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Desmopressin (d-DAVP) Effects on Platelet Rheology and von Willebrand Factor Activities in Uremia

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Bleeding times, von Willebrand activities, and platelet retentions were examined before and following d-DAVP in 13 uremic patients. Shortening of the bleeding time from 16.6 ± 2.2 (SEM) to 6.8 ± 0.7 min was seen in six patients. However, bleeding times remained ≥20 min in the remaining seven individuals. The only baseline parameter that correlated with response to d-DAVP was the amount of blood loss (mg/min) during the bleeding time test. Responders had normal blood loss values averaging 6.2 ± 1.5 mg/min. By contrast, these values were elevated in 6/7 of the non-responders and averaged 28.4 ± 5.9 mg/min ($P = 0.01$). Von Willebrand activities increased following d-DAVP in the responders but not in the non-responders. Platelet retention was uniformly low in all patients and improved from 21.0 ± 7.0% to 75.0 ± 7.9% ($P = <0.001$) following d-DAVP in responders but not non-responders. To further define the retention abnormality in uremia, the two-stage platelet retention assay was performed prior to d-DAVP. Most of the patients (9/12) had both first- and second-phase abnormalities. Therefore, the retention defect in uremia appears to be more complex than that seen in von Willebrand's disease (2nd phase abnormality only). Nevertheless, d-DAVP seems to improve platelet rheology in uremic individuals whose von Willebrand activities increase with d-DAVP.

Key words: uremia, bleeding times, platelet retention, von Willebrand factor

INTRODUCTION

The synthetic vasopressin derivative desmopressin (1-deamino-8-D-arginine vasopressin; d-DAVP) shortens the bleeding time in patients with uremia [1,2]. However, the mechanism of the bleeding time improvement is unknown. Although the platelet function defect in uremia is complex, a constant feature is impaired platelet retention and/or adhesion [3–6]. In addition, cryoprecipitate has been reported to improve bleeding times in uremia [7]. These observations suggest that von Willebrand factor may play a role in uremic platelet dysfunction. However, no consistent von Willebrand factor alterations have been found [1,8–10].

Therapy with d-DAVP has been successful in a variety of thrombocytopathic disorders. These include von Willebrand's disease, platelet storage pool disease, aspirin-induced platelet dysfunction, and isolated prolonged bleeding times [11]. In addition, d-DAVP has been shown to improve bleeding times and platelet retention in a series of mild bleeding disorder patients, characterized as having a second-phase platelet retention defect [12]. These patients had no plasma von Willebrand factor abnormalities, even though their second-phase retention abnormality was similar to that seen in von Willebrand's disease [12].

The present studies examined the effect of d-DAVP on the bleeding time, platelet retention, and von Willebrand factor activities in a series of uremic patients. In addition, the behavior of uremic platelets in the two-stage retention assay of McPherson and Zucker [13] was evaluated prior to drug administration.

MATERIALS AND METHODS

Laboratory Studies

Bleeding times. Template bleeding times were performed using a commercial bleeding time device (Simplate II, General Diagnostics, Morris Plains, NJ) to make two horizontal incisions with the use of venostasis [14]. In addition, the amount of effluent blood (expressed as

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mg/min) was determined using preweighed filter papers as described previously [15].

Von Willebrand activities. Factor VIII coagulant activity (VIII:C) was evaluated in a standard one-stage activated partial thromboplastin time assay [16]. Von Willebrand antigen (vWF:Ag) was measured using Laurell's gel electrophoresis and antiserum supplied by Helena, Beaumont, TX [17]. Ristocetin cofactor activity was measured using lyophilized platelets and ristocetin supplied by Bio Data, Hatboro, PA. Concentrations of ristocetin cofactor were determined from the slopes of the aggregation curves obtained with test plasma using a Bio Data aggregometer [18].

Platelet retention assays. The one-stage platelet retention assay was performed using whole blood without anticoagulant and a constant infusion pump (Sienco, Inc., Morrison, CO) as described by Rossi and Green [19]. In addition, heparinized blood was used to examine the behavior of platelets for possible functional defects in the first or the second phases of the two-stage platelet retention assay of McPherson et al. [13].

