Inhibition of Human Platelet Aggregation by Plasmin Digests of Human Factor VIII

By M. B. Donati, G. de Gaetano, J. Vermylen, and M. Verstraete

Human factor VIII, upon prolonged incubation at 37°C with plasmin, inhibits platelet aggregation induced by ADP, adrenaline, or collagen. The human factor VIII digests are not anticoagulant in the thrombin time or partial thromboplastin time. Factor VIII split products may interfere in vivo with platelet function and thus could contribute in some way to the hemorrhagic diathesis of fibrinolytic states.

WE HAVE RECENTLY shown[1] that purified, fibrinogen-free *bovine* factor VIII (F VIII) aggregates human platelets, a finding also reported by Forbes and Prentice.[2] This activity would be due to interaction of exposed carbohydrate side chains containing galactose but not terminated by sialic acid with a sialyltransferase in the platelet membrane.[3] This situation would be normally present in bovine F VIII and absent in human F VIII, which cannot aggregate human platelets, unless previously treated with neuraminidase.[3] When bovine F VIII was incubated with plasmin, its coagulant activity disappeared much earlier than the aggregating activity. At the stage of extensive degradation, when the F VIII lysate had totally lost its aggregating capacity, it developed an inhibitory effect on platelet aggregation induced by undegraded F VIII, adenosine-5′-diphosphate (ADP) or collagen; it had, however, no anticoagulant effect on the thrombin time or the partial thromboplastin time of normal plasma.[1] The aim of this paper is to briefly report inhibition of human platelet aggregation by *human* F VIII digested by plasmin.

MATERIALS AND METHODS

F VIII was purified by gel filtration of human chylomicron-poor cryoprecipitate (Belgian Red Cross) on agarose (Bio-Gel A-5M, Bio Rad Laboratories) as previously described for bovine F VIII.[1] The F VIII coagulant activity, measured by the method of Vermylen and Verstraete,[4] was eluted in a single peak coinciding with the void volume. This peak did not contain fibrin(ogen) complexes, as shown by the absence of clottable protein[5] and of staphylococcal clumping activity[6]; furthermore, no fibrinogen-related antigen was found by the Tanned Red Cell Hemagglutination Inhibition Immunoassay.[7] The starting material contained about 15 F VIII U/ml, whereas the eluted fractions contained 1.5 2.0 F VIII U/ml. Purified human fibrinogen and human plasmin were kindly provided by Kabi (Stockholm); plasmin contained 35 Casein Units Sgouris/ml (= 39.2 CTA units plasmin/ml). ADP, adrenaline and collagen were prepared as described

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Table 1. Effect of Overnight Incubation at 37°C on F VIII and Clottable Protein

	F VIII	F VIII + Plasmin	Fibrinogen	Fibrinogen + Plasmin
	U/ml		Clottable Protein (mg/ml)	
Before incubation	1.0	1.0	3.0	3.0
After incubation	0.4	0.01	2.65	< 0.08

previously.[8] Platelet aggregation was followed in a Born MKI aggregometer at 37°C and the variations of light transmission automatically recorded as already described.[9] One milliliter F VIII (containing one F VIII unit), 1 ml fibrinogen (containing 3 mg clottable protein) or 1 ml elution buffer were incubated overnight at 37°C with or without 0.7 C.U. Sgouris of plasmin.

RESULTS

The effect of incubation of F VIII and clottable protein is summarized in Table 1. Neither purified F VIII nor purified fibrinogen (either fresh or incubated overnight at 37°C) were capable of modifying platelet aggregation. In contrast, F VIII lysate inhibited platelet aggregation induced by ADP (Fig. 1), adrenaline, or collagen. Plasmin digests of purified fibrinogen also showed a strong inhibitory effect on platelet aggregation induced by the above-mentioned substances, a finding first reported by Kowalski et al.[10] Plasmin itself had no effect in the platelet aggregation test. The F VIII digest did not prolong the thrombin time or partial thromboplastin time of normal plasma.

Pasquini and Hershgold[11] first reported the lack of anticoagulant activity of human F VIII digests and on this basis suggested that F VIII split products, possibly formed in fibrinolytic states, would not contribute significantly to the hemorrhagic diathesis in these conditions. This conclusion may seem premature in view of the in vitro interference of F VIII split products with platelet function

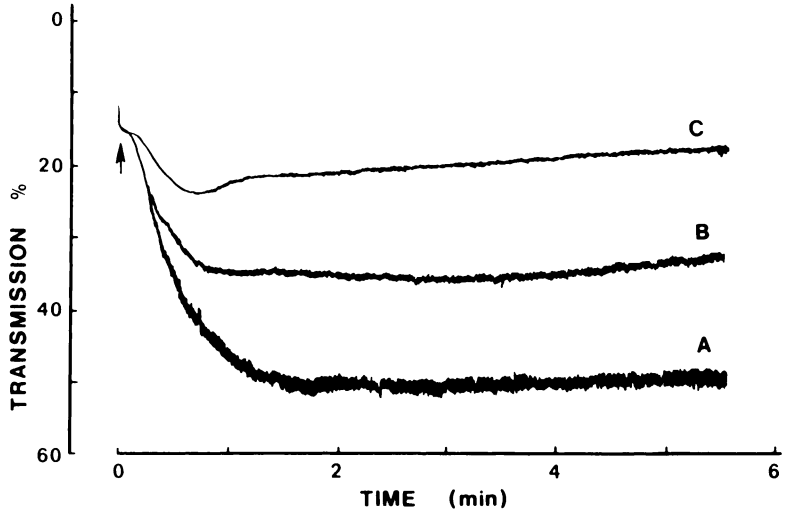

Fig. 1. Aggregation of citrated human platelet rich-plasma by 1 μM ADP in the presence of: A, buffer; B, plasmin-treated human F VIII diluted 1 to 10 in platelet-rich plasma; C, plasmin-treated human F VIII diluted 1 to 5. 1 ml human factor VIII (containing 1 factor VIII unit) was preincubated overnight at 37°C with 0.7 C.U. Sgouris of plasmin. The arrow indicates the moment of the addition of ADP.

reported here. Studies are in progress in our laboratory with more purified preparations of human F VIII; moreover, an attempt is being made to determine the size of the inhibitory split products.

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