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Acute von Willebrand Factor Secretion from the Endothelium In Vivo: Assessment through Plasma Propeptide (vWf:AgII) Levels

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Summary

Elevated plasma concentrations of von Willebrand factor (vWf) are increasingly recognized as a cardiovascular risk factor, and are used as a marker of endothelial activation. However, the factors which determine the rate of vWf release from the endothelium in vivo have not been defined clearly. In addition, vWf plasma levels may also be influenced by adhesion of vWf to the vascular wall or to platelets, and by its rate of degradation. The propeptide of vWf (also called vWf:AgII) is stored and released in equimolar amounts with vWf. In the present study we attempted to determine whether this propeptide could be a more reliable marker of endothelial secretion than vWf itself. To accomplish this we developed an ELISA based on monoclonal antibodies. The propeptide levels in normal plasma were found to be 0.7 μg/ml, more than 10 times lower than vWf itself. Administration of desmopressin (DDAVP) induced a rapid relative increase in propeptide (from 106 to 879%) and in vWf (from 112 to 272%). However, the increases in vWf and propeptide were equivalent when expressed in molar units. A time course study indicated a half-life of the propeptide of 3 h or less. In a baboon model of disseminated intravascular coagulation (DIC) induced by FXa, vWf increased by less than 100%, whereas the propeptide concentrations increased by up to 450%. In view of the massive thrombin generation (as assessed by fibrinogen depletion), the increases in vWf are small, compared to the strong secretory response to thrombin and fibrin previously observed in vitro. Our results suggest that due to its rapid turnover, the propeptide could provide a sensitive plasma marker of acute endothelial secretion.

Introduction

Plasma von Willebrand factor (vWf) is increasingly recognized as a predictor of cardiovascular mortality. As shown by the ECAT study and other reports, elevations in plasma vWf , along with tissue plasminogen activator (t-PA) and fibrinogen (1, 2), predict myocardial infarction in patients with angina pectoris. Plasma vWf is elevated in severe hypertension (3), diabetic nephropathy (4, 5) and end-stage renal failure (6). It is a predictor of the excess cardiovascular disease associated with these conditions, and is often considered as a marker of endothelial activation. vWf is synthesized, stored and secreted by megakaryocytes/platelets and by endothelial cells (7). Since the plasma pool mainly originates from endothelial cells (8), it can be assumed that plasma vWf levels are determined by the rate of endothelial release (8).

In endothelial cells vWf is stored in specialized secretory granules, called Weibel-Palade bodies (7), and released after stimulation by thrombin or other mediators of thrombosis and/or inflammation (e.g. histamine, complement C5a and C5b-9) (9-12). Thrombin may act directly on its receptor on endothelial cells, or indirectly by generating fibrin, which in turn induces endothelial secretion (10). In addition, hormones such as epinephrine and vasopressin can induce an increase in plasma vWf. Indeed, the vasopressin analog DDAVP is used clinically to increase plasma vWf and to shorten bleeding time (13-15). We have recently observed that epinephrine can induce vWf release from Weibel-Palade bodies in vitro after activation of β-adrenergic receptors, although it is a much less potent stimulus than thrombin (Vischer and Wollheim, submitted). The relative contribution of these factors to the determination of plasma vWf concentration is presently unknown.

The assessment of endothelial vWf secretion has several potential limitations. Fluctuations in plasma vWf are usually small, and tend to overlap with normal values, which display a fairly wide range (16). vWf is released from endothelial cells on the baso-lateral side (17). A part of the secreted vWf may therefore remain trapped in the sub-endothelium at the site of release (18), and fail to reach the bloodstream. The fraction retained could be modified in vascular disorders. Further, vWf levels may be affected by changes in its clearance. If it is released in a thrombogenic context, vWf may be rapidly consumed in the process of platelet aggregation (19). Accelerated proteolytic degradation of vWf, which circulates as complex multimers, has been suggested in patients with severe arterial disease (20). Plasma vWf concentrations are influenced by blood groups, presumably because the protein carries AB0 antigens which influence its clearance rate (21, 22). Finally, the long half-life of vWf in the circulation (approx. 12 h) (23) implies that even extensive release by the endothelium may result in only modest relative increases in plasma vWf.