The two-stage retention assay entails the initial passage of 1 ml of blood (A) through the columns, followed by a rinse with saline (2 ml). A second sample of blood (B) is then passed through the column. If platelets from sample A have adhered, the platelets in sample B are highly retained on the column. Measurements are made of the number of platelets retained on the column from the effluent second and fifth ml of the second sample of blood. The ability of patient platelets (blood A) to initially coat the beads, and the ability of patient platelets (blood B) to interact with the platelets supplied by normal blood (blood A) were both evaluated.

Statistics

All analyses were performed using a MacIntosh Plus computer with Stat View 512+ (Calabasas, CA) software. Comparisons of group means were made using the unpaired t-test. Platelet function data before and following drug administration were analyzed using the paired t-test.

Patients

Thirteen adult patients (age = 23–80 years) with uremia and prolonged bleeding times participated in this study. Written informed consent was obtained from each patient prior to participating in this study. The protocol was approved by the Protection of Human Subjects Committee of Montefiore Hospital.

All patients received an infusion of desmopressin in an attempt to decrease their bleeding times. Baseline studies included bleeding time, one- and two-stage platelet retention analysis, and von Willebrand activities. One hr following drug infusion, the above assays (except the two-stage retention assay) were repeated in 12/13 of the patients. Post-d-DAVP values were not obtained in patient number 13 because of technical difficulties.

The clinical characteristics of the patients are summarized in Table I. Their underlying renal diseases included diabetic nephrosclerosis (six patients), chronic glomerulonephritis (five patients), nephrosclerosis (one patient), and focal glomerulonephritis (one patient). Seven patients were undergoing chronic hemodialysis and two were undergoing peritoneal dialysis. The remaining four patients were not on dialysis. Serum creatinine values ranged from 7.0 to 25.0 mg/dl (median = 8.0 mg/dl).

TABLE I. Clinical Characteristics*

Renal disease	Dialysis	Cr (mg/dl)	HCT (%)	Medications	Response
1. DM	N	12.8	23	Furosemide, captopril	R
2. CGN	HD	8.3	25	Furosemide, atenolol	R
3. DM	N	7.0	23	Acetylsalicylic acid, metoprolol hydrochlorothiazide, enalapril	R
4. CGN	PD	25.0	30	Furosemide, minoxidil, atenolol	R
5. DM	PD	7.0	26	Digoxin	R
6. DM	N	6.0	22	Furosemide, nifedipine, digoxin	R
7. NS	HD	11.0	34	Nifedipine	NR
8. DM	HD	8.0	27	Clonidine, minoxidil	NR
9. CGN	HD	8.0	24	Furosemide, clonidine, verapamil, metoprolol hydrochlorothiazide	NR
10. DM	HD	12.0	29	Methyldopa, chlorpromazine	NR
11. CGN	HD	8.0	24	Cyclophosphamide, methylprednisolone	NR
12. CGN	HD	7.0	20	Cyclophosphamide, methylprednisolone	NR
13. FG	N	16.0	19	Prazosin, nifedipine	NR

*DM, diabetes mellitus; NS, nephrosclerosis; CGN, chronic glomerulonephritis; FG, focal glomerulosclerosis; N, none; PD, peritoneal dialysis; HD, hemodialysis; R, responder; NR, non-responder.

Their hematocrit levels were low and ranged from 19 to 34%.

Medications consisted primarily of diuretics, antihypertensive medications, and cyclophosphamide and methylprednisolone. One patient (a responder) was taking aspirin. Responses to desmopressin are also indicated in Table I.

Desmopressin (d-DAVP) Infusion

Desmopressin (Rorer Pharmaceuticals) was administered in 50 ml of isotonic saline, by continuous intravenous infusion over 30 min. Each patient received 10 $\mu g/M^2$ (maximum dose = 24 μg) [20].

RESULTS

The patients in this series were arbitrarily divided into responders and non-responders based upon their venostasis template bleeding time results after d-DAVP (Fig. 1). The responders (six patients) either had a decrease in their bleeding time by greater than or equal to 10 min or had normalization of their bleeding time. The non-responders had bleeding times >20 min before and after d-DAVP. All of the non-responders and four of six of the responders had baseline values >20 min.

The only baseline parameter that correlated with response was measured blood loss during the bleeding time test (Fig. 2). All of the responders had normal blood loss values (<10.5 mg/min) [15]. By contrast, six of seven non-responders had increased blood loss values. The mean blood loss in the non-responders was 28.4 ± 5.9 (SEM) mg/min compared to 6.2 ± 1.5 in the responders ($P = 0.01$).

