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A DIFFERENCE IN THE HALF-LIFE OF ANTIHEMOPHILIC FACTOR AS MEASURED BY PROCOAGULANT AND IMMUNOLOGIC TECHNIQUES

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ABSTRACT Antihemophilic factor (AHF, factor VIII) levels were measured by a standard coagulation method and by an immunologic technique before and after infusion of AHF concentrates into patients with classic hemophilia. After infusion of AHF concentrates, the half-life of the AHF procoagulant (i.e., clot-promoting) activity varied from 12 to 14 hr, whereas that of the antigen ranged from 24 to 40 hr. The half-life of the antigen was similar in patients with and without circulating anticoagulants to AHF. The data are compatible with the suggestion that the antigen may be carried on a precursor molecule which the patient with hemophilia produces but cannot convert to the functional clot-promoting agent. Other explanations of the observations are, however, recognized.

INTRODUCTION

Classic hemophilia is a familial hemorrhagic disorder characterized by an X-chromosome-linked recessive mode of inheritance and by a deficiency in plasma antihemophilic factor (AHF, factor VIII)[1] procoagulant or clot-promoting activity. Recently, employing highly purified preparations of normal human AHF, a precipitating antibody to normal AHF has been prepared. Using this antibody, we have observed that the plasma of all patients with classic hemophilia contained normal or increased amounts of a molecule precipitated by this antibody. In contrast, the plasma of patients with von Willebrand's disease was deficient both in AHF clot-promoting activity and this antigen (1, 2). These results have been confirmed by a number of studies (3–6).

Treatment of bleeding in patients with hemophilia requires the infusion of AHF concentrates twice daily to ensure hemostasis. This schedule is necessary because the clot-promoting material has a short half-life in the circulation after infusion into hemophilic patients (approximately 12 hr) (7, 8). We report here studies on the half-life of AHF procoagulant (i.e., clot-promoting) activity and of the AHF-like antigen after infusions of AHF concentrates into five patients with classic hemophilia, two of whom had developed powerful circulating anticoagulants to AHF. These observations indicate that the half-life of the antigenic protein is invariably longer than that of the AHF clot-promoting activity and is similar in the presence or the absence of circulating anticoagulants to AHF.

METHODS

Purified AHF was prepared from normal human plasma by successive precipitations with ethyl alcohol at −3°C, and with polyethylene glycol at room temperature, followed by filtration through agarose (Sepharose 4B) columns (2). This achieves purification of AHF of up to × 20,000. Antiserum to this purified AHF was raised as described in white female New Zealand rabbits (2). Antiserum thus obtained often produced three precipitin lines on routine immunoelectrophoresis of human serum, although contaminating proteins were not detectable by double diffusion in the preparations used as antigens. Absorption of the antiserum with an AHF-

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[1] *Abbrevintion used in this paper:* AHF, antihemophilic factor.

poor fraction of human plasma resulted in an antiserum with the following characteristics: (*a*) in specific assays the antiserum destroyed the functional clot-promoting activity of normal human AHF; (*b*) on double diffusion and immunoelectrophoresis the antiserum produced one line against normal human plasma and against the purified AHF; (*c*) use of the antiserum to quantify the antigen by the immunoelectrophoretic method described previously (2) detected normal quantities of antigen in patients with classic hemophilia and reduced or absent antigen in patients with von Willebrand's disease; in normal people and those with von Willebrand's disease the levels of antigen correlated well with those of functional AHF clot-promoting activity measured in specific assays. The antisera used in these studies fulfilled all three of these criteria. The term used to describe the antigen detected by the antisera, "AHF-like antigen," it must be emphasized, need not refer specifically to the functional normal molecule. It refers to the antigen, quantities of which correlate with those of AHF clotting activity in normal people and those with von Willebrand's disease, (2) but which is present in normal amounts in patients with classic hemophilia, who by definition are deficient in AHF clot-promoting activity.