vWf is synthesized as a large 360 kD precursor, pro-vWf. After undergoing dimerization and glycosylation in the endoplasmic reticulum and the Golgi apparatus, the precursor reaches the trans-Golgi network where it undergoes multimerization of the dimers and cleavage into mature vWf and an unusually large, 97 kD propeptide. Both fragments are directed to the Weibel-Palade bodies, as they remain associated by non-covalent, pH-dependent interactions. Stimulation of exocytosis therefore causes the release of equimolar amounts of vWf and

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propeptide (7, 24, 25). The propeptide has a simpler molecular structure than vWf itself (e.g. it does not undergo multimerization) and seems not to adhere to platelets or to vascular structures [although it contains an integrin binding RGD sequence (7)]. The plasma propeptide levels could therefore be an attractive candidate marker of endothelial secretion in vivo. McCarrroll et al. (26) used the difference between mature vWf and propeptide levels to identify patients with type II von Willebrand disease, attributing the lower levels of vWf to an accelerated clearance.

The present study was undertaken to investigate whether the plasma propeptide could be a better marker of endothelial exocytosis than vWf itself. We constructed a quantitative ELISA procedure to measure plasma propeptide levels and tested it in two model situations of acute vWf release, i.e. the response to the injection of DDAVP in normal individuals and an experimental disseminated intravascular coagulation (DIC) in the baboon.

Methods

Propeptide ELISA

We set up a sandwich-type ELISA based on monoclonal antibodies. Monoclonals BR5 and 8H10 have been previously characterized (27) and were kindly provided by Dr. P. Fay (Rochester NY). Monoclonal 3H4 was generated by injecting balb/c mice with WP body fractions (28). The purified antibody stains Weibel-Palade bodies by indirect immunofluorescence in cultured human umbilical vein endothelial cells (HUVECs) and recognizes unreduced purified propeptide by Western Blot (not shown).

96-well plates were coated with antibodies BR5 and 3H4 0.75 µg/ml each in carbonate buffer ($NaCO_3$ 50 mM, pH 9.6), 100 µl/well. After 4 washes with TBS-T (Tris 20 mM, NaCl 140 mM, Tween-20 0.1%, pH 7.4), the remaining protein binding sites were blocked with 150 µl/well blocking solution (TBS-T + 5% dry skim milk). Samples and standards diluted in blocking solution were added and incubated for 2 h at 37° C. After 4 washes with TBS-T, antibody 8H10 conjugated to alkaline phosphatase diluted 1:800 (approx. 0.25 µg/ml) was added for 90 min at 37° C. Bound alkaline phosphatase activity was detected using p-nitrophenyl phosphate as a substrate and measuring optical density at 405 nm.

Purified plasma propeptide was generously provided by Dr. P. J. Fay (29). The protein content was determined by the Bradford assay, using bovine serum albumin as a standard. This preparation contained a single 97 kDa band on silver staining of an overloaded gel (2 µg/lane). The purified protein was used as a standard, allowing us to determine the propeptide concentration in normal pooled plasma (NPP). For routine assays, a standard curve was constructed with NPP. Each sample was tested at 4 dilutions. Results are expressed either as a percentage of NPP, or converted to µg/ml (assuming a concentration of 0.72 µg/ml in NPP, see results). Results for baboon propeptide are always expressed as a percentage of a reference baboon plasma.

A range of normal values was determined on citrated plasma from 30 healthy individuals (age range 20-60 years).

vWf was measured by ELISA as previously described (30). Mean vWf levels in 22 baboon samples was 121 ± 19% (mean ± SD) compared to human pooled plasma, and the (log (OD) v. log (conc.)) relations of human and baboon samples were parallel. Unless stated otherwise, results are expressed as means ± SEM. Statistical comparison between groups was done using the paired, two-tailed Student's t-test.

