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Short Communications

Availability of Endothelial von Willebrand Factor and Platelet Function in Diabetic Patients Infused with a Vasopressin Analogue

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Summary. Acute release of von Willebrand factor and its immunologically detected correspondent, factor VIII-related antigen, from the endothelial cells into the circulation was induced by an infusion of 1-deamino-8-D-arginine vasopressin in eight normal subjects and eight insulin-dependent diabetic patients. The patients had higher basal levels of von Willebrand factor (140 ± 13%) and VIII-related antigen (112 ± 18%) than the normal subjects (95 ± 5% and 70 ± 8%, respectively). 1-deamino-8-D-arginine vasopressin induced a two-fold rise of these levels in both groups, von Willebrand factor/VIII-related antigen remaining higher in the diabetic patients throughout the experiment. In the normal subjects, platelet retention in glass bead columns increased soon after the infusion but returned to basal values after 2 h. In the patients, it remained high at the end of the infusion. No changes in platelet aggregation occurred. These results suggest that increased plasma von Willebrand factor/VIII-related antigen in diabetic patients is accompanied by an increased endothelial content of this factor.

Key words: Plasma coagulation factors, von Willebrand factor, platelet adhesiveness, platelet aggregation, argipressin, diabetes mellitus, diabetic microangiopathy.

The plasma levels of von Willebrand factor (vWF), a glycoprotein synthesized and stored in the endothelial cells [1], are increased in patients with diabetic microangiopathy [2–4]. This abnormality is associated with hyperpermeability of the retinal capillaries to fluorescein [5] and could result from widespread endothelial damage in diabetes. The causes for increased plasma vWF levels are unexplained. The availability of other substances synthesized in the endothelium, such as prostacyclin and plasminogen activator, is reduced in the presence of diabetic microangiopathy [6, 7]. vWF could either leak from damaged endothelial cells, be more actively synthesized, or cleared more slowly from the circulation. Little is known about the possible relationships between the plasma levels of vWF and platelet adhesion and aggregation, which have been reported to be abnormal in diabetes by some investigators [2, 4, 8, 9], but not by others [9–12]. vWF is necessary for platelet adhesion to subendothelial structures [13] and to glass surfaces [14]. Enhanced ADP-induced platelet aggregation has been reported to accompany increased vWF in diabetic patients [15].

In this study, patients with insulin-dependent diabetes and normal subjects were given 1-deamino-8-D-arginine vasopressin (DDAVP), a vasopressin analogue that releases vWF from endothelial cells [16]. The aims of the study were to investigate (a) the endothelial availability of vWF in patients with an increased plasma pool of this factor and (b) the behaviour of platelet adhesiveness and aggregation following changes of plasma vWF levels in these experiments.

Subjects and Methods

Eight normal subjects and eight patients with insulin-dependent diabetes gave their informed consent to participation in the study, in accordance with the Declaration of Helsinki (1975). The experimental protocol was approved by the Hospital Ethical Committee.

Eight healthy men were studied (mean age 29, range 23–39 years, ponderal index 104 ± 4, mean ± SEM). The ponderal index was calculated as a percentage of the mean ideal body weight for subjects of medium frame, according to the 1959 Metropolitan Life Insurance Co. Tables [17]. Eight diabetic men were studied (mean age 28, range 24–36 years, ponderal index 106 ± 4, mean duration of disease 17 years, range 6–28 years, mean daily insulin dose 68 ± 11 U). Two patients had no diabetic retinopathy (as assessed by ophthalmoscopy, colour photography, and fluorescein angiography), five had mild background retinopathy and one had been treated by photocoagulation for proliferative lesions. None of the patients had signs of impaired renal function or had received therapy apart from insulin for at least 15 days before the infusion.