The assay of AHF clot-promoting activity, together with details of the methods outlined above have been described (2). The assay technique for AHF-like antigen in the patients with classic hemophilia employed a modification in that whole plasma samples were applied to the wells in place of ethanol concentrates of plasma. As indicated previously this detects quantities of antigen over 25% of the standard pool when whole plasma is employed (2); in contrast to our previous report, duplication of results using whole plasma as source of antigen is excellent. At the normal or high levels of antigen which exist in the patients with classic hemophilia, therefore, it was unnecessary to concentrate plasma for the test; this avoids the possible influence of widely varying fibrinogen levels (before and after infusion of cryoprecipitate) in precipitating varying proportions of plasma AHF-like antigen into the concentrates.

1 U of AHF clot-promoting activity is that amount present in 1 ml of pooled whole normal plasma (2). 1 U of AHF-like antigen is also that amount present in 1 ml pooled whole normal plasma (2). As has already been emphasized, the two techniques do not necessarily measure identical molecular entities, and the two unitages need not, therefore, be interchangeable.

Collection of the blood samples and preparation of the plasma was as previously described (2).

Patients with classic hemophilia were admitted to the hospital for treatment of a variety of bleeding episodes. In these patients the diagnosis had been established on a basis of typical family and personal history of bleeding disorder, AHF clot-promoting activity levels of less than 0.01 U/ml plasma and normal bleeding times. The three patients without circulating anticoagulants, A, B, and C, had sustained minor head injuries in car accidents. Patients D and E, with circulating anticoagulants, had multiple hemarthroses and a large hematoma of the right gluteal region respectively. All were over 19 yr of age and had received multiple infusions of plasma and cryoprecipitate in the treatment of previous bleeding episodes. Patients A, B, and C received single infusions of cryoprecipitate, D a single infusion of glycine precipitated AHF (Hemophil, Hyland, Laboratories, Los Angeles, Calif.), and E received two infusions of cryoprecipitate. In patients D and E, treatment was discontinued when it became evident that an anticoagulant was present.

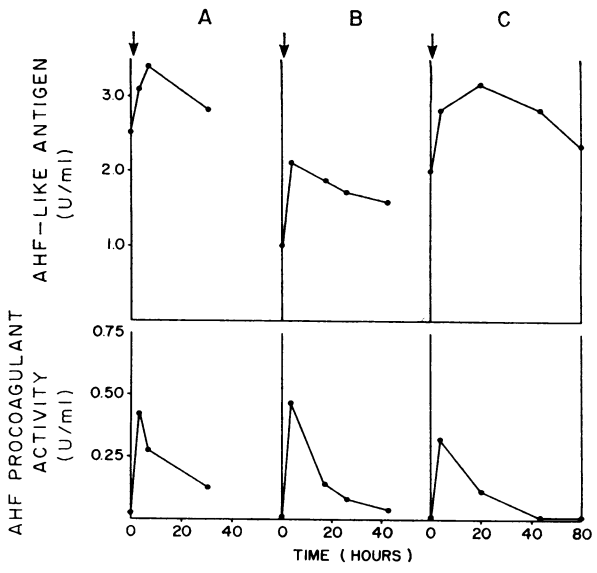

FIGURE 1 Levels of AHF procoagulant activity and AHF-like antigen before and after infusions of cryoprecipitate into three hemophiliacs without circulating anticoagulants. Infusions of cryoprecipitate are indicated by the arrows. Patient A, (weight 68 kg) received 25 bags cryoprecipitate, patient B, (weight 90 kg) received 35 bags and patient C, (weight 59 kg) received 23 bags. The half-life of AHF procoagulant activity was between 12 and 14 hr in all three patients, while that of AHF-like antigen was 24, 40, and 32 hr in A, B, and C, respectively. Each bag of cryoprecipitate was prepared from approximately 225 ml of fresh plasma (11).

The half-life[2] of the AHF clot-promoting activity or antigen was calculated by inspection of the plots of values observed at various times after the infusions. Relatively few observations were possible in four of the patients; in these, the first posttransfusion specimen was obtained between 15 and 30 min after completion of the infusions and the half-life was calculated from the peak values observed. In patient D, a specimen was obtained immediately following administration of the concentrate and a second 25 min later; in this patient, a biphasic decline in antigen levels was observed as has been suggested by other studies (9). The half-life in this patient therefore was calculated from the determinations made 25 min after completion of treatment (that is, at the time when the first postinfusion specimens were obtained from the other patients).