DDAVP Studies

Nine normal healthy volunteers (4 men and 5 women, median age 24, range 21-44) received a single intravenous infusion of desmopressin (Minirin, Ferring AG), given at a dose of 0.4 µg/kg body weight intravenously over 30 min, as previously described (31). The volunteers gave informed consent and the protocol was approved by the local ethics committee. Blood was drawn before and 1 h after the end of the infusion. vWf and propeptide were measured on citrated plasma. Five additional volunteers were injected with desmopressin subcutaneously, and blood was collected before and 1, 3, and 6 h after the injection.

Disseminated Intravascular Coagulation (DIC) Studies

Baboons (papio ursinus) weighing 25-30 kg were quarantined for three months on arrival and screened for evidence of disease before being released from quarantine. They were housed individually in a facility accredited by the French ministry of Agriculture, and fed with commercial primate chow, fresh fruit and tap water ad libitum. On the day of the studies, five animals were injected with factor Xa 0.45 U/kg together with phosphatidylcholine/phosphatidylserine (PC/PS) 56 nmol/kg (Group A, low dose). A second group of 6 animals was injected twice with FXa 1.2 U/kg and PC/PS 200 nmol/kg at times 0 and 5 h (group B, high dose). Three animals in each group were injected with interleukin-6 (IL-6) 8 h before the administration of FXa, as a part of a wider study designed to assess the effect of IL-6 on the coagulation and fibrinolytic responses in DIC. IL-6 pretreatment had no effect on vWf and propeptide levels, and the results from the two subgroups are therefore pooled in the present report. Blood was collected from a posterior saphenous vein before and 2, 5, 10, 20, 30 min and 1 h after each injection of FXa, and subsequently at least every 2 h up to 12 h. Later samples were drawn after 24 and 48 h. The high dose FXa protocol caused a venous collapse after the first injection, preventing blood sampling for the first 30 min. This problem did not occur after the second injection, allowing normal sampling. All injections and blood sampling were done under light general anesthesia (ketamine 7 mg/kg).

Fibrinogen was measured by a thrombin-initiated clotting rate assay (Clauss's assay), using reagents obtained from Diagnostica Stago (Asnières, France). Thrombin-antithrombin III complexes and prothrombin fragment 1+2 were measured by ELISA (Enzygnost TAT and Enzygnost 1+2, Behring). Fibrinopeptide A was measured by using a competitive ELISA after removal of fibrinogen by Bentonite absorption (Asserachrom FPA, Diagnostica Stago).

Results

Characteristics of the Propeptide ELISA

The assay for the propeptide (vWf:AgII) is a capture ELISA, using 2 monoclonal antibodies as a catching layer, and a third monoclonal antibody coupled to alkaline phosphatase for detection. We observed a linear relationship between plasma dilutions and optical density (OD_{405}) over a range of normal pooled plasma (NPP) dilutions from 1:10 to 1:640 (Fig. 1A). We also used purified propeptide as a standard, and found a propeptide concentration of 720 ± 43 ng/ml (n = 5) in normal pooled plasma. This value is at least ten fold lower than most estimates of plasma vWf (10 µg/ml) (16, 20). If the molar ratio of vWf (260 kD) and the propeptide (97 kD) is taken into account, the concentration of propeptide is still approximately 5 times lower than vWf. The propeptide signal detected in NPP at a dilution of 1:320-1:640 corresponds to a sensitivity of <2.5 ng/ml (<25 pM, assuming a molecular weight of 97 kD). The correlation coefficient between repeat measurements was 0.93.

We determined the propeptide plasma levels in 30 normal individuals (Fig. 1B). The values ranged from 69 to 167% (102 ± 23%, mean ± SD). These results indicate a smaller variation among normal individuals for the propeptide than for vWf (range 47-185%, mean ± SD, 109 ± 33). We also studied 14 patients with type III von Willebrand's disease (plasma vWf less than 1%). Propeptide levels were below the limit of detection (less than 3%) in all patients, confirming the specificity of our assay (Fig. 1B).

