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Plasmin Generation and Fibrin(ogen)olysis Following Desmopressin Infusion

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Desmopressin acetate (DDAVP) is known to stimulate the release of tissue-type plasminogen activator (t-PA) from endothelial cells, but it is unclear whether the increased t-PA actually elicits the plasmin generation and fibrin(ogen)olysis in the circulating blood. We measured plasma levels of plasmin-α_2-plasmin inhibitor complex, fibrinogen degradation products (FgDP) and fibrin degradation products (FbDP) following desmopressin infusion in 19 patients with bleeding disorders or thrombophilia. Administration of desmopressin (0.3–0.4 μg/kg) produced a 4.0-fold increase in plasmin-α_2-plasmin inhibitor complex at 30 min, whereas neither FgDP nor FbDP was elevated significantly. These findings indicate that desmopressin infusion provokes the generation of plasmin in vivo, but most of the plasmin generated is complexed to α_2-plasmin inhibitor and does not degradate fibrin or fibrinogen.

Key words: DDAVP, plasmin, plasmin-α_2-plasmin inhibitor complex, fibrin degradation products, tissue-type plasminogen activator

INTRODUCTION

Administration of 1-deamino-8-D-arginine vasopressin (DDAVP, desmopressin acetate), a synthetic analogue of the antidiuretic hormone L-arginine vasopressin, produces a marked increase in factor VIII and von Willebrand factor in blood [1–4]. Desmopressin is established in the treatment of patients with mild and moderate hemophilia A and von Willebrand's disease [5]. A massive release of tissue-type plasminogen activator (t-PA) from endothelial cells is also provoked [2,4,6]; this agent is sometimes employed as a systemic stimulus for evaluating the fibrinolytic potential in patients with thrombotic disorders [7]. Furthermore, some investigators have attempted to use desmopressin as a vehicle for inducing fibrinolysis in thrombotic patients [8,9]. The release of t-PA into the circulating plasma results in no or only a minimal variation of plasma fibrinogen, plasminogen, and α_2-plasmin inhibitor [3,6,7]. However, the actual degree of the resultant plasmin generation is not known. In this paper, we evaluated the systemic activation of fibrinolysis induced by desmopressin infusion by employing specific and sensitive methods for the assessment of plasmin generation and fibrin(ogen)olysis.

MATERIALS AND METHODS

Patients and Study Design

Eighteen patients with bleeding disorders (three with hemophilia A, four with von Willebrand's disease, and 11 with miscellaneous conditions associated with prolonged bleeding time), and one with thrombophilia of an undetermined etiology were studied. With their informed consent, desmopressin acetate (Ferring Pharmaceuticals AB, Malmö, Sweden) was infused intravenously at a dose of 0.3–0.4 μg/kg in 20 ml saline over 20 min. Blood samples were collected prior to and at 30 min, 1, 2, and 6 hr after the infusion. Additionally, plasma samples were obtained at 24 hr in four of these patients.

Blood Collection and Plasma Preparation

Venous blood was collected into siliconized tubes containing 1/10 vol of 0.129 mol/L trisodium citrate, and

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centrifuged at 2,000*g* for 20 min at 4°C to obtain platelet-poor plasma. Assays were performed on fresh samples or samples stored at −70°C until use.

Assay Methods

Plasmin-α_2-plasmin inhibitor complex was measured by a one-step sandwich enzyme-linked immunosorbent assay (ELISA) (PIC test, Teijin Ltd., Tokyo, Japan) using polyclonal antiplasminogen antibody-coated polystyrene ball and peroxidase-conjugated monoclonal anti-α_2-plasmin inhibitor antibody [10,11]. Fibrinogen degradation products (FgDP), fibrin degradation products (FbDP), and fibrinogenolysis products plus fibrinolysis products (TDP) were quantitated with a sandwich-type ELISA based on the use of monoclonal antibodies (Fibrinostika; Organon Teknika, Turnhout, Belgium) [12–15], and the results were expressed in terms of ng fibrinogen equivalents per ml of plasma.

t-PA and type 1 plasminogen activator inhibitor (PAI-1) were measured immunologically with ELISA kits obtained from Fujirebio Inc. (Tokyo) and Monozyme ApS (Charlottenlund, Denmark), respectively. Plasma euglobulin clot lysis time was measured by a slight modification of the method of Nilsson and Olow [16], the end points being recorded using an automatic clot-lysis recorder (Riko Trading Co., Tokyo). The results of euglobulin clot lysis time was expressed as units (euglobulin fibrinolytic activity) derived from the formula $10^6/T^2$, where T is the euglobulin clot lysis time (in min). von Willebrand factor antigen (vWf:Ag) was assayed by an ELISA (Asserachrom vWF; Diagnostica Stago, Asnieres, France).

