothrombin continues to be slow in recalcified plasma of both hemophilia A and B, and with the whole blood of hemophilia A. But there is a difference between the two inasmuch as prothrombin is largely consumed in hemophilia B blood when platelet cofactor I is added.

Materials and Methods

Platelet Cofactor I. This was prepared from bovine plasma as described by Seegers, Landaburu and Fenichel (1) and modified as described more recently (4). The platelet cofactor I protein was dissolved in water at a concentration of 600 U/ml. This represents about 0.2 mg/ml.

Platelet Factor 3. This activity was concentrated from bovine platelets according to the method of Alkjaersig, Abe and Seegers (5). In terms of their assay the activity was 3,600 U/ml. This material is probably lipoprotein and loses its platelet factor 3 activity upon extraction with fat solvents. Such extracts do, however, still contain procoagulant activity that can be measured as "partial thromboplastin" in the sense of Brinkhous or in "thromboplastin generation" (6).

Threone. Mixtures of platelet cofactor I and platelet factor 3 are by definition threone activity. In this study we mixed equal quantities of the platelet factor 3 and platelet cofactor I described above.

Experimental

Hemophilia A. The 8 year old boy was bleeding at the time a blood sample was taken. One ml of blood was added to 0.1 ml of physiological saline solution, or platelet cofactor I or platelet factor 3 or threone. In the latter case, however, 0.2 ml of solution was used. At various times the reactions were stopped by adding anticoagulant (0.15 ml of 3.2% Na citrate). After centrifuging the samples were frozen and subsequently thawed to measure the prothrombin concentration by the modified 2-stage method (7). Plasma was obtained by mixing blood with 3.2% sodium citrate in the proportion of 9 to 1. After centrifugation we recalcified the plasma with addition of the test material as follows: plasma 0.2 plus saline 0.3 plus calcium chloride 0.2 plus test material 0.1 ml (except threone 0.2 ml). The prothrombin utilization was only extensive with threone and even then only strikingly so with the plasma (Table). The dilution of the plasma probably accounts for some utilization of prothrombin in the control analysis.

Prothrombin utilization as measured by 2-stage in hemophilia

Material Added	Time (Min.)	Hemophilia A Prothrombin in U/ml		Hemophilia B Prothrombin in U/ml	
		Blood	Plasma	Blood	Plasma
Saline Control	0		156		338
	20		209		173
	40		165		142
	60	238	111	308	98
	120		85		93
Platelet Cofactor I	0		337		352
	20		337	77	191
	40		227	53	169
	60	270	182	38	84 (?)
	120		107	19	142
Platelet factor 3	0		350		467
	20		187		191
	40		120		169
	60	212	107	170	129
	120		62		124
Threone	60	139	none	32	none

Hemophilia B. The blood was from a man 50 years old. He was not actively bleeding at the time the blood sample was taken. Previously there were many bleeding episodes. The experiments were performed as with hemophilia A. As with hemophilia A the utilization of prothrombin was poor except with threone. Then there was also another difference. Platelet cofactor I alone improves prothrombin utilization with the whole blood.

Discussion

In the first prothrombin utilization experiments ever performed Brinkhous (3) found prothrombin utilization after a hemophiliac was transfused with 300 ml of whole blood. Our platelet cofactor I concentrate is a very potent procoagulant in various clotting tests, but is not very effective with hemophilia, unless this happens to be hemophilia B blood, and unless platelet factor 3 is also present. Many authors state that hemophilia A is a blood clotting defect in which there is a deficiency of antihemophilic factor. This hypothetical

factor has not been obtained in pure form and thus no one has described its properties well enough to enable a comparison with platelet cofactor I. Certainly platelet cofactor I alone is not sufficient to correct the defect. It may very well be that the initial burst of thrombin which accounts for the clotting contributes to the rapid destruction of platelet cofactor I for it has been found that the disappearance of this activity occurs in the presence of a plasma factor(s) and thrombin (8).

Very few procoagulants or anticoagulants are specific. Consequently we are not surprised to find that there is prothrombin utilization in hemophilia B blood when platelet cofactor I alone is added. There is also no difficulty in seeing prothrombin utilization in all tested situations with hemophilia A and B on the basis of non-specificity of these procoagulants. It has already been considered that inhibitor(s) found in hemophilia A plasma account for the fact that platelet cofactor I is more effective in the normal individual than in the hemophiliac.

Threone is a more powerful procoagulant mixture than platelet cofactor I or platelet factor 3 alone and readily breaks through whatever barrier is in hemophilia A or B.

The creation of an excellent platelet cofactor I preparation and a platelet factor 3 preparation opens many new possibilities in the diagnosis and classification of hemophilia. By simply taking these reagents and adding them to blood or plasma, with or without further analysis, a classification and grading is possible that can be added to conventional methods.

Summary

Prothrombin is utilized in hemophilia B blood when a platelet cofactor I concentrate is added. It is not well utilized when the same preparation is added to hemophilia B plasma, hemophilia A plasma, or hemophilia A blood. Platelet factor 3 concentrates did not promote prothrombin utilization. Mixtures of platelet cofactor I and platelet factor 3 (threone) give prothrombin utilization under all conditions tested in hemophilia A and B.

Résumé

L'addition d'un cofactor plaquettaire I concentré favorise la consommation de la prothrombine dans le sang d'un hémophile B mais pas du plasma d'un hémophile B, hémophile A ou du sang d'un hémophile A. Une préparation concentrée du facteur 3 est sans influence sur la consommation de la prothrombine. Des

mélanges de cofacteur plaquettaire I et de facteur plaquettaire 3 (thréone) corrige la consommation de la prothrombine dans l'hémophilie A et B dans toutes les conditions expérimentales.

Zusammenfassung

Prothrombin wird in Hämophilie-B-Blut verbraucht, wenn ein Thrombozyten-Cofaktor-I-Konzentrat zugesetzt wird. Es wird nur schlecht verbraucht, wenn dasselbe Präparat zu Hämophilie-B-Plasma, Hämophilie-A-Plasma oder Hämophilie-A-Blut zugesetzt wird. Thrombozytenfaktor-3-Konzentrate fördern den Prothrombinverbrauch nicht. Mischungen von Plättchen Cofaktor I und Thrombozytenfaktor 3 (Threone) ermöglichen einen Prothrombinverbrauch unter allen untersuchten Bedingungen bei Hämophilie A und B.

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