We observed a weaker signal with baboon plasma than with human plasma (Fig. 1A). Although all three antibodies tested individually react with baboon propeptide, the likeliest explanation is that the anti-

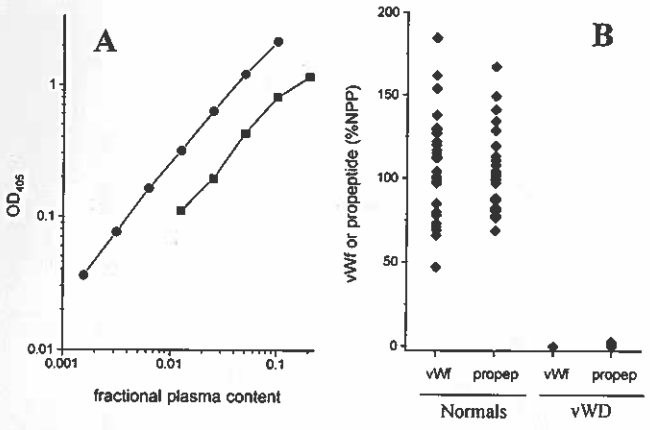

Fig. 1 Characterization of the propeptide assay. A) Standard curves for the propeptide ELISA, based on human (circles) and baboon (squares) plasma. The optical density (OD_{405}) is plotted against the fractional plasma content of diluted samples. The results shown are the mean of 3 parallel determinations done under identical conditions. B) Plasma propeptide and vWf values from 30 normal individuals and 14 patients with severe type III von Willebrand's disease

body combination used has a lower affinity for baboon than for human propeptide. Since no purified standard was available, results for baboon propeptide are always expressed as a percentage of a reference baboon plasma.

Plasma Propeptide Increases after DDAVP Administration

DDAVP is known to increase plasma vWf levels. In vitro, propeptide is released along with vWf from the endothelial cells. The plasma propeptide should therefore also increase after the administration of DDAVP. vWf and propeptide were measured in 9 normal individuals before and 1 h after the i.v. injection of DDAVP (Fig. 2). As expected, the plasma vWf level rose from 110 ± 12% to 272 ± 10% (mean ± SEM, $p < 0.0001$) (Fig. 2A). There was a much more marked rise in propeptide, from 106 ± 8 to 879 ± 67% ($p < 0.0001$) (Fig. 2B). When expressed as actual concentrations (using the propeptide concentration of normal pooled plasma (0.72 μg/ml) as a conversion factor), propeptide rose from 0.77 ± 0.06 to 6.33 ± 0.48 μg/ml 1 h after the DDAVP injection, representing a mean increase of 5.56 ± 0.47 μg/ml. This value compares with a mean increase in vWf of 16.20 ± 1.09 μg/ml (assuming a concentration of 10 μg/ml in the pooled plasma used as a standard (16, 23). Expressed in molar units, the mean increases were 57 nM for propeptide and 62 nM for vWf, assuming molecular weights of 97 and 260 kD respectively. These two values are quite similar, and compatible with the in vitro observation that vWf and propeptide are stored and released in a 1:1 ratio. Thus although the amounts released from the endothelium are quite similar, the increase in propeptide level is much more impressive than the increase in vWf when expressed as a percentage of normal plasma.

To estimate the turn-over of the propeptide, 5 normal individuals were injected with DDAVP, and propeptide and vWf were measured after 1, 3 and 6 h (Fig. 3). Following a mean initial 4.8 fold increase after 1 h, the propeptide values decreased by >70% over the next 5 h. Although these experiments do not allow a formal determination, we can estimate the circulating half-life to be in the order of 3 h. The decrease in vWf level was much less pronounced, <18% between 1 h and 6 h, in accordance with the much longer half-life reported for vWf of approximately 12 h (23).