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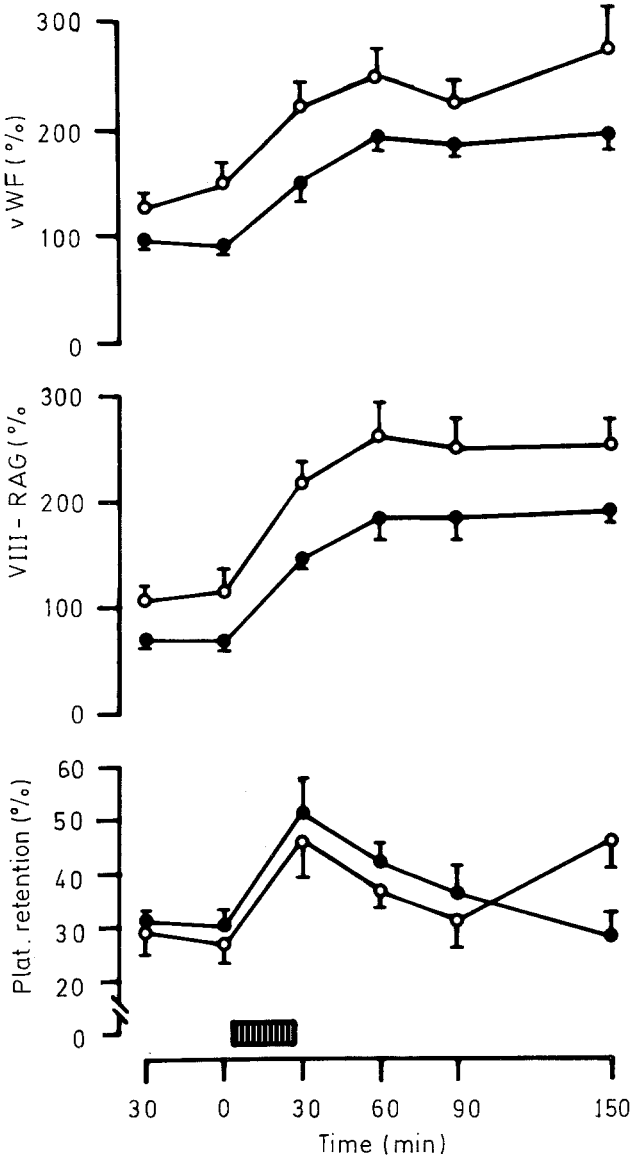

Fig. 1. Effect of DDAVP infusion (hatched area) on plasma levels of vWF, VIII-RAG and on platelet retention in eight normal subjects (●——●) and eight patients with insulin-dependent diabetes (○——○)

Experimental Procedure

The experiments started at 09.00–09.30 h, the patients remaining in a recumbent position for 3 h. A 19-G Butterfly needle (1.1 mm) was inserted in an antecubital vein and utilised for both sampling and the infusion, in order to minimise vascular trauma. The needle was kept open by a continuous flow of minimal quantities of normal saline (< 100 ml were infused throughout the study, to avoid water loading).

DDAVP (0.4 μg/kg, Ferring Pharmaceuticals, Twickenham, UK), diluted up to 30 ml in normal saline, was administered as a slow IV injection between times 5 and 25 min of the experiment. Blood samples were taken without stasis at times −30, 0, 30, 60, 90 and 150 min.

vWF was measured as described previously [18], by ristocetin-induced agglutination of fixed-washed platelets suspended in dilutions of the test plasma. Factor VIII-related antigen (VIII-RAG), the immunologically detected correspondent of vWF, was measured by immunoelectrophoresis using Laurell's technique [19]. The values of vWF and VIII-RAG were expressed as percentages of the activity of a standard pooled plasma from 20 healthy donors defined as 100% vWF/VIII-RAG. Platelet adhesiveness was measured as retention in glass bead columns, using commercial kits. Native non-anticoagulated blood was passed at constant velocity through an Adeplat T glass bead column (Semmelweis, Milan, Italy) using an Adeplat pump (Semmelweis, Milan, Italy). Platelet counts were performed by a Coulter Counter (Coulter Electronics, Luton, UK) on blood samples collected before (C_{100}) and after (C_X) passage through the columns. The percentage of platelet retention was expressed by the formula:

$(C_{100} - C_X)/C_{100} \times 100$.

