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Modification of the Phospholipids Structure of Platelets Through the Action of Heparin

by

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The action of heparin on the aggregation of the platelets was studied in a previous paper (2). The basis of this action was discussed in the above work and it is observed how heparin, when administered in small doses, produces a deficient plasmatic coagulability and, on the other hand, does not modify the aggregation capacity of the platelets. Whereby it is concluded thats its antiaggregation action is not a consequence of its antithrombotic action.

The lipolytic activity of heparin (8) is also known, which is achieved by activating a plasmatic lipase (18). However, based on previous facts, we have thought that perhaps the antiaggregation action of heparin is due to a modification of the phospholipid structure of the platelet, secondary to its lipase action. Therefore, in this work, we have studied the platelet phospolipids before and after incubating a platelet rich plasma with heparin in order to observe the possible alterations produced.

Material and Methods

Human blood extracted with a 3.8 % solution of sodium citrate is used. One part anticoagulant and nine parts of blood.

Plastic or silicon treated material was used for all manipulations of the platelets.

Once the sample was obtained, it was centrifuged at a rate of 400 × g to obtain plasma containing around 400.000 platelets per mm^3 and only 2.000 to 5.000 red cells. In order to eliminate this red cells, the supernatant plasma is centrifuged at 2.000 × g for 20 minutes and the precipitate is gently homogenised with a hypotonic solution formed by 15 ml of distilled water and 10 ml saline solution and then centrifuged at 2.000 × g for 20 minutes, whereby a precipitate practically free from red cells is obtained. This precipitate is washed trice times in a saline

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solutions and finally is rediluted with an equal volume of saline solution to the volume of plasma initially used. Two samples are taken from this solution to determine the proteins and the rest is used for the determination of phospholipids.

The proteins are determined according to the method of LOWRY *et al.* (11).

The phospholipids are determined according to the usual method for determining phosphorous and multiplying the micrograms found by 25 (1). The method actually used is the following: A certain amount of the platelets suspension in saline solution is mixed with an equal amount of 0.6 molar perchloric acid, gently homogenised and centrifuged at 2.000 × g for 20 minutes. The supernatant liquid is decanted and 2 ml of chloroform-etanol-hydrochloric acid (20/10/0.1) is added to the precipitate which is then left to rest for 30 minutes. Then 8 ml of 0.1 N hydrochloric acid is added while shaking vigourously and then centrifuging at 2.000 × g for 30 minutes.

The aqueus part is drawn off and the rest is poured into a graduated tube, measuring the exact volume obtained. In a later stage the phosphorus is determined by introducing 0.5 ml of the chloroform solution into a tube, along with 2 drops of ammonium molybdate 5 % solution and 1.2 ml of 70 % perchloric acid solution.

The sample is heated in an oven for 15 minutes at 200° C, removed and left to cool. Then 0.4 ml of 5 % ammonium molybdate solution is added, and 0.4 ml of Fiske and Sobbarow's reagent and distilled water is used to bring the addition up to 10 ml, with gentle shaking. Then it is placed in a water bath at 100° C for 15 minutes, left to cool and read in the spectrophotometer at 830 mμ.

The results are ploted against a master curve and the amount obtained is multiplied by 25 to obtain the μg of phospholipids contained in the initial chloroform solution.

Results

In order to check the action of heparin, a sample of platelet rich plasma is incubated with the required amount of heparin and left at 37° C for two hours, after which the proteins and phospholipids, in this sample and in another blank lacking in heparin are determined. The results obtained are given in Table I. With heparin, there is a significant loss of phospholipids ($p < 0.01$).

TABLE I

Action of heparin on the phospholipids of platelets suspended in their own plasma. In the first group of experiments, the phospholipids and proteins of normal platelets are determined and the relation between both is found. In the second group, these platelets are incubated with 80 u of heparin per ml and the phospolipids and proteins are also determined. After incubation of the platelets with heparin, a significant reduction of the phospholipids/protein quotient ($p<0.01$) is found.

Without heparin			With heparin		
Proteins μg/ml	Phospholipids μg/ml	Prot. phosp.	Proteins μg/ml	Phospholipids μg/ml	Prot. phosp.
120	26	0.22	120	13	0.11
103	20	0.23	102	8	0.08
102	25	0.25	106	12	0.12
158	48	0.32	135	31	0.24
123	37	0.30	117	17	0.15
135	36	0.27	132	36	0.28
85	25	0.30	96	9	0.10
102	23	0.23	98	13	0.14
28	7	0.25	28	7	0.25
67	18	0.21	94	12	0.13
108	29	0.27	94	15	0.16
125	26	0.21	120	13	0.11
128	32	0.25	125	31	0.25
135	21	0.19	111	14	0.13
125	22	0.18	123	17	0.14
133	30	0.23	132	15	0.12
118	23	0.20	120	18	0.15
97	33	0.35	16	4	0.25
105	24	0.23	100	13	0.13
115	25	0.22	102	10	0.10
111	26	0.23	103	15	0.14
$\bar{x}$		$\sigma=0.044$			$\sigma=0.05$

In order to complete this study, a second group of experiments was performed in which the platelets were incubated with different amounts of heparin to see if there was any correlation between the volume of heparin and the phospholipids increases parallelly with the amount of heparin used (Table II).