Plasma vWf and Propeptide Levels in Experimental DIC

We next investigated the changes in vWf and its propeptide following activation of coagulation. We chose a DIC model in the baboon, which allows the time-controlled generation of intravascular thrombin by injection of FXa. Five baboons were injected with a single dose of FXa (0.45 U/Kg), together with phospholipids. This resulted in a very rapid and transient increase in the plasma levels of markers of thrombin generation. Prothrombin fragment 1+2 increased sharply to a maximum at 2 min after the injection of FXa, and then decreased very rapidly, to return to basal levels after 3-4 h. A very similar profile was observed with the thrombin-antithrombin complex (Fig. 4A). We also observed a rapid increase in fibrinopeptide A (FPA), together with a significant decrease in fibrinogen levels (from 1.95 ± 0.17 to 0.98 ± 0.17 g/l after 10 min, $p = 0.001$) (Fig. 4B). Fibrinogen levels recovered over the subsequent 12 h and were increased over prestimulation values by 24 h. These data confirm that an injection of FXa induces intravascular generation of thrombin, which in turn induces the formation of fibrin. The period of fibrin generation is very brief, as is indicated by the fibrino-

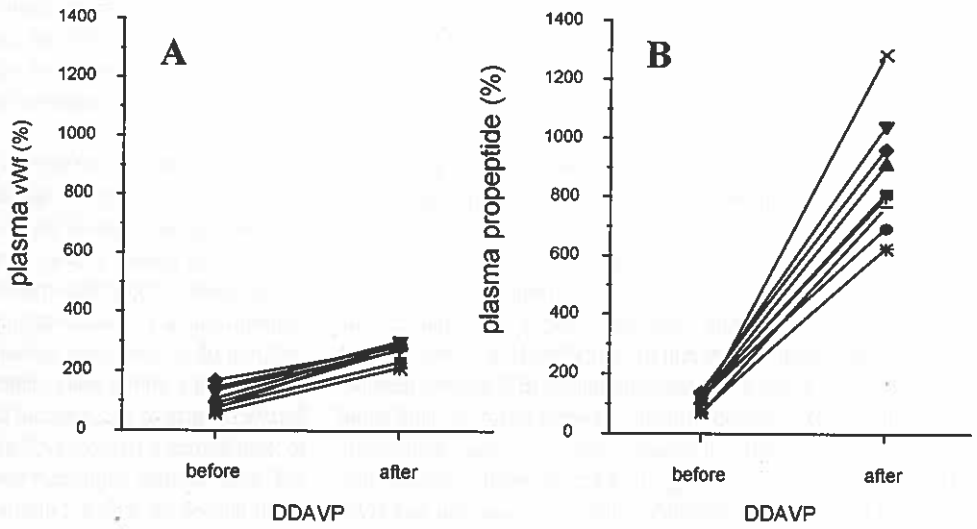

Fig. 2 Effect of DDAVP on plasma levels of vWf and propeptide. Nine normal volunteers received an intravenous infusion of DDAVP 0.4 μg/kg over 30 min. vWf (panel A) and propeptide (panel B) were measured by ELISA on citrated plasma obtained before and one hour after the infusion. Values are shown for each individual donor. Results are expressed as a percentage of normal pooled plasma

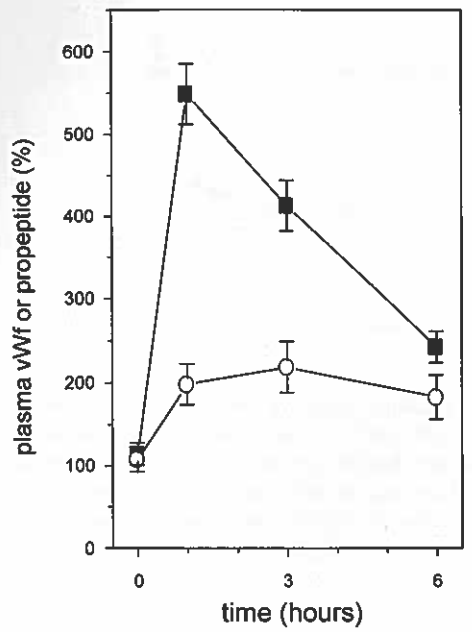

Fig. 3 Effect of DDAVP on plasma levels of vWf and propeptide: time course