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Coumarin Therapy and Platelet Aggregation*

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Summary: Platelet aggregation has been related to blood coagulation studies in patients on nicoumalone, a coumarin anticoagulant. Aggregation studies were performed by means of Chandler's tube and the adenosine diphosphate (A.D.P.)-induced optical density method. Platelet aggregation in Chandler's tube has been shown to be quite different from A.D.P. aggregation and to be dependent on the "intrinsic" (blood) clotting system. When the intrinsic system was depressed by coumarin anticoagulant, aggregation was delayed in Chandler's tube, but patients with a predominantly "extrinsic" (tissue) system defect gave normal results even when their prothrombin time was excessively prolonged. In contrast there was an increased response to A.D.P. in the anticoagulated patients.

The study emphasizes the different mechanisms of platelet aggregation, which we have referred to as coagulation-induced and A.D.P.-induced aggregation. It also shows the limitations of routine control of oral anticoagulants by prothrombin time alone, as the coagulation-induced platelet aggregation appears to be quantitatively related to the overall level of clotting factors in the intrinsic system and independent of the extrinsic system.

Introduction

As well as their action on blood clotting, oral anticoagulants may have additional effects which might contribute to or even explain their role in the prevention of thrombosis. One possible important action may be on platelets.

Anticoagulant treatment reduces both "extrinsic" (tissue) and "intrinsic" (blood) systems of prothrombin conversion. The observation of abnormal platelet function in haemophilia (Hellem and Owren, 1964) suggested to us that platelet aggregation may be abnormal in patients on anticoagulants when their intrinsic system is depressed. We therefore studied the coagulation system in a group of patients receiving a coumarin drug, and tried to correlate these results with platelet aggregation.

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Method of Study

A group of 30 patients on nicoumalone (Sinthrome) was studied. Platelet aggregation studies and coagulation factor assays were performed on the same blood specimens. A parallel group of healthy adults was also studied.

The following coagulation tests were performed: prothrombin time (Quick test), cephalin time, and factor II, VII, VIII, IX, and X assays. Platelet aggregation was studied by Chandler's tube and adenosine diphosphate (A.D.P.)-induced platelet aggregation by an optical density method. A normal range was obtained for Chandler's tube aggregation from 53 normal adults (24 males, 29 females) and for the A.D.P.-induced aggregation from 63 adults (27 males, 36 females).

Technique

Coagulation Studies.—Prothrombin time (Quick test) (Poller, 1964), cephalin time (Hjort *et al.*, 1955) activated by the addition of kaolin, factor II (Pechet, 1964), factor VII (Poller and Thomson, 1964), factors VIII and IX (Egeberg, 1961) with the addition of kaolin, and factor X (Denson, 1961). The results were expressed as seconds and compared with the parallel normal controls, except for factor VIII assays. Results of factor VIII were expressed as a percentage (Poller and Thomson, 1964).

Platelet Aggregation Studies.—(1) Chandler's tube method (Cunningham *et al.*, 1965, modified). Platelet-rich plasma, prepared by centrifugation at 105 *g* for 10 minutes, was rotated in a tube (internal diameter, 1 cm., length 60.8 cm.) at an angle of 44° at 34 r.p.m. in a 37° C. incubator. Then 0.5 ml. of M/4 calcium chloride and 10 ml. of 0.9% sodium chloride were mixed and 5 ml. of platelet-rich plasma was added. (2) A.D.P.-induced aggregation (O'Brien *et al.*, 1966, slightly modified). The difference between the initial and final optical density readings was measured. This figure was adjusted to give the change corrected for a platelet count of 400,000/cu. mm.

Results

Three of the 30 patients were not sufficiently anticoagulated —that is, were not in the therapeutic range on the prothrombin scale (15–30% activity with the Manchester reagent)—and were therefore excluded. Six patients were over-anticoagulated and were also eliminated. One patient with very lipaemic plasma had to be excluded because of difficulty in interpretation. Three

TABLE I

Group	Total	Mean Prothrombin Time (sec.)	Mean Cephalin Time (sec.)	Mean Factor II (sec.)	Mean Factor VII (sec.)	Mean Factor IX (sec.)	Mean Factor X (sec.)	Mean Factor VII (% of Normal)
1	15	31·6 (S.D. 4·1)	65·8 (S.D. 3·7)	33·6 (S.D. 3·1)	29·2 (S.D. 3·5)	79·6 (S.D. 3·1)	27·0 (S.D. 4·7)	104 (S.D. 5·82)
2	5	34·5 (S.D. 9·5)	46·3 (S.D. 3·5)	—	38·2 (S.D. 10·6)	69·3 (S.D. 8·5)	20·6 (S.D. 1·9)	113 (S.D. 1·78)

patients though over-anticoagulated, had pure factor VII deficiencies and were therefore of sufficient interest for inclusion. The remaining 20 patients were divided into two groups: (1) 15 therapeutic patients with prolonged cephalin times—that is, gross intrinsic clotting system reduction—and (2) five patients with normal cephalin times—that is, no gross intrinsic defect. The diagnoses of the 20 patients were as follows: myocardial infarction in 14, other arterial occlusions in 4, venous thrombosis in 1, and pulmonary embolism in 1.