Von Willebrand activities were measured before and after d-DAVP in the responders and in the non-responders (Table II). No differences were seen in the baseline levels in the two response groups. The responders showed significant increases in the levels of VIII:C, vWF:Ag, and RCoF post-d-DAVP compared to pre-d-DAVP (paired t test analysis). By contrast, no significant changes were seen in these levels in the non-responders.

Retention of platelets on a glass bead column was measured before and following drug administration (Fig. 3). Glass bead retention was uniformly depressed in all of the patients. The baseline values were similar in both the responders and the non-responders. This parameter improved from 21.0 ± 7.0% to 75.0 ± 7.9% in the responders. Values were unchanged in the non-responders.

Two-stage retention assays were performed in 12/13 of the uremic patients in this study before the infusion of d-DAVP. All of the patients exhibited second-phase retention abnormalities (Fig. 4). In addition, most (8/12) also had defective behavior in the first phase as well.

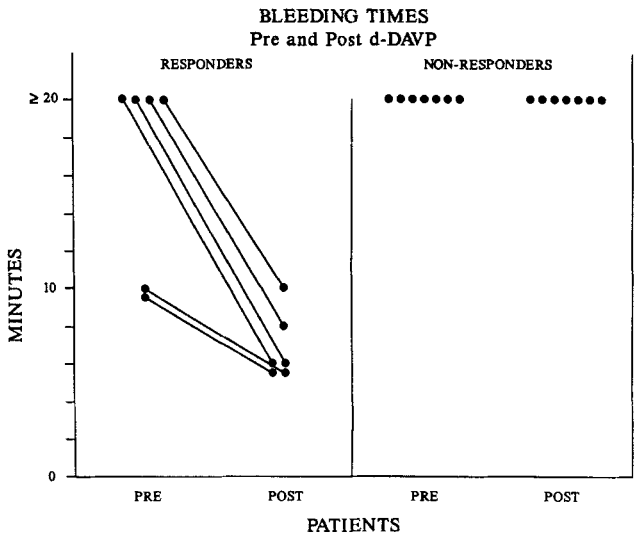

Fig. 1. The uremic patients were divided into responders and non-responders. Six patients had either normalization or a decrease in their bleeding time by ≥10 minutes (left panel). The non-responders (right panel) had no change in their bleeding times following d-DAVP. Pre and post are before and after d-DAVP.

DISCUSSION

The present studies evaluated the response to d-DAVP in a series of uremic individuals. Emphasis was placed on examining the nature of the rheologic defect, its response to desmopressin, and the effect of the drug on von Willebrand activities. Abnormal platelet retention was seen uniformly in all patients. The behavior of their platelets in the two-stage retention assay indicated that uremic platelets function defectively in both phases. This is in contrast to von Willebrand's disease where an isolated second-phase defect has been found [12,13]. In this series, shortening of the bleeding time was observed in 6/13 (38%) of the patients. It should be noted that many of these patients were on medications with possible anti-platelet effects.

Glass bead retention improved in the responders and was associated with an increase in von Willebrand activities, despite the fact that these levels were normal or high before drug administration. By contrast, the non-responders did not increase their plasma von Willebrand levels nor improve their platelet retention. In addition, excessive blood loss during the bleeding time test was noted in the non-responders compared to the responders. Even though the rheologic defect is more complex in uremia than von Willebrand's disease, improvement may relate (at least in part) to the ability of endothelial cells to release factor VIII stores. It is possible that excessive