ADP-induced platelet aggregation was assessed as previously described [12] by determining the minimum (threshold) concentration of ADP able to elicit irreversible aggregation in platelet-rich plasma kept at 37 °C and stirred at 1,100 rev/min in an aggregometer (Payton Associates, Buffalo, New York). ADP was used in final concentrations of 0.4, 1.2, 2.0 and 10.0 μmol/l.

Platelet-rich plasma was left for 10 min in the aggregometer in the same conditions described above, to note the occurrence of spontaneous aggregation. Platelet aggregation tests were carried out in six normal and five diabetic subjects.

Aspirin Administration

The DDAVP infusion was repeated in one normal subject who had been administered aspirin, 900 mg 12 h before and 900 mg 1 h before the experiment.

Statistical Analysis

The values of vWF, VIII-RAG and retention at the different times of the experiment were compared with the mean of the baseline values by Student's paired t-test.

vWF and VIII-RAG values were distributed differently in the normal and diabetic subjects. Therefore, to compare the two sets of results, logarithmic transformation of the data was carried out before analysis by Student's unpaired t-test. All the results are expressed as mean ± SEM.

Results

The effects of DDAVP on vWF/VIII-RAG and platelet retention are summarised in Figure 1. In all the normal and diabetic subjects, DDAVP induced a significant increase of plasma vWF/VIII-RAG levels, which reached a plateau from 60 to 150 min.

In the normal subjects, vWF reached mean levels of 196 ± 11% after 60 min, corresponding with 205% of the mean basal values (95 ± 5%, $p < 0.001$). Factor VIII-RAG behaved similarly, increasing from 70 ± 8% to 186 ± 16% (264% of basal value, $p < 0.001$). Platelet retention increased at 30 min ($p < 0.02$) and 60 min ($p < 0.01$), returning to basal values at 90 and 150 min. No consistent changes occurred in spontaneous and ADP-induced platelet aggregation.

The mean basal values of vWF were higher in the diabetic (140 ± 13%) than in the healthy subjects ($p < 0.01$). Following DDAVP, vWF increased to 245 ± 22% after 60 min (182% of basal, $p < 0.001$). Factor VIII-RAG levels were also higher in the patients (112 ± 18%) but the difference was not significant. VIII-RAG reached a plateau at 257 ± 28% (230% of basal value, $p < 0.001$).

The mean levels of both vWF and VIII-RAG re-

mained higher in the diabetic patients at 30 min ($p < 0.05$) and in the plateau phase after 60 min ($p < 0.05$).

Platelet retention behaved similarly to that observed in the healthy subjects. At 150 min, it was higher in the patients (46 ± 6%) than in the healthy subjects (28 ± 7%; $p < 0.05$). Platelet aggregation in the diabetic patients did not differ from the normal subjects and did not change following DDAVP administration.

Aspirin administration did not modify the increase of plasma vWF/VIII-RAG induced by DDAVP, nor the behaviour of platelet retention throughout the experiment. ADP-induced platelet aggregation showed inhibition of the release reaction following aspirin ingestion.

In the healthy subjects, the systolic blood pressure decreased by 5–6% of the basal values at 60, 90, and 150 min ($p < 0.01$). The diastolic blood pressure decreased by 12% of basal at 30 min ($p < 0.025$) and by 9–10% at 60 and 90 min ($p < 0.001$). Heart rate increased by 12% at 30 min ($p < 0.01$) and by 6% at 60 min ($p < 0.025$).

In the diabetic patients, the systolic blood pressure decreased by 4% at 30 and 60 min ($p < 0.01$), and the heart rate increased by 17% ($p < 0.01$). No significant changes occurred in diastolic blood pressure. No changes of these parameters were observed in normal subjects when saline was infused instead of DDAVP.

