

Ristocetin: A Means of Differentiating Von Willebrand's Disease Into Two Groups

By Margaret A. Howard, R. J. Sawers, and B. G. Firkin

Earlier studies have shown that patients with von Willebrand's disease are considered to have normal platelets, but they lack at least one plasma protein. Results are presented indicating that ristocetin, known to aggregate normal platelets, fails to cause platelet aggregation in the group of von Willebrand's disease patients exhib-

iting no platelet adhesiveness. It is postulated that there may be two groups of patients within von Willebrand's disease and, further, that ristocetin will provide a useful approach to study the plasma deficiency or abnormality in von Willebrand's disease.

RISTOCETIN is an antibiotic of unknown chemical structure that causes platelet aggregation in man. Its precise mode of action is unknown, although it does induce a release reaction and aggregation can be partially blocked with agents such as adenosine.⁶ Of particular interest is the fact that patients with congenital thrombasthenia will aggregate with ristocetin.⁷ Platelet aggregation has been found to be normal in a number of the classical bleeding disorders (such as hemophilia, afibrinogenemia, and Christmas disease) and in patients receiving warfarin.⁶ Abnormalities with ristocetin aggregation have been observed, however, in acquired platelet defects in patients with infectious mononucleosis, idiopathic thrombocytopenia, and acute leukemia. However, in these situations other defects in platelet aggregation to collagen or ADP are usually present.^{2,3} In these instances, the defect is believed to involve the platelet intrinsically, since it is not corrected by the addition of normal plasma.^{2,3,11}

Criteria for the diagnosis of von Willebrand's disease are still disputed.¹³ With regard to platelet function, it has been observed that lowered or absent platelet adhesiveness is common in patients with von Willebrand's disease,^{1,5,9} while other platelet function tests, including aggregation, are normal.^{10,12}

This communication reports observations that suggest that patients with von Willebrand's disease may be separated into two groups. Ristocetin aggregation was abnormal in the group of patients with von Willebrand's disease exhibiting no platelet adhesiveness. On the other hand, platelets from patients exhibiting low normal adhesiveness all aggregated with ristocetin.

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MATERIALS AND METHODS

Patients selected for this study fulfilled the following criteria for von Willebrand's disease: (1) a family history of an autosomal dominant trait of easy bruising, prolonged bleeding following trauma, mucosal hemorrhages, and/or excessive menstrual loss; (2) a prolonged skin bleeding time on at least one occasion; and (3) a markedly reduced plasma factor VIII level.

Platelet aggregations were performed as described previously.⁶ Ristocetin was supplied by Abbott Laboratories, Chicago, Ill., and was dissolved in normal saline to produce a final concentration in platelet-rich plasma (PRP) of 1.0 mg/ml. Adenosine diphosphate (ADP) was obtained from Sigma and dissolved in normal saline to produce a final concentration of 5×10^{-6} M. Results are expressed as a percentage and calculated using the following formula:

$$\frac{\text{Optical Density of (PRP minus PRP agg.*)}}{\text{Optical Density of (PRP minus PPP*)}} \times 100$$

Washed platelets were prepared from whole blood collected into 4% ethylene diametetraacetic acid (EDTA) in 0.154 M Tris, EDTA, NaCl, glucose buffer⁸ (pH 7.4). After four washes, the platelets were resuspended in the same buffer lacking EDTA to give a final platelet count of 280,000/cu mm.

Platelet adhesions were performed using the method described by Salzman,⁹ with the normal range for this laboratory being 20%–80%.

Factor VIII assays were performed following the method described by Hardisty and McPherson.⁴

RESULTS

Table 1 contains the results of factor VIII assays, tests of platelet adhesions and of platelet aggregations with both ADP (5×10^{-6} M) and ristocetin (1.0 mg/ml) on 14 patients with von Willebrand's disease. All 14 patients exhibited normal aggregation to ADP (5×10^{-6} M), while aggregation with ristocetin (1.0 mg/ml) by nine of the patients was either absent or markedly reduced. These findings were consistently obtained when several patients were reexamined on a number of occasions. It may be of interest that three of the five patients with "normal" aggregation to ristocetin were at the lower limit of the normal range (Table 1).