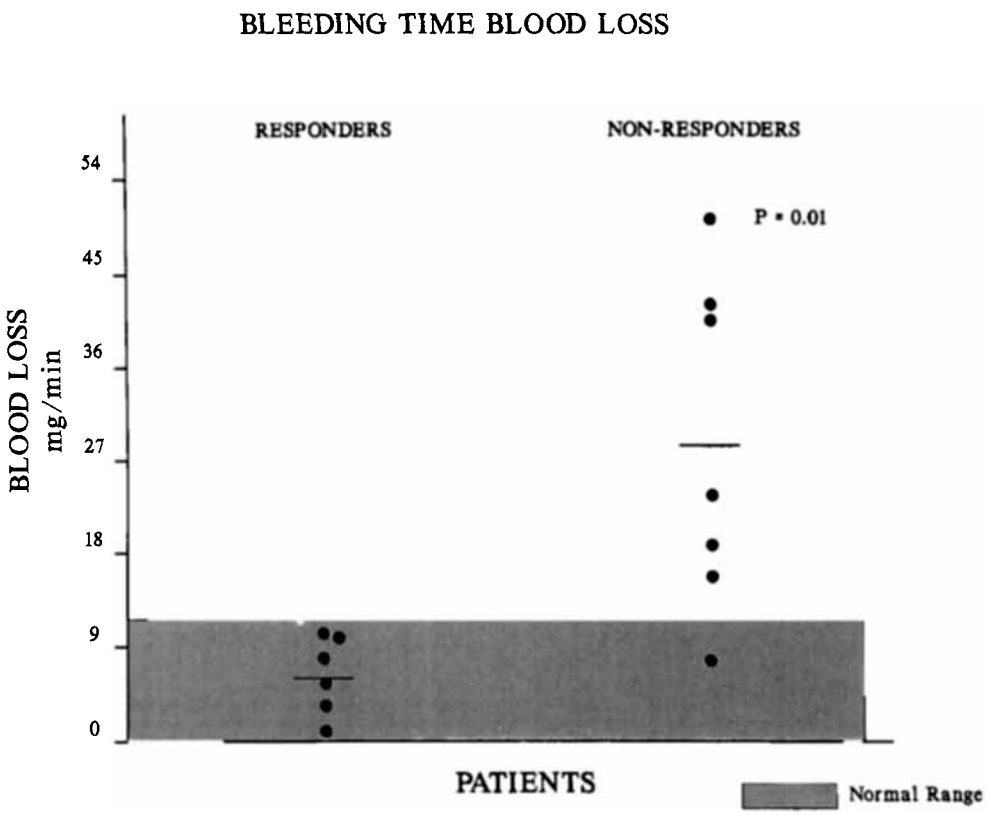

Fig. 2. The amount of blood loss (mg/min) measured during the bleeding time is depicted for the responders and non-responders. The horizontal lines represent means. $P = 0.01$ by Student's t test. The shaded area represents the normal range (x ± 2 SD of 16 normal subjects).

TABLE II. VWF Activities Pre- and Post-d-DAVP in the Responders and Non-Responders

	Responders		Non-responders	
	Pre x ± SEM	Post x ± SEM	Pre x ± SEM	Post x ± SEM
VIII:C (U/ml)	1.90 ± 0.26	2.80 ± 0.32*	2.47 ± 0.23	2.80 ± 0.32
vWF:Ag (U/ml)	1.90 ± 0.26	2.42 ± 0.28**	2.53 ± 0.46	2.39 ± 0.42
RCoF (U/ml)	1.84 ± 0.35	2.56 ± 0.33**	2.23 ± 0.37	2.77 ± 0.43

*$P = 0.02$.
**$P = 0.01$.

blood loss in the non-responders reflects vascular damage and correlates with endothelial cell d-DAVP unresponsiveness.

The uremic platelets functioned poorly in both phases of the two stage retention assay. Studies by McPherson et al. [13,21] have examined the factors that are important for both phases of this assay. Adenosine diphosphate and von Willebrand factor appear to be important for continued platelet-platelet interactions in the second phase. In contrast, fibrinogen appears to be crucial for normal first-phase adherence phenomena. These findings suggest that uremia may produce defects in both the interaction of fibrinogen and of von Willebrand factor with their receptors. Recently Escolar et al. [22] have