TABLE II

Action of varying amounts of heparin on the phospholipids of platelets suspended in their own plasma. The values given indicate the phospholipid/protein ratios obtained in each experiment. A significant loos of phospholipids ($p < 0.01$) is observed as the amount of heparin used is increased.

Exp. núm.	Without heparin	With heparin		
		800 u./ml	400 u./ml	200 u./ml
1	0.25	0.15	0.20	0.23
2	0.30	0.15	0.17	0.26
3	0.18	0.11	0.15	0.16
4	0.30	0.25	0.27	0.27
5	0.21	0.13	0.15	0.17
6	0.21	0.13	0.16	0.18
7	0.19	0.09	0.13	0.15
8	0.18	0.17	0.15	0.16
9	0.23	0.12	0.15	0.13
10	0.28	0.16	0.17	0.23
$\bar{x}$	0.23	0.14	0.17	0.19

As we said at the beginning, the clarifying action of the heparin was achieved by activating a plasmatic lipase (18). In order to demonstrate whether the action of the heparin on the platelet phospholipids is carried out directly on the platelets themselves or indirectly through the activation of this plasmatic lipase, the platelets were incubated after having been subjected to several washing (usually seven) in a saline solution until a supernatant liquid lacking in proteins is obtained. These platelets, washed and resuspended in a saline solution, are divided into two lots, one incubated with heparin and the other blank. After being maintained at 37° C for two hours, the proteins and phospholipids are determined by the above mentioned method and it is observed (Table III) that there is no loss of phospholipids in the sample incubated with heparin, which backs up the hypothesis that the heparin acts through a plasmatic lipase.

TABLE III

Action of heparin on the phospholipids of platelets washed and suspended in a saline solution. The value given indicate the phospholipid/protein ratio before and after incubation with heparin. No significant difference is observed between these experiments.

Exp.	Without heparin	With heparin 800 u./ml
1	0.23	0.24
2	0.19	0.19
3	0.22	0.20
4	0.22	0.22
5	0.28	0.27
6	0.29	0.29
7	0.17	0.19
8	0.20	0.21
9	0.20	0.20
10	0.20	0.19
$\bar{x}$	0.22	0.22

Discussion

Although the data taken from the literature are not in complete agreement, it seems that heparin exercises a definite antiaggregation action on the platelets (2). However, what still remains to be made clear are the fundamentals of this action.

Within this phenomenon of platelet aggregation, one of the most important links in the chain, is the membrane of the thrombocytes. This has certain receivers on it (3), which join up with a plasmatic protein (5), tenned by some as the von Willebrandt factor (9). This in turn would join up with ADP so as to complete the bond of the interplatelet link in this way through a calcic bridge (6). Thus perfect funtioning of these receivers is a must for

normal aggregation of the platelets (3, 4).

At the same time as these active receivers, the electrical charges of the platelet membrane are important in the phenomenon of aggregation, since it is known that they perform a definite role in the majority of the aggregation processes of the blood cells (12). Therefore, the anti-aggregation action of heparin could be based on a loss of phospholipids (15, 17). Which, on the one hand, would produce an alteration of the platelet membrane (13) with a possible modification of the active receivers, which in turn agraes with the fact that heparin is capable of altering diverse cellular membranes (10) and, on the other hand, could cause modifications of the electrical charges in the platelet itself.

Thus, as we have said above, this alteration of platelet phospholipids is a result of the activation of a plasmatic lipase by heparin, which would cause the hydrolysis of these phospholipids by separating them from their protein base which may be related with the presence in the platelets of an inhibitor of this plasmatic lipase which appears after the action of the heparin (7,14) and which is different from the factor 4 or antiheparinic (16). It may be that this antilipase action is secondary to a consumption of this lipase in the hydrolysis of the phospholipids in the platelets.

In this paper, it has been shown that heparin produces a significant loss of phospholipids (p 0.01) and that this action is performed through the activation of the mentioned plasmatic lipase. As a result of this, it is suggested that this phospholipid loss alters the receivers of the platelet membrane and, or, the electrical charges of the platelet with the consequent alteration of the aggregation that this may produce.

Summary

In this paper the author studied the alterations which take place in the phospholipids of the platelets after incubation of the latter with heparin. It appears that this action is secondary to the activation of a plasmatic lipase.

At the same time some theorical speculations are made to establish a possible union between the reduction of the aggregation of platelets by heparin and this loss of phospolipids that it causes.

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