Normal values obtained from healthy subjects at rest were <0.5 μg/ml for plasmin-α_2-plasmin inhibitor complex, 198–690 ng/ml for FgDP, 89–562 ng/ml for FbDP, 343–1,242 ng/ml for TDP, 1.4–10.8 ng/ml for t-PA, <41 ng/ml for PAI-1, and 0.49–1.81 U/ml for vWf:Ag.

Statistical Analysis

Data are expressed as mean ±SD. The significance of differences between groups was tested by one-way analysis of variance. Differences between pre- and post-infusion values were assessed by Student's paired t-test. Regression analysis was performed by the method of least squares, and the correlation coefficient (r) was calculated.

RESULTS

Fibrinolytic responses to desmopressin infusion for all subjects are summarized in Table I. Administration of desmopressin produced at 30 min a mean 1.7-fold increase in plasma t-PA antigen together with a 2.6-fold increase in vWf:Ag. The euglobulin fibrinolytic activity was markedly elevated from a mean preinfusion value of 15 ± 27 units to 1,428 ± 746 units at 30 min ($P < 0.001$). A modest decrease in PAI-1 antigen was noted. At the same time, a 4.0-fold increase in plasmin-α_2-plasmin inhibitor complex was found, whereas the elevation of FgDP, FbDP, and TDP was not statistically significant (Table I). Subsequently, the elevated t-PA antigen and euglobulin fibrinolytic activity decreased gradually and returned to the baseline values at 6 hr; the t-PA level at 6 hr was 92 ± 7% of the initial value and euglobulin fibrinolytic activity was 1,062 ± 1,984 units at 1 hr, 171 ± 185 units at 2 hr, and 26 ± 17 units at 6 hr. PAI-1 antigen reached the preinfusion value at 1 hr (Table I). Mean plasma levels of plasmin-α_2-plasmin inhibitor complex and vWf:Ag were still elevated at 6 hr (plasmin-α_2-plasmin inhibitor complex, 351 ± 477% of initial; and vWf:Ag, 182 ± 36%), but reached nearly the baseline values at 24 hr (plasmin-α_2-plasmin inhibitor complex, 154 ± 103% of initial; and vWf:Ag, 122 ± 15%).

When the subjects were analyzed according to the disease categories, the baseline TDP value was relatively high in patients other than hemophilia A or von Willebrand's disease; 350 ± 75 ng TDP/ml in hemophilia A, 283 ± 71 ng/ml in von Willebrand's disease, and 628 ± 183 ng/ml in others ($P < 0.01$). Hemophiliacs showed a less marked elevation of vWf:Ag after desmopressin infusion. For example, the percent increase in vWF:Ag at 2 hr was 133 ± 16% in hemophilia A, 293 ± 60% in von Willebrand's disease and 202 ± 56% in others ($P < 0.05$). However, there was no statistical difference in the fibrinolytic responses to desmopressin between groups studied here.

The interrelationship between plasmin-α_2-plasmin inhibitor complex and other variables is shown in Table II. t-PA and euglobulin fibrinolytic activity were significantly correlated with plasmin-α_2-plasmin inhibitor complex at 1 hr. However, plasma concentration of plasmin-α_2-plasmin inhibitor complex did not show any correlation with t-PA, PAI-1, euglobulin fibrinolytic activity, or vWf:Ag at baseline, 30 min, or 2 hr after desmopressin.

DISCUSSION

The fibrinolytic system is initiated by the conversion of plasminogen to plasmin by plasminogen activators (PA), mainly by t-PA synthesized in the vascular endothelial cells. Under basal conditions, there is no or little plasma PA activity, since t-PA circulates mainly as a complex with PAI-1 and free t-PA is extremely low in the circulating plasma [17]. The plasmin generated is neutralized by complexing with the natural inhibitor α_2-plasmin inhibitor [18,19]. The presence of plasmin-α_2-plasmin inhibitor complex in plasma is therefore a direct indicator of in vivo generation of plasmin. Some

TABLE I. Changes in Fibrinolytic Parameters and vWf:Ag Following Desmopressin Infusion in the Patients†

Variable	Initial value	Changes after desmopressin (% initial)[a]		
		at 30 min	at 1 hr	at 2 hr
t-PA	10.9 ± 5.5 (ng/ml)	165 ± 37***	133 ± 25***	116 ± 24*
PAI-1	24.9 ± 22.3 (ng/ml)	82 ± 18**	95 ± 40	111 ± 59
PIC	0.61 ± 0.51 (μg/ml)	403 ± 381**	312 ± 326*	344 ± 432*
FgDP	203 ± 96 (ng/ml)	127 ± 64	131 ± 65	122 ± 51
FbDP	228 ± 177 (ng/ml)	103 ± 28	105 ± 29	112 ± 34
TDP	524 ± 215 (ng/ml)	123 ± 44	114 ± 40	114 ± 36
vWf:Ag	1.03 ± 0.65 (U/ml)	263 ± 162**	268 ± 163***	210 ± 69***