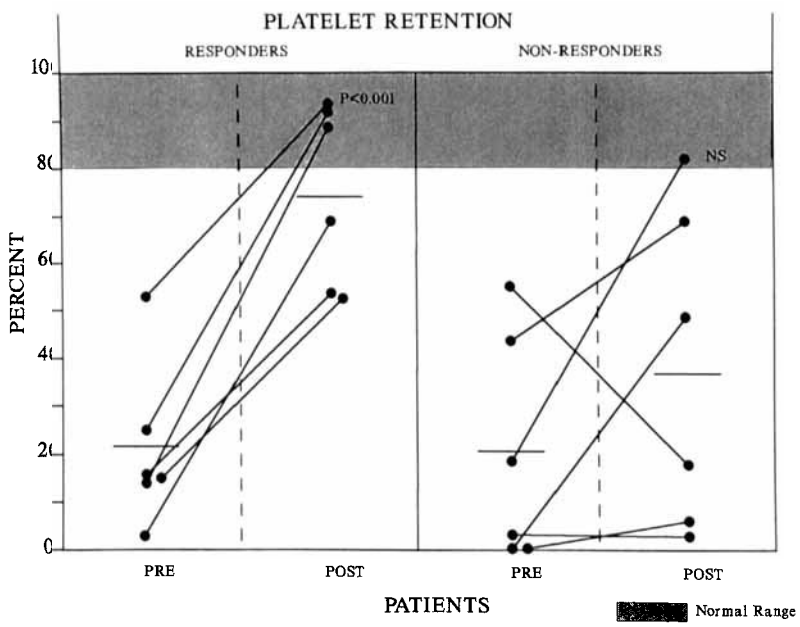

Fig. 3. One-stage glass bead retention (percent) is shown pre and post-d-DAVP in the responders (left panel) and non-responders (right panel). Individual patients are depicted with the direction of change. The bars represent the means for the groups. *P* values were determined from paired t tests. The shaded area represents the normal range (x ± 2 SD, n = 31).

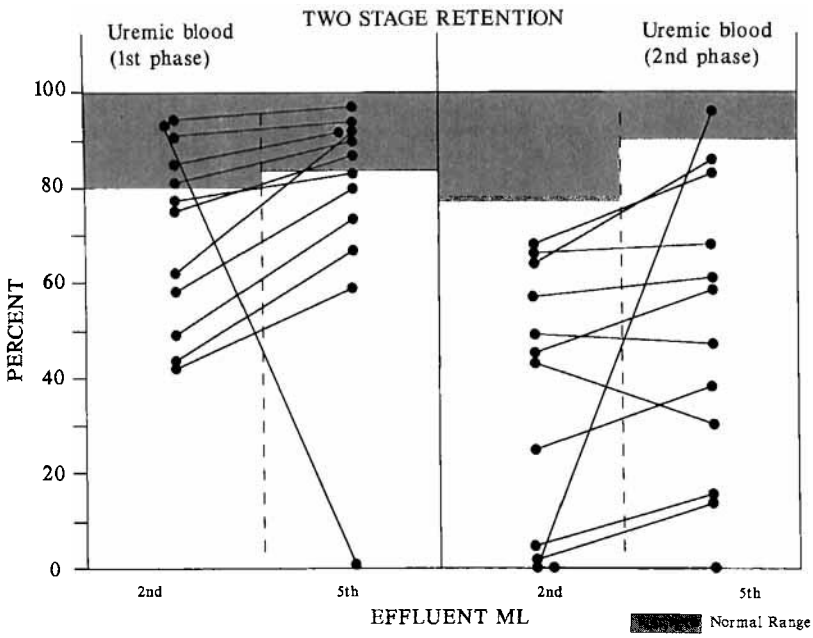

Fig. 4. Two-stage retention assay in uremics before d-DAVP. Percent platelet retention is shown on the abscissa. The aliquots (2nd and 5th) are shown on the ordinate. The first two panels show the results with uremic blood in the first phase and the last two panels with uremic blood in the second phase. The shaded area represents the normal range (x ± 2 SD, n = 10).

shown that uremic platelets are defective in their ability to interact with von Willebrand factor via the GPIIb/IIIa receptor.

Information has been confusing and contradictory regarding von Willebrand factor abnormalities in uremia. Both low [8] and normal to high levels [9,10] of plasma vWF have been reported. In addition, there are conflicting reports of normal [1] and abnormal [10] von Willebrand multimers. Normal to high levels of vWF were seen in this series; the multimeric structure was not analyzed.

Von Willebrand factor levels were increased following d-DAVP and correlated with improvement in glass bead retention in the responders. These changes were not seen in the non-responders. Previous reports have indicated that these levels tend to increase with d-DAVP in uremics [1,2]. Glass bead retention has not previously been studied following d-DAVP in this patient population.

Recently there has been evidence that high levels of von Willebrand factor in vitro can overcome a relative adhesive defect in uremia [6]. However, these studies indicated that uremic plasma depleted of von Willebrand factor also exhibited this adhesion defect. This suggests that there is an unknown plasma factor in uremia that inhibits platelet adhesion. Nevertheless, the present results suggest that improved adhesion may occur post-d-DAVP in uremic individuals, whose endothelial cells can respond by release of VIII stores.

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