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Research Article

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Use of Human Factor VIIa in the Treatment of Two Hemophilia A Patients with High-Titer Inhibitors

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ABSTRACT Two patients with hemophilia A complicated with high-titer alloantibodies have been treated by repeated infusions of microgram quantities of pure human Factor VIIa. Patient 1 was presented with a gastrocnemius muscle bleeding that involved the knee joint. Upon treatment with Factor VIIa the circumference of the muscle decreased and joint mobility increased substantially. Patient 2 was given Factor VIIa concurrent with tranexamic acid in association with the extraction of two primary molars. No significant gingival bleeding occurred after Factor VIIa and tranexamic acid treatment. Furthermore, no deleterious side effects or increase of the alloantibody level were observed in either patient throughout the Factor VIIa infusion. These results, although limited and preliminary in nature, suggest that trace quantities of Factor VIIa can act as a Factor VIII bypassing activity and restore hemostasis in these patients.

INTRODUCTION

Patients with severe hemophilia A complicated with high titers of antibodies against Factor VIII:C_{Ag} represent a serious therapeutic problem. Recently, the use concentrates of the unactivated and activated prothrombin-complex have met with limited success in some cases (1-5), while in others, evidence of disseminated intravascular coagulation or thromboembolic complications were observed (6-8). The putative hemostatic agent(s) in these preparations has never been identified, although Factor VIIa has been suggested as one possibility (9). Factor VIIa has an absolute requirement for tissue factor for expression of its proteolytic activity. In addition, Factor VIIa is not inhibited to any significant extent by antithrombin III in the absence of heparin (10). Thus, it is possible that upon administration, Factor VIIa may find its way

unabated to the site of trauma, complex specifically with tissue factor at the site, and rapidly initiate the formation of thrombin by the extrinsic pathway. This mechanism would "by-pass" the intrinsic pathway, which requires normal levels of Factor VIII and Factor IX. With homogeneous preparations of Factor VIIa, our goal was to test this hypothesis in hemophilia A patients complicated with alloantibodies. We report herein the treatment of two patients with severe hemophilia A with alloantibodies against Factor VIII:C_{Ag} by the infusion of microgram quantities of homogeneous preparation of human Factor VIIa.

METHODS

Coagulation assays

Activated partial thromboplastin time (General Diagnostics Automated APTT, General Diagnostics, Div. of Warner Lambert Co., Morris Plains, NJ) was performed according to the manufacturer's specifications. Factor XII, Factor XI, Factor V, Owren's P + P (prothrombin plus Factor VII plus Factor X), fibrinogen, and fibrinogen/fibrin degradation products were assayed as previously described (11). Prothrombin time, thrombin time, and reptilase time were performed as described (11). Factor VIII coagulant activity (VIII:C) and Factor VIII-related antigen (VIII:R_{Ag}) were assayed according to Nilsson (12) and Holmberg and Nilsson (13), respectively. Antithrombin III and α_2 -antiplasmin were assayed by electroimmunoassay (14) as well as by amidolytic assay using S-2238 and S-2251, respectively. Factor X and prothrombin were assayed by one-stage assays according to Bachmann et al. (15) and Hjort et al. (16), respectively. Factor IX coagulant activity (IX:C) was assayed by a one-stage assay (12) and Factor IX antigen (IX:Ag) was assayed by the immunoradiometric assay as described by Holmberg et al. (17). Factor VII was assayed by a one-stage assay using hereditary Factor VII-deficient human plasma and human thromboplastin (10). One unit of Factor VII activity is arbitrarily defined as that amount of activity present in 1 ml of normal, pooled human plasma. The inhibitory activity of the plasma was expressed in Bethesda units (BU)¹ (18).

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¹ Abbreviations used in this paper: BU, Bethesda units; BW, body weight.