The results of the coagulation studies are given in Table I. Prothrombin times of both groups 1 and 2 were grossly prolonged as compared with the parallel normal group. The cephalin time results in group 1 were grossly prolonged (intrinsic defect), but group 2 results were within the normal range (no gross intrinsic system defect). In group 1 the results of factor II, VII, IX, and X assays were grossly prolonged. In the extrinsic group (group 2) factor VII only was significantly depressed. In contrast factor VIII assays showed a significantly raised level ($P=0.05$) in group 1, whereas in group 2 there was a gross increase.

Platelet aggregation results are given in Table II (1) *Chandler's tube:* Group 1 (prolonged cephalin times) differed significantly from the normal group and also from group 2 (normal cephalin times). Group 2 did not differ significantly from normal. Group 1 was subdivided. In the eight patients whose cephalin time exceeded 60 seconds the platelet aggregation with Chandler's tube technique was significantly longer than in the seven remaining patients whose cephalin time was less than 60 seconds (see Table III). (2) *A.D.P.-induced aggregation:* Group 1 differed significantly from the normal group, though it did not differ significantly from group 2. In group 2 the mean aggregation was also increased but not significantly different from normal.

TABLE II

Group	No. of Patients	Chandler's Tube Mean Aggregation Time (min.)	A.D.P. Aggregation Mean Adjusted O.D. Change
1	15	14·7 (S.D. 4·7)	10·4 (S.D. 4·2)
2	5	8·2 (S.D. 1·4)	10·6 (S.D. 6·3)
Normal ..	53	6·9 (S.D. 1·4)	—
Normal ..	63	—	7·5 (S.D. 2·7)

TABLE III.—*Results of Platelet Aggregation Studies (Chandler's Tube) in Group 1 Patients*

	No.	Chandler's Tube. Mean Aggregation Time (min.)	S.D.
Patients with cephalin time > 60 sec.	8	17·4	4·1
Patients with cephalin time < 60 sec.	7	11·7	3·6

Discussion

Using two completely different techniques we have been able to contrast platelet aggregation, which is apparently dependent on "intrinsic" blood coagulation, with A.D.P.-induced platelet aggregation. We shall therefore refer to coagulation-induced and A.D.P.-induced systems. There have been conflicting reports on the effects of coumarin drugs on platelets. A decrease in adhesiveness of the platelets has been reported by various workers (Spooner and Meyer, 1944; Weiner *et al.*, 1948; Murphy and Mustard, 1961). An increase in the early stages of oral anticoagulant treatment was, however, reported by Horlick (1961). The overall impression has been that aggregation of platelets is not reduced by oral anticoagulants. In contrast, Knieriem and Chandler (1967) described increased A.D.P.-induced aggregation during coumarin administration. Excessive dosage with coumarin-indanedione drugs is known to cause purpura and prolonged bleeding-times. The finding of reduced platelet aggregation in Chandler's tube by Cunningham *et al.* (1965), when the patients were at therapeutic levels of prothrombin activity, seemed to require elucidation.

The demonstration in the present study of reduced platelet aggregation in Chandler's tube system confirms the findings of Cunningham *et al.* (1965), but we have shown that this occurs only when the intrinsic coagulation system is depressed. Even if the patient is over-anticoagulated according to the prothrombin time results, if the intrinsic system is not appreciably depressed platelet aggregation with Chandler's tube is normal. This means that during the first 48 to 72 hours of oral anticoagulant therapy there is no appreciable change in coagulation-induced platelet aggregation, as factor VII, which affects the extrinsic clotting system alone, may be the only blood coagulation factor showing appreciable reduction. Of the group 2 patients showing a pure extrinsic deficiency, three, though over-anticoagulated from their prothrombin time, showed normal platelet aggregation. If reduction in platelet aggregation plays a part in bleeding during anticoagulant treatment, this might explain why haemorrhage is uncommon during the first two to three days of therapy when patients are commonly overdosed.

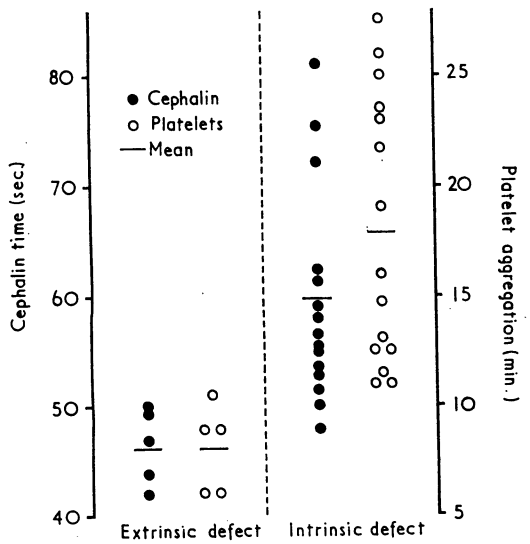
Our Chandler's tube results support the concept that platelet surface clotting systems have a key role in haemostasis and are dependent on intrinsic clotting factors (Hellem and Owren, 1964). These views were based on observations that used the secondary bleeding-time in congenital coagulation disorders. Quantitative comparison with congenital coagulation disorders is difficult, however, owing to the broader spectrum of the coumarin defect. Hellem and Owren (1964) also found that the secondary bleeding-time was restored in severe haemophilia by the small amount of factor VIII adsorbed on platelet concentrates. Moreover, Borchgrevink (1961) described a normal secondary bleeding-time in three patients with factor VII deficiency.

