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Different Properties of Factor VIII in Von Willebrand's Disease with Respect to Recovery in Cryoprecipitate

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Keywords. Von Willebrand's disease · Hemophilia-A · Factor VIII · Factor VIII-related antigen · Bleeding factor · Cryoprecipitate

Abstract. The recovery of factor VIII-activity in cryoprecipitate prepared from plasma from six patients with von Willebrand's disease was low as compared to the recovery from normal and hemophilia-A plasma. The possible cause is briefly discussed.

Using a standardized method to prepare cryoprecipitate from small plasma samples it was found that the recovery of factor VIII-activity (F. VIII-act.) was low from plasma collected from patients with von Willebrand's disease (vWd). The results are reported and discussed.

Materials and Methods

Platelet-poor plasma (PPP). 5 ml of blood was collected into polystyrene tubes containing 0.1 ml sodium citrate 0.55 M, centrifuged immediately (10 min, 1,000 g, room temperature) the plasma separated and centrifuged again (25 min, 20,000 g, 4°C).

Preparation of cryoprecipitate. Immediately after the second centrifugation run, 2-ml samples of PPP were pipetted into polycarbonate centrifuge tubes, quickly frozen at -30 °C in an ethanol/water mixture, stoppered and stored at -70 °C for 1-7 days. The samples were then thawed for 1 h at 2 °C in a continuously stirred ethanol/water mixture. (Preliminary experiments showed that thawing for 45-120 min gave reproducible results.) The tubes were then centrifuged (15 min, 20,000 g, 2 °C) and the supernatant poured off. Remnants of supernatant were carefully removed with filter paper. The cryoprecipitate

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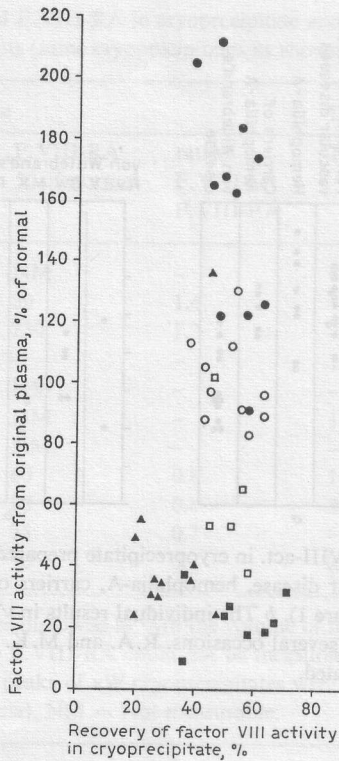


Fig. 1. Recovery of F. VIII-act. in cryoprecipitate prepared from normal, hemophilia-A and vW plasma. Proven carriers of hemophilia-A, who were known to have a low F. VIII-act. and patients with cardiovascular disease known to have a high F. VIII-act. were included to obtain a continuous scale of F. VIII-act. in the plasmas studied. \circ = Normals; \bullet = patients with cardiovascular diseases; \blacksquare = hemophilia-A; \square = carriers of hemophilia-A; \blacktriangledown = von Willebrand's disease.

was dissolved in 0.4 ml veronal buffer (pH 7.42). In 8 duplicate preparations of cryoprecipitate of plasma (4 vWd and 4 normals) the mean error of recovery was 4.6%. F. VIII-act. in supernatant and cryoprecipitate were measured immediately.

Factor VIII-related antigen (F. VIII-RA) was measured by the Laurell technique using rabbit anti-human factor VIII antiserum (Nordic, Tilburg). Pooled normal plasma was used as a standard. *F. VIII-act.* was assayed according to VELTKAMP *et al.* [14] as modified by VELTKAMP *et al.* [15]. *Bleeding time* according to IVY [3]. *Retention of platelets in a glass-bead column* was measured according to SALZMAN [8].