In calculating the half-life of the antigen it was assumed that the plasma concentration of the patients' own circulating antigen remained constant throughout the period of study, and provided, in effect, a base line for the calculation. This assumption was probably reasonable in this study, since in those patients observed for a sufficient length of time (D and E), the antigen level stabilized at approximately preinfusion values. In individuals subject to major trauma, not

[2] Strictly speaking, the term "half-disappearance time" is more appropriate than "half-life." The observations measured the time elapsing until one-half of the infused material was no longer detectable in the plasma. Presumably, this time reflects not only biological degradation or excretion, but also the rate of distribution to tissues other than plasma.

the case in these patients, this assumption may not prove valid.

RESULTS

Fig. 1 illustrates the data obtained in the three patients with classic hemophilia uncomplicated by anticoagulants. In all three, the half-life of AHF clot-promoting activity was close to that observed by many workers, ranging from 12 to 14 hr. In contrast, the half-life of the infused antigenic material was considerably longer than that of procoagulant activity, and ranged from 24 to 40 hr. Fig. 2 presents the observations in the patients with anticoagulants to AHF. As would be expected, the levels of AHF clot-promoting activity were not influenced by the infusions. Levels of AHF-like antigen, however, rose after infusion of the concentrates. The half-life of the antigen infused appeared to be similar to that in the three uncomplicated cases, 24 and 27 hr respectively, subject to the conditions for the calculation detailed in the methods section for patient D.

Table I presents the levels of AHF clot-promoting activity and antigen in 12 cryoprecipitate solutions. Fresh normal plasma is arbitrarily assigned 1 U of clot-promoting activity and 1 U of antigen/ml; as pointed out earlier, the two units may not reflect the same molecular entities. The preparations of the cryoprecipitates contained relatively more antigen than clot-promoting activity.

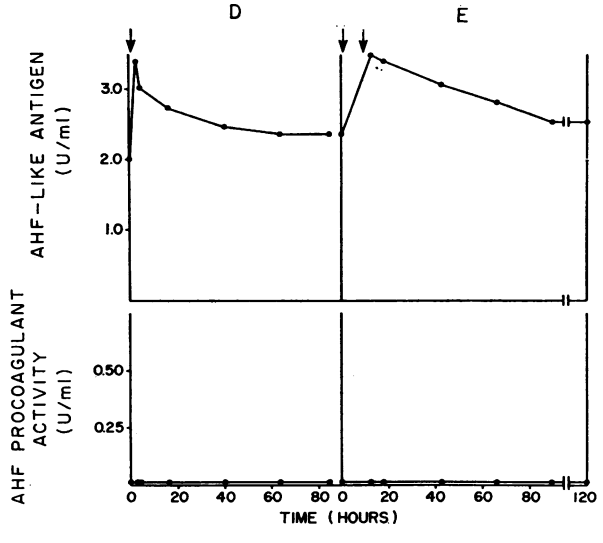

FIGURE 2 Levels of AHF procoagulant activity and AHF-like antigen before and after infusions of concentrate of AHF into two hemophiliacs who had developed circulating anticoagulants to AHF. The infusions are indicated by arrows. Patient D, (weight 50 kg) received glycine-precipitated AHF (Hemophil) equivalent by assay of procoagulant activity to 1700 ml plasma. Patient E, (weight 55 kg) received two infusions of cryoprecipitate, each of 12 bags, as indicated. No rise in level of functional AHF was observed in either patient. The half-life of the AHF-like antigen was 24 hr in D and 27 hr in E.