DDAVP induced flushing of the face, neck and chest, lasting about 2 h and not inhibited by aspirin, in all the normal and diabetic subjects.

Discussion

DDAVP has been shown to release vWF/VIII-RAG, factor VIII-antihaemophilic and plasminogen activator from endothelial storage sites [16, 20]. This drug is used as an alternative to plasma derivatives in the treatment of patients with mild von Willebrand's disease or haemophilia, in the preparation for minor surgery [21]. In some countries, DDAVP is administered to blood donors in order to harvest factor VIII-enriched plasma derivatives [22]. This study shows that vWF/VIII-RAG plasma levels may rise in diabetic patients to higher values than in normal subjects after the administration of DDAVP. Vascular stress in diabetic microangiopathy could result in the increased content (and possibly increased rate of synthesis) of this factor in the endothelium. At present, the possibility that the clearance of vWF/VIII-RAG is reduced in diabetes cannot be evaluated. This observation also suggests that diabetic patients pre-treated with DDAVP may be more suitable than normal subjects as donors of vWF/VIII-RAG-enriched plasma derivatives. In the past, plasma from diabetic patients had been reported to correct some platelet abnormalities of von Willebrand's disease more effectively than normal plasma [23], possibly due to its higher content of vWF/VIII-RAG.

The mechanism of action of DDAVP is unknown. The drug has minimal vasoactive properties, yet it has been reported to be more effective than adrenaline and lysine- or arginine-vasopressin in mobilising endothelial molecules [24]. Small, though significant, changes of blood pressure and heart rate, together with marked flushing, were observed in all subjects in this study, suggesting that DDAVP is active at some vascular sites (i.e. the skin of the face, neck and chest) and that the endothelium may be differentially affected by the action of the analogue.

In spite of the different plasma levels of vWF/VIII-RAG, platelet retention in glass bead columns was similar in the normal and diabetic subjects before the infusion. As suggested previously [25], the decrease of platelet retention observed in normal subjects at 90 and 150 min may be caused by an inhibitory mechanism(s) counteracting the state of platelet hyperadhesiveness observed at 30 and 60 min in association with rising levels of vWF/VIII-RAG. This anti-adhesive mechanism(s) may be effective in maintaining normal platelet retention in diabetes despite high vWF/VIII-RAG levels. The observation that platelet retention was higher in the patients at 150 min suggests that this anti-adhesive mechanism(s) may fail to compensate for a further increase of vWF/VIII-RAG as induced by DDAVP. Further work is necessary to characterize this anti-adhesive mechanism(s). Prostacyclin, a potent anti-aggregating/anti-adhesive prostaglandin synthesized by the endothelial cells [26] could be released with vWF/VIII-RAG and inhibit platelet retention. This hypothesis was regarded as unlikely in a previous paper [25], which showed that the levels of 6-oxo-$PGF_{1\alpha}$ (a by-product of prostacyclin) did not increase following DDAVP, and is conclusively ruled out in the present study. In fact, aspirin administered to a normal subject in a dose capable of inhibiting the synthesis of prostacyclin over 12 h before study [26], did not modify the platelet retention pattern following DDAVP.

Spontaneous and ADP-induced platelet aggregation were similar in the normal and diabetic subjects and did not change following DDAVP, confirming other evidence that these parameters of platelet function are not influenced by the circulating levels of vWF/VIII-RAG [3].

In conclusion, this study shows that plasma vWF/VIII-RAG levels similarly increase in response to DDAVP in diabetic patients and normal subjects. High levels of vWF/VIII-RAG in the plasma of these patients may reflect a proportionally increased availability of this factor from the vessel wall and not simply leakage from damaged endothelial cells. High vWF/VIII-RAG levels may lead to abnormal regulation of platelet adhesiveness in diabetes.

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