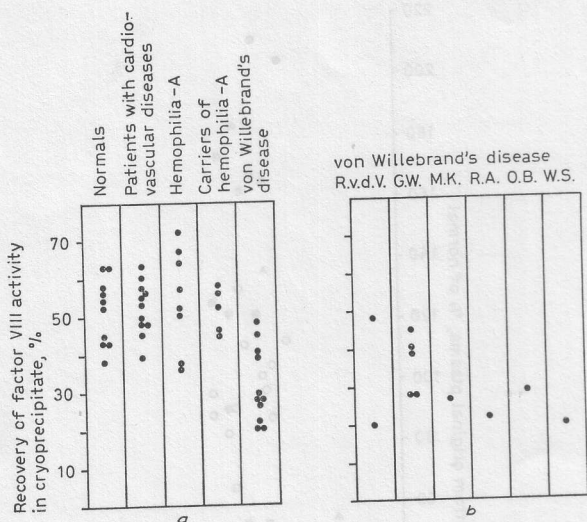


Fig. 2a. Recovery of F. VIII-act. in cryoprecipitate prepared from plasma of normals, patients with cardiovascular disease, hemophilia-A, carriers of hemophilia-A and vWd (same data as shown in figure 1). b The individual results in vWd are shown. R. v. d. V. and G.W. were studied on several occasions. R.A. and M.K. are mother and daughter, the other patients are unrelated.

Results

Recovery of F. VIII-act. in cryoprecipitate prepared from vW plasma was found to be low as compared to the recovery from plasma from normals and hemophilia-A patients (fig. 1, 2a). The F. VIII-act. left in the supernatant was correspondingly higher in vWd (table I). There was no correlation between the F. VIII-act. in the original plasma and the recovery of it in the cryoprecipitate (fig. 1). In figure 2b the recovery of F. VIII-act. in cryoprecipitate is shown separately for each of the six vW patients studied. All vW patients had low F. VIII-act. (see fig. 1), a strongly prolonged bleeding time and a low retention of platelets in the glassbead column. G.W. once showed a normal F. VIII-act. in her plasma (135% of normal) when she had an infectious disease. F. VIII-RA was measured in cryoprecipitate and supernatant (table I). In no instance could F. VIII-RA be measured in the supernatant. In cryoprecipitate the ratio biological activity/F. VIII-RA ranged from 0.7 to 1.4.

Table I. F. VIII-act. and F. VIII-RA in cryoprecipitate and its supernatant prepared from 6 vW patients (same cryoprecipitates as shown in figures 1 and 2)

Patient	Cryoprecipitate			Supernatant	
	F. VIII-act. % of normal	F. VIII-RA % of normal	ratio F. VIII-act F. VIII-RA	F. VIII-act. % of normal	F. VIII-RA % of normal
R. v. d. V.	57	NM	-	8	NM
	42	30	1.4	-	-
G. W.	304	230	1.3	-	-
	80	-	-	18	NM
	50	NM	-	-	-
	42	NM	-	14	NM
	42	NM	-	-	-
M. K.	48	60	0.8	18	NM
R. A.	60	80	0.8	32	NM
O. B.	49	75	0.7	-	-
W. S.	65	90	0.7	22	NM

The cryoprecipitates are dissolved in $\frac{1}{5}$ th the volume of the original plasma. In some of the cryoprecipitates the F. VIII-RA could not be measured, which may be due to the fact that the precipitation peaks of vW cryoprecipitates were often faint as compared to the standard (normal plasma). NM = Not measurable.

Discussion

There is accumulating evidence that F. VIII-act. is bound to more than one molecular form [1, 2, 4, 7, 9, 10, 12, 13, 16, 17] although it is questionable whether (part of) these reported forms reflect the native state of F. VIII.

In former experiments it was found by us that F. VIII-act. in plasma *in vitro* drops to about 50% of its initial value in about 10 h while during the following 4 days practically no further drop in activity was seen [10, 12]. The results were interpreted in this manner that F. VIII-act. was bound to two molecular forms which were interdependent [12]. One form then is labile (or converted to the other form *in vitro*), while the other is quite stable. In some patients with vWd the initial drop in F. VIII-act. was higher, being about 70% of the initial value. This was true for their own as well as transfusion-induced F. VIII-act. [10] suggesting different properties of F. VIII in those vW patients investigated as compared to normal F. VIII.