It can be seen from Table 1 that a correlation exists between platelet aggregation toward ristocetin (1.0 mg/ml) and platelet adhesiveness to glass beads. No obvious correlation is evident between the plasma factor VIII levels and platelet function.

Washed, normal platelet resuspended in buffer alone did not aggregate with ristocetin, but the addition of 0.2 ml of normal PPP resulted in aggregation; conversely, no aggregation occurred when von Willebrand's plasma was added to the platelet suspension. Washed platelets from a patient with von Willebrand's disease (M.B.), whose PRP did not aggregate with ristocetin, were similarly tested and were found to aggregate as well as the normal control on the addition of normal PPP, but not her own plasma (Fig. 1). Plasma and serum from other patients in the same category as M.B. also failed to stimulate ristocetin aggregation.

*PPP, platelet-poor plasma; PRP agg., OD of the PRP solution following aggregation of platelets.

Table 1. Platelet Aggregation

Patients	Adhesion (%)	Ristocetin (%)	ADP (%)	Factor VIII (%)
CONTROL	20-80	45-90	35-95	50-150
J.S.	22	80	68	33
C.H.	17	60	85	30
M.U.*1	15	41	61	20
G.U.*1	17	48	64	17
N.S.	25	45	50	4
M.B.	0	0	100	18
G.P.	0	0	47	12
C.M.*2	0	0	83	10
J.M.*2	0	0	76	5
T.C.*2	0	0	76	15
D.W.*2	0	0	93	30
B.K.	0	18	75	15
B.W.*3	0	19	100	2
J.W.*3	6	7	89	15

*Indicates related patients.

DISCUSSION

The results suggest that there may be two groups of patients within the label of von Willebrand's disease. These two groups may represent two distinct abnormalities or, alternatively, the results may be explained in terms of a differing degree of severity of the same basic abnormality.

Examination of Table 1 indicates that related patients demonstrated the same pattern of abnormalities. Family No. 2 was particularly important, as C.M. was the grandfather of J.M. and T.C., who were first cousins.

It is well recognized that platelets from Von Willebrand's patients exhibit normal aggregation to a wide range of agents.^{10,12} Therefore, the correlation between lack of platelet response to ristocetin and lack of platelet adhesion suggests that aggregation by ristocetin may involve a plasma factor required for platelet adhesion to glass beads that is absent or abnormal in patients

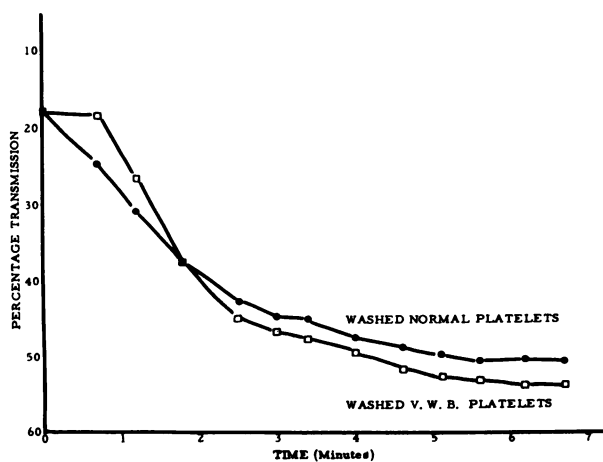


Fig. 1. Effect of normal platelet-poor plasma on aggregation of platelets from a patient (M.B.) with von Willebrand's disease (V.W.B.)

with von Willebrand's disease. This is supported by the finding that the addition of normal plasma to PRP from patients with von Willebrand's disease enabled the platelets to aggregate in response to ristocetin and that washed platelets from a patient with von Willebrand's disease aggregated normally with ristocetin when small amounts of normal plasma were added.

These studies indicate that ristocetin provides a useful method for study of the underlying plasma deficiency or abnormality in von Willebrand's disease that is manifested as decreased platelet adhesiveness. Ristocetin may also be of diagnostic value in confirming the presence of von Willebrand's disease.

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