Preparation of human Factor VIIa

Factor VII was purified from human citrated fresh-frozen plasma through the DEAE-Sepharose step essentially as described by Broze and Majerus (10). Fractions from the DEAE-Sepharose containing Factor VII activity were pooled, concentrated to ~30 ml by ultrafiltration, and dialyzed overnight against 0.05 M Tris-HCl (pH 8.0)/0.15 M NaCl/10 mM benzamidine. The retentate was applied to a QAE-Sephadex A-50 column (1.6 × 35 cm) previously equilibrated at 4°C with 0.05 M Tris-HCl (pH 8.0)/0.15 M NaCl/1 mM benzamidine. Factor VII was eluted from the column with a linear gradient of CaCl₂ consisting of 150 ml of equilibrating buffer and 150 ml of equilibrating buffer containing 10 mM CaCl₂. The fractions containing Factor VII were combined, made 10 mM in EDTA, and concentrated to 10 ml by ultrafiltration. The concentrate was dialyzed overnight at 4°C against 25 mM Tris-HCl (pH 8.0)/25 mM glycine/20 mM benzamidine/5 mM EDTA, and subsequently subjected to preparative electrophoresis as described (19). Fractions were assayed immediately after elution from the preparative electrophoresis column, and those containing Factor VII activity pooled, and concentrated to ~0.5 mg/ml by ultrafiltration. Factor VII isolated by this procedure appeared as a single band by SDS-polyacrylamide gel electrophoresis in the presence or absence of reducing agent. The molecular weight noted by this technique was 50,000 and the specific clotting activity was 2 U/μg. Single-chain Factor VII was converted to the two-chain Factor VIIa by limited proteolysis using human Factor XIIa at an enzyme-to-substrate weight ratio of 1:100. Two-chain human α-Factor XIIa was generously provided by Dr. Kazuo Fujikawa, University of Washington. The Factor XII was isolated from human plasma as described (20), and converted to two-chain Factor XIIa by human kallikrein in the presence of dextran sulfate (21). Before activation Factor VII was dialyzed at 4°C against 20 liter of 0.01 M Tris-HCl (pH 8.0)/0.15 M NaCl. The dialyzed Factor VII was then incubated at 37°C with the appropriate amount of human Factor XIIa. Complete conversion of Factor VII to Factor VIIa occurred within 2 h under these conditions, and coincided with the formation of a two-chain Factor VIIa molecule consisting of a heavy chain ($M_r = 34,000$) and a light chain ($M_r = 24,000$) held together by a disulfide bond(s) (Fig. 1). The specific coagulant activity of Factor VII increased to a maximum of 50 U/μg after activation by Factor XIIa. Factor VIIa was then sterilized by Millipore filtration (Millex-HA 0.45, μm membrane, Millipore Corp., Bedford, MA) in the hospital pharmacy. Approximately 20–25% of the Factor VIIa activity was lost during the sterilization-filtration process. Portions of the sterilized preparations were analyzed for pyrogens (the National Bacterial Laboratory, Stockholm, Sweden) and for HbsAg using the Ausria II assay (Abbott Laboratories, North Chicago, IL). The sterilized Factor VIIa was stored at -80°C in 0.5-ml aliquots (~10,000 U per vial) before use. Before infusion, each preparation was diluted with sterile saline to the concentration desired.

Protocol for the infusion of human Factor VIIa

These studies were conducted with the approval of the Human Subjects Committee of the University of Lund, Malmo, Sweden, and followed guidelines established by the Declaration of Helsinki. Informed consent was obtained from each patient and their guardian before treatment. Before infusion into patients, the thrombotic potential of the

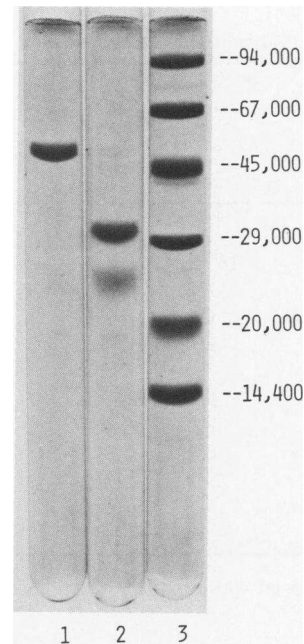


FIGURE 1 SDS polyacrylamide gel electrophoresis of human Factor VIIa. Sample 1, 15 μg of unreduced Factor VIIa; sample 2, 15 μg of reduced Factor VIIa; sample 3 contains a mixture of reduced standard proteins that include phosphorylase *b* (94,000), bovine serum albumin (67,000), ovalbumin (45,000), carbonic anhydrase (29,000), soybean trypsin inhibitor (20,000), and α-lactalbumin (14,400). Electrophoresis was carried out in 10% polyacrylamide gels as previously described (19).