WEISS and KOCHWA [16] reported that normal plasma contained two molecular forms of F. VIII, a lower and a higher molecular weight form, while cryoprecipitate only contained the high molecular weight form. These results, combined with the finding that the F. VIII-act. in cryoprecipitate in comparable conditions as in plasma (normal cryoprecipitate dissolved in hemophilia-A plasma) is fairly stable [11] led us to assume that this high molecular weight form of F. VIII might be related or identical with the form which was recognized by us as stable. Following this reasoning and together with our former finding in vWd, differences in cryoprecipitation of vW plasma were to be expected. In fact, low recovery of F. VIII-act. and F. VIII-RA in several vW patients were reported [5]. Cryoprecipitates were made under standardized conditions of six patients with vWd – several of them on different occasions – and compared with normals and hemophilia-A patients. In the vW patients the recovery was distinctly lower.

These results may be explained on basis of a hypothesis on the synthesis of F. VIII and the Bleeding factor (or von Willebrand factor) which was proposed by us in 1967 [10] to account for our finding that *in vitro* about 50% of the F. VIII-act. disappears quickly while the remaining activity is stable [10, 12] and at the same time could explain all phenomena seen in vWd and hemophilia-A. It was assumed that F. VIII was composed of two subunits, i.e. subunit α (α for hemophilia-A), deficient or defective in hemophilia-A and subunit β (β for Bleeding factor), deficient or defective in vWd. Subunits α and β are coded by a gene on the X or an autosomal chromosome respectively. Subunit β regulates the rate of synthesis or at least the release of subunit α . The Bleeding factor activity was supposed to reside on the β subunit. The F. VIII-RA as described later [18] was thought to be identical with the β subunit. F. VIII-act. may be bound to the free α subunit, the combined α and β subunit and/or polymers of it $(\alpha_n\beta_m)_x$, thus explaining the existence of two (or more) interdependent molecules showing F. VIII-act. Recently a hypothesis has been proposed by BLOOM *et al.* [1] which has many similarities to ours. The high molecular weight and low molecular weight subunits in their hypothesis being comparable to our β and α subunit, respectively.

A deficient or defective β subunit – resulting in some type of vWd – may change the relative concentrations of the different forms of biological active F. VIII, or alternatively change the specific biological F. VIII-act. of one form, resulting in different recovery of the F. VIII-act. in cryoprecipitate. All vW patients investigated by us belonged to the same type of vW with regard to low F. VIII-act., strongly prolonged bleeding time and low retention of platelets in the glassbead column.

The recovery of F.VIII-RA in cryoprecipitate may depend on the type of vWd, namely the kind of defect of β subunit or deficiency of it. KERNOFF *et al.* [6] reported a consistent low recovery of F.VIII-RA in cryoprecipitate prepared from plasma of one patient with vWd as compared to normal and hemophilia-A plasma. F.VIII-act. was not measured. In five other vW patients the results were found to be more variable with what was thought to be probably due to the difficulty measuring low levels of F.VIII-RA. In our vW patients the ratio biological F.VIII-act./F.VIII-RA in the cryoprecipitate ranged from 0.7 to 1.4 (table I). Whether this broad range represents real differences or is due to technical difficulties is not known. One might expect that differences in molecular form will be reflected in the ratio biological activity/F.VIII-RA. It has to be recognized, however, that different molecular forms may differ in specific biological F.VIII-act. Furthermore, it is not known what fraction of the total β subunit (i.e. of the total F.VIII-RA measured) occurs in a state not related to F.VIII-act., e.g. free and/or bound to inactivated α subunits. On the other hand, in the active $(\alpha_n\beta_m)_x$ form, antigenic determinants of the β subunit may be concealed.

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