†Results are shown as means ± SD. PIC denotes plasmin-α_2-plasmin inhibitor complex.
[a]Postinfusion values are expressed as a percentage of preinfusion vlaues (100%).
*$P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

TABLE II. Correlation of Plasmin-α_2-Plasmin Inhibitor Complex With Other Parameters Before and at Various Times After Desmopressin Infusion in the Patients†

Variable	Before	Time after desmopressin (hr)		
		0.5	1	2
t-PA	0.130	0.056	0.512*	0.346
PAI-1	0.106	−0.181	−0.257	0.111
EFA	−0.091	−0.501	0.925**	−0.382
vWf:Ag	−0.211	−0.030	−0.294	−0.305

†Correlation coefficients are given.
EFA denotes euglobulin fibrinolytic activity.
*$P < 0.05$, ** $P < 0.001$.

parts of plasmin generated would participate in fibrinolysis and/or fibrinogenolysis. It is well known that desmopressin induces a massive release of t-PA from endothelial cells [2,4,6], but it is not clear whether its release actually promotes fibrinolysis in vivo. Lowe et al. [20] showed an elevation of B β_{15-42} peptides after desmopressin infusion, suggesting the increased plasmin activity in plasma. We evaluated in the present study the effects of desmopressin administration to the fibrinolytic system by measuring plasmin-α_2-plasmin inhibitor complex and fibrin(ogen)olysis products in plasma.

As expected, desmopressin induced an increase in t-PA, euglobulin fibrinolytic activity, and vWf:Ag in plasma (Table I). There was a fall of PAI-1 immediately after the infusion, probably as a result of complex formation with t-PA, becoming less immunoreactive in its antigenic assay and being cleared from the circulation. Similar findings have been observed by others [21,22]. We demonstrated here that desmopressin infusion actually results in an elevation of plasmin-α_2-plasmin inhibitor complex, indicating directly the generation of plasmin in the circulating blood (Table I). However, FgDP, FbDP, or TDP did not increase significantly, suggesting that the plasmin generated is mostly complexed to α_2-plasmin inhibitor and does not enhance the fibrinolysis or fibrinogenolysis under conditions studied here. These findings imply that the concomitant use of antifibrinolytic agents (tranexamic acid or ε-aminocaproic acid) would be usually unnecessary even when desmopressin is administered into patients with hemostatic defects.

On the other hand, Nilsen et al. [8] have tried to reduce the incidence of postcatheterization thrombosis by desmopressin in patients undergoing diagnostic percutaneous catheterization. They found a moderate reduction in the frequency of thrombosis (from 86% in the placebo group to 53% in the desmopressin-treated group), but the reduction attained no statistical significance. In addition, this reduction was restricted to small single-vessel thrombi, and major thrombosis could not be prevented. Fibrin or fibrinogen degradation products were not measured in their study. In another trial, as a potential thrombolytic agent, desmopressin was given supplementary to standard heparin treatment in patients with acute deep vein thrombosis [9]. They observed a 19% reduction in thrombus size after desmopressin administration. However, since they did not use a control group, it is obscure whether this reduction is superior to that expected after heparin alone. In their patients, the levels of fibrinogen/fibrin degradation products remained unchanged during desmopressin treatment. We could not find any increase in FbDP in our patients with initially normal FbDP levels (Table I). From our studies, however, it is impossible to predict whether fibrin(ogen)olysis can occur after desmopressin administration in case of subjects with preformed thrombosis or ongoing thrombus formation. Further studies are required in this respect.

The magnitude of plasmin generation when judged from plasma levels of plasmin-α_2-plasmin inhibitor complex is correlated with t-PA levels only at 1 hr after

desmopressin infusion (Table II). Poor correlation of plasmin-α_2-plasmin inhibitor complex with t-PA at other times and with other parameters would be related to the complex regulation of fibrinolysis mentioned above [18,19]. In addition, our results indicate that we cannot surmise precisely the magnitude of plasmin generation following desmopressin from the plasma level of t-PA or fibrinolytic activity in the euglobulin fraction of plasma. The relatively long half-life of plasmin-α_2-plasmin inhibitor complex in blood of approximately 12 hr [23] as compared with t-PA [6] would be, at least in part, responsible for the sustained elevation of plasmin-α_2-plasmin inhibitor complex observed after desmopressin infusion (Table I).

CONCLUSION

Desmopressin administration provokes an increase in plasma t-PA and plasmin generation in vivo. Most of plasmin generated is complexed to the natural inhibitor α_2-plasmin inhibitor and does not degradate fibrin or fibrinogen at least in patients studied here.

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