TABLE I

*The AHF Procoagulant Activity and the AHF-Like Antigen Present in Preparations of Cryoprecipitate**

Specimen	AHF procoagulant activity	AHF-like antigen
	U/ml	*U/ml*
1	10.8	36.0
2	9.2	24.8
3	13.6	32.0
4	8.4	24.0
5	7.2	24.8
6	20.0	44.0
7	9.2	32.0
8	6.2	20.4
9	7.2	32.0
10	2.4	10.4
11	4.4	12.0
12	2.9	10.6

* In these studies, specimens of cryoprecipitate were tested for procoagulant and antigen activity after individual bags of the material had been dissolved for a variety of reasons (administration to patients, purification of constituents, etc). Different volumes of solvent were therefore used and the results are presented simply to emphasize the greater quantities of antigenic material as compared with procoagulant in each preparation. The absolute differences in the values of different observations depend simply on dilution and are irrelevant.

DISCUSSION

Our observations on hemophilic patients indicate that the half-life of the AHF-like antigen is considerably longer than that of the AHF functional clot-promoting activity. Only one previous study with which we are familiar employed an immunological technique to determine the half-life of infused AHF. Information not available at the time of that study now raises questions as to the specificity of the antibody employed, but nevertheless Adelson and his colleagues, using such a technique, also reported a half-life of AHF considerably longer than that 12 hr usually observed when clot-promoting activity assays alone are employed (9).

The data included in Table I indicate that a large proportion of the antigenic material present in the cryoprecipitate preparations is not associated with clot-promoting activity as it is in fresh normal plasma. This may represent destruction of the clot-promoting activity as a result of the preparation of the precipitates, or preferential precipitation of the antigen, if the clot-promoting agent and the antigen should prove to be separate entities in normal plasma. If the first alternative is correct the data suggest that approximately two-thirds of the clot-promoting

activity has been lost during the manipulation of the plasma as judged by the relative amounts of clot-promoting activity and antigen. This is in agreement with our finding that preparations of cryoprecipitate currently in use supply rather less than the half of the original plasma clot-promoting activity which has been reported by some workers.

Previous studies have indicated that the plasma of hemophilic patients contains a material antigenically identical with that in normal plasma when tested with the rabbit antiserum to purified normal AHF (1–3, 5, 6). This suggests that hemophiliacs produce a functionally defective but antigenically detectable form of AHF. Alternatively, the antibody may detect, in addition to the functionally active material, a precursor form of AHF which shares the antigenic site with the completed molecule. Perhaps the hemophilic subject produces the precursor in normal quantity but is deficient in an agent which converts it to the active form. The infused cryoprecipitates present the patient with quantities of antigenic material considerably in excess of functional clot-promoting activity. The longer half-life for the antigen would be expected if the hemophilic patient was unable to utilize the antigenic material by converting it to active clot-promoting agent. It is recognized, however, that other possible causes of the prolonged half-life of the antigenic material exist; for example, alteration of the molecule in preparation of cryoprecipitate may prolong its half-life.

The long half-life of the antigenic material in the hemophiliac is in sharp contrast to that in the patient with von Willebrand's disease in whom antigen is rapidly cleared from the blood, with a half-life of less than 12 hr, as if these patients were utilizing or destroying the protein in some manner not available to the hemophiliac (10). Thus, it is clear that patients with hemophilia and von Willebrand's disease deal with the protein detected by our rabbit antibody in markedly different fashion. We wish to emphasize, however, that although these results may be interpreted on the precursor hypothesis outlined, not all the present observations are thus simply explained and alternative interpretations are recognized.

The similarity in the half-life of the antigen in the presence and the absence of circulating anticoagulants to AHF serves to emphasize the dissimilarity of these human anticoagulants (which are, in all probability, autologous antibodies) and the heterologous antibody to purified AHF prepared in rabbits. Human anticoagulants do not demonstrate precipitin activity, although the most active ones we have studied are more powerful inhibitors of the AHF clot-promoting activity than are our best preparations of rabbit antiserum. Indeed, the rabbit antiserum may not be directed against the functional site at all, but may render AHF inactive simply by precipitation. It is, therefore, not surprising to observe the presence of antigen in patients with anticoagulants nor to note the similar half-life of the antigen in these individuals and in uncomplicated hemophiliacs. If the hemophiliac cannot utilize the protein normally, while the anticoagulant inactivates AHF without forming a precipitin, one would anticipate this result.

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