Factor VIIa preparations was examined in healthy dogs by essentially the same protocol described for the analysis of prothrombin complex concentrates (11). No attempt was made to remove the trace amounts of Factor XIIa present in the Factor VIIa preparations. Accordingly, these studies examined the thrombotic potential of Factor XIIa as well as Factor VIIa. Each preparation of Factor VIIa was given to a mongrel (16–17 kg) in a dose of 50–100 U Factor VII/kg body wt (BW). The concentrate was given intravenously in 3 ml of sterile saline without anesthesia. The sampling was performed by intermittent venous puncture before the injection, and 15 min, 1, 4, and 24 h after injection. Each time, 30 ml of blood was withdrawn and anticoagulated with 0.038% sodium citrate. After infusion of Factor VIIa, absolutely no change in the coagulation or fibrinolytic profile was observed in the dog plasma except the expected rise in plasma Factor VII (Table I). Furthermore, the dogs did not exhibit any untoward side effects during or after the injection of Factor VIIa.

Case reports

Case 1. A 13-yr-old male with severe hemophilia A was admitted to the hospital with bleeding in the left gastrocnemius muscle. The circumference of the left leg just below the knee was 33.5 cm as compared with 30 cm for the right

TABLE I
Coagulation Analysis in Two Dogs Given 100 U VIIa/kg BW and 50 U VIIa/kg BW, Respectively

	100 U/kg BW					50 U/kg BW				
	Before	After				Before	After			
		15 min	1 h	4 h	24 h		15 min	1 h	4 h	24 h
Platelets, $10^9/liter$	—	320	270	330	311	404	315	348	316	320
APTT, s	18 (18)*	18 (18)	17 (18)	17 (18)	17 (18)	18 (18)	18 (18)	18 (18)	17 (18)	18 (18)
VIII:C, %	153	103	97	100	105	87	80	83	62	110
IX:C, %	180	167	169	101	156	135	106	108	98	103
P&P, %	116	128	172	180	152	136	175	152	162	150
VII, %	83	500	430	235	102	90	235	242	235	109
X, %	116	158	152	155	148	140	165	135	130	135
V, %	100	110	95	105	90	100	95	110	90	110
Fibrinogen, g/liter	2.8	1.5	2.4	2.3	2.9	1.7	2.5	2.5	1.9	1.8
Ethanol gel test	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg
α_2 -macroglobulin, %	80	80	79	78	74	106	80	73	77	89

* The numbers given within parentheses represent the APTT for normal dog plasma. Neg, negative.

leg. Secondary to the muscle bleeding the patient also exhibited an extension defect of 90° in the left knee. Laboratory records indicated that his antibody titer varied from 0.3–3.0 BU/ml and increased 50-fold upon administration of Factor VIII concentrates. The patient had repeated joint bleedings over the past 7 yr and was treated with a combination of high doses of Factor VIII and cyclophosphamide on eight different occasions. He has also been treated with activated prothrombin complex concentrates (50 U/kg BW FEIBA [Immuno, Vienna] or 50 U/kg BW Autoplex [Hyland, Costa Mesa, CA] in association with minor bleedings, particularly in the ankle joint. During most of these episodes, he experienced some improvement over a period of 24–28 h after the infusion. In one such episode, he experienced mild side effects including flushness in the face.