If it were assumed that the main benefit of oral anticoagulants in prevention of thrombosis depends on reduction of coagulation-induced platelet aggregation, this might support the view that the cephalin time is a better method than the prothrombin time for controlling long-term anticoagulated patients (Eastham, 1968). The fact that coagulation-induced platelet aggregation is not normally measured and that only an extrinsic system test is performed routinely illustrates the shortcomings of conventional control of oral anticoagulants. Further study will be required to determine whether it is necessary to perform Chandler's tube platelet aggregation studies or whether the cephalin time is a sufficient guide to changes in coagulation-induced platelet aggregation.

There seems to be a quantitative relationship between the prolongation of the cephalin time and the platelet aggregation results. When the patients with long cephalin times were compared with those with only moderately prolonged times the results differed significantly (see Chart). When the mean results are taken in conjunction with individual results they suggest that the cephalin time is a reasonable though only approximate guide to coagulation-induced platelet aggregation. The relationship between the coumarin factors involved in the intrinsic system and increases of factor VIII is very complex, and therefore the individual clotting factor assays did not reflect

the alteration in platelet aggregation as well as the cephalin time, which measures the overall "intrinsic" changes.

Of the five anticoagulated patients with normal cephalin times two had appreciable depression of factors IX and X. In these patients, however, there was a very high level of factor VIII, which appears to have compensated for the reduction of the other clotting factors. It is relevant to note that the anticoagulated patients with normal cephalin times had, as a group, significantly raised levels of factor VIII. All the patients in this small group, however, were within the first 24 to 48 hours of treatment after a thrombotic episode, and the raised levels of factor VIII may be evidence of a hypercoagulable state, present at the time of the vascular occlusion. Previously we have shown that factor VIII levels rise in patients during long-term therapy (Poller and Thomson, 1964). In our present study the first group of anticoagulated patients was a mixed one of short-term and long-term patients. Nevertheless, it was interesting to find that the increase in factor VIII levels was statistically significant, though not as great as in the patients within a few hours of a thrombotic episode. It is thus possible that there may be a fluctuating level of factor VIII in thrombotic patients treated with anticoagulants.



Relationship of platelet aggregation times to the extrinsic and intrinsic clotting systems.

It thus seems likely that the reduction of one factor in the cephalin time test may be compensated by increases of another. This emphasizes the importance of the raised levels of factor VIII in "long-term" anticoagulant patients, suggesting the need for a greater degree of anticoagulation when these factor VIII increases appear. The present evidence supports the more widespread routine use of the cephalin time test in controlling long-term anticoagulants as it measures the overall "intrinsic" defect. The further suggestion of a quantitative relationship between cephalin time and platelet aggregation also supports its routine performance.

A.D.P. aggregation represents a reversible early stage of platelet aggregation (Hellem, 1960), and comparison with the phase of coagulation-induced aggregation is of interest. The results with the A.D.P. aggregation in our study were in sharp contrast to those with the coagulation-induced system. Instead of a reduction with the A.D.P. method, platelet aggregation was significantly increased in group 1. In group 2 it was also increased, but the smaller number and the scatter of results obtained with this method made it difficult to achieve a significant result.

Our results have shown that A.D.P.-induced aggregation as measured in an optical density system is significantly increased in patients who are receiving anticoagulant therapy after a thrombotic episode. An increase in the sensitivity of platelets to A.D.P. was noted by Hampton and Mitchell (1966), using an electrophoretic technique, in patients suffering from acute myocardial infarction. O'Brien *et al.* (1966) also observed increased sensitivity to A.D.P., and concluded that the administration of oral anticoagulants to patients with myocardial infarction had no effect on A.D.P.-induced aggregation of platelets. This suggested that something inherent in the blood of these patients was responsible for the increased A.D.P. sensitivity rather than the anticoagulant. The raised levels of serum lipids associated with ischaemic heart disease (Haslam, 1964) or the appearance of a lysolecithin in the plasma of these patients (Bolton *et al.*, 1967) was suggested as the reason for the increase in platelet sensitivity to A.D.P. In contrast Knieriem and Chandler (1967), using an A.D.P.-induced technique with Chandler's tube, also found an increase in the duration of A.D.P. aggregation in normal adult volunteers receiving coumarin anticoagulant, indicating that the effect is not due to a thrombotic tendency.

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