Factor VIIa dosage and hemostatic response. The patient initially was given a dose of 50 U Factor VIIa/kg BW equivalent to 40–50 µg of total protein (in 5 ml of sterile saline). Following administration, the patient's plasma Factor VII level increased from 90 to 135% (Table II). The following day his muscle was markedly softer and less tender (circumference of 33 cm) and the mobility in the left knee joint was almost completely restored (extension defect of 10°). Another dose of Factor VIIa was administered (100 U/kg BW) upon which his Factor VII level rose from 90 to 200% (Table II). The following day, the patient walked without difficulty (leg circumference 32 cm) and was then discharged from the hospital. After extensive physical activity, rebleeding occurred in the same muscle 36 h later and another dose of Factor VIIa (100 U/kg BW) was administered. 4 h after this treatment, his muscle was considerably less tender and clearly softer. The next day the swelling had virtually disappeared (circumference 31 cm), and the joint mobility was completely restored. Aside from the expected rise in plasma Factor VII, no changes in the patient's coagulation or fibrinolytic profile occurred throughout the Factor VIIa treatment (Table II).

Case 2. A 12-yr-old male with severe hemophilia A complicated with antibodies against Factor VIII:C (12 BU/ml)

was admitted to the hospital with bleeding from two primary molars. The patient also had a moderate bleeding in his left knee joint with marked discomfort in the patella region. This patient has been treated with high doses of Factor VIII combined with cyclophosphamide on four different occasions in association with various bleedings, particularly during intraarticular injection of radioactive gold and teeth extractions. Although a good hemostatic effect was achieved, a marked anamnestic rise in the antibody titer against Factor VIII occurred (up to 60 U/ml BU). In addition, activated prothrombin complex concentrate, FEIBA (50 U/kg BW or 100 U/kg BW), was given on two occasions in association with minor and moderate joint and muscle bleeding. A positive effect of this treatment was dubious in this patient. Nonactivated Factor IX concentrate was also given on two occasions in association with minor joint bleedings. No hemostatic effect was observed with such a treatment, and the patient refused further treatment with this concentrate. The patient has experienced extensive bleeding problems coincident with the loss of six primary teeth necessitating multiple blood transfusions in spite of antifibrinolytic therapy given intravenously every 6 h for periods of 2–3 wk. On each occasion he was unable to attend school for at least 2 wk and was virtually unable to feed himself adequately.

Factor VIIa dosage and hemostatic response. The patient initially was given a dose of 50 U Factor VIIa/kg BW (40–50 µg of protein in 1.3 ml of sterile saline). The fully visible bleeding from the teeth stopped promptly and examination of the knee joint 1 h after the infusion revealed a complete absence of pain in the patella region. Another dose of Factor VIIa (50 U/kg BW) was given 2 h after the first injection, followed by an equal dose 16 h later. No bleeding from the teeth occurred throughout the treatment period. The circumference of the left knee at two different levels was on admission 29.5 and 30.5 cm, respectively. 24 h later the corresponding circumferences were 28.0 and 29.0 cm. No changes occurred in the patient's coagulation or fibrinolytic profile except for a slight rise in the plasma Factor VII level. The second treatment with Factor VIIa concen-

TABLE II
Coagulation Pattern in Patient 1 before and after the Treatments with Human Factor VIIa
at Two Concentrations (50 U/kg and 100 U kg BW)

	Day 1		Day 2			Day 5		Day 6
	Before treatment	1 h after VIIa	24 h after VIIa	1 h after VIIa	Before treatment	1 h after VIIa	24 h after VIIa	
		50 U/kg	50 U/kg	100 U/kg		100 U/kg	100 U/kg	
Platelet counts, 10 ⁹ /liter	295	290	275	260	315	305	290	
APTT, s	68 (31)*	61 (31)	72 (31)	78 (31)	77 (32)*	78 (32)	72 (32)	
VIII:C, %	<2.5	<2.5	<2.5	<2.5	<2.5	<2.5	<2.5	
VIII:Ag, %	90	90	90	80	110	100	80	
IX:C, %	145	128	92	82	100	93	105	
XII, clotting assay, %	193	153	133	108	101	103	90	
immunochemical, %	110	110	110	100	110	100	110	
Prothrombin time, s	15.2	13.2	15.0	13.9	15.2	13.2	16.6	
P & P, %	87	120	90	132	96	132	94	
VII, %	90	<u>135</u>	98	<u>200</u>	95	<u>200</u>	93	
V, %	104	102	91	98	110	96	100	
X, %	98	98	98	100	88	90	85	
Fibrinogen, g/liter	5.5	ND†	5.0	ND†	5.3	ND†	4.7	
Ethanol gel test	Neg	Neg	Neg	Neg	Neg	Neg	Neg	

Neg, negative.

* Numbers in parentheses represent the APTT for normal human plasma at the time of measurement.

† ND, not determined.

trate occurred 1 wk later when the patient was presented with a huge fragile blood clot in the cavity formed by the spontaneous loss of a primary molar ~12 h earlier. Due to its enormous size, the clot had to be removed. At this point, dental consults advised the extraction of two additional primary molars. Accordingly, the patient was given another dose of Factor VIIa (100 U/kg BW) and the two molars were removed under local anesthesia. The cavities created by the extracted teeth were filled with gauze soaked with tranexamic acid. Another dose of Factor VIIa (50 U/kg BW) was administered 4 h after the extraction. Tranexamic acid (10 mg/kg BW) was administered intravenously at this time, and the same dose repeated every 6 h for 2 d. Thereafter, tranexamic acid was given orally in a dose of 60 mg/kg BW three times daily for an additional 8 d. No further bleeding from the cavities was noted following extraction of the two molars. In contrast to the large clot observed upon admission, no such clot ever formed in the cavities created by the extraction of the two molars in conjunction with Factor VIIa and tranexamic acid treatment. The clot observed in these cavities was of a normal appearance, and by the second day the tissue had retracted nicely.

RESULTS

Infusion of purified human Factor VIIa into two patients with hemophilia A with antibodies against Factor VIII:C produced no immediate adverse effects such as chills, nausea, flush, or urticaria. Furthermore, no evidence of consumption coagulopathy, disseminated intravascular coagulation, or increase in factor VIII:C

antibody titer were observed in either patient throughout the treatment. Table II contains the coagulation pattern of patient 1 before and after the administration of Factor VIIa. The coagulation pattern of patient 2 was virtually identical (data not shown). No hepatitis has occurred thus far in either patient (one patient followed for 1.5 yr and the other for 3 mo) following the last infusion of Factor VIIa.

The postinfusion recovery of Factor VIIa (measured 15 min after infusion) ranged from 38 to 45%, assuming no extravascular equilibration and 40 ml blood/kg BW. Depending on the injection dose, the plasma Factor VII activity of each patient returned to preinjection levels in ~5–8 h. A shortening of the prothrombin time in the patient's plasma by an average of 2 s occurred invariably on each administration. The effect of Factor VIIa on the muscle bleeding (patient 1) and the knee joint bleeding (patient 2) was evaluated by the decrease in leg circumference 24 h after the administration. As for the muscle bleeding, a softening of the tissue and a decreased tenderness were evident within 2 h after the administration of Factor VIIa. A subjective disappearance of the discomfort associated with the knee joint was also noted within a few hours after infusion of Factor VIIa.

Perhaps the most dramatic evidence of the hemostatic effectiveness of Factor VIIa was associated with

the mucocutaneous bleedings following the loss of a primary tooth in patient 2. In this episode, the patient had spontaneously lost a primary molar and developed a loose clot in the cavity. This clot was typical for patients with hemophilia in that it was huge, of fragile consistency, and hanging from the back of the throat. Signs of an ongoing diffuse bleeding around this clot were also fully visible upon admission. Following extraction of two additional molars, and the administration of Factor VIIa and tranexamic acid, a small clot of a normal appearance could clearly be seen in the cavities and no diffuse bleeding ever occurred later than 30 min after the extraction.

DISCUSSION

A number of investigators have reported on the successful use of both ordinary prothrombin complex concentrates (1, 2) and so-called activated prothrombin complex concentrates (3-5) in the treatment of hemophilia A patients complicated with inhibitors. However, such concentrates also have been reported to be thrombogenic (6-8). Activated prothrombin complex concentrates contain Factors IX, X, VII, and prothrombin in both activated and zymogen forms. Thus far, no consensus has been reached on which of these factors possess Factor VIII bypassing activity, and which factor (or factors) is responsible for the thrombogenic properties.

Factor VIIa converts Factor X to Factor Xa in the presence of tissue factor and calcium ions (22), thus establishing a Factor VIII bypassing mechanism for thrombin formation. As pointed out by Seligsohn et al. (9), prothrombin complex concentrates appear to contain a rather high ratio of Factor VIIa/Factor VII. Even higher ratios were observed in activated prothrombin complex concentrates. Furthermore, these investigators found an increase in plasma Factor VII activity in hemophilia B patients after infusion of prothrombin complex concentrates. On the other hand, they could not demonstrate any significant amounts of Factor IXa or Factor Xa in vivo in the same patients. These findings point to Factor VIIa as the active hemostatic principle and as responsible for the Factor VIII bypassing activity.

Judging from our results in the two hemophilia A patients with inhibitors against Factor VIII:C, purified Factor VIIa also seems to be effective in restoring hemostasis in such patients. Patient 2 had extensive bleeding problems in the past coincident with the loss of five of six primary teeth, necessitating multiple blood transfusions on three of these occasions. It is noteworthy to mention that patient 2 lost three teeth during Factor VIIa therapy, two of which still had

significant roots and were extracted. In spite of this, the patient was back home after 4 d of hospitalization without having experienced any measurable bleeding. The treatment was sustained by the administration of tranexamic acid according to the protocol recommended at tooth extractions in uncomplicated hemophilia treated with Factor VIII/IX (23). This very patient had been treated with tranexamic acid in exactly the same way during all of his previous teeth bleeding episodes. In spite of this treatment, bleeding episodes occurred in all events before the administration of Factor VIIa. Tranexamic acid most probably contributed to the hemostatic effect observed with Factor VIIa by inhibiting local fibrinolysis as suggested by Bjorlin and Nilsson (23). Hanna et al. (5) noted a similar sustaining effect of a fibrinolytic inhibitor, epsilon amino caproic acid, on the hemostatic efficacy of activated prothrombin complex concentrates at surgery in three severe hemophilia A patients with antibodies against Factor VIII. Treatment with Factor VIIa most probably would be augmented by concurrent administration of a fibrinolytic inhibitor, since it is unlikely that the same degree of hemostasis can be achieved by Factor VIIa as that observed with Factor VIII in uncomplicated hemophilia A patients.

Patient 1 in our study also experienced a rebleeding two days after hemostasis had been restored by Factor VIIa. A somewhat similar observation was made by Kingdon and Hassell (24) in their dog model following administration of an activated prothrombin complex concentrate (Autoplex, Hyland, Costa Mesa, CA). These investigators were able to overcome this rebleeding tendency by increasing the dose. In treating our second patient, we also administered Factor VIIa for 3 d (two doses a day). Thus, in order to achieve satisfactory hemostasis with Factor VIIa, it may be advisable to give several doses of at least 100 U/kg BW at intervals of 6-10 h together with tranexamic acid.

No deleterious side effects were observed after administration of Factor VIIa in either patient. No increase in plasma Factor X activity was observed, indicating that Factor VIIa was not activating circulating Factor X. Furthermore, no evidence of an activated coagulation system was found as shown by the absence of fibrin monomers, as well as unchanged levels of fibrinogen, antithrombin III, or α_2 -macroglobulin. Although we feel confident that the administered Factor VIIa played a significant role in the hemostasis of our two patients, we also recognize that it is far too premature to draw any sweeping conclusions regarding the safety and efficacy of Factor VIIa administration in all hemophilia A patients complicated with alloantibodies. We hope our findings will stimulate further research on Factor VIIa as a potential Factor VIII by-

passing material for the management of a very difficult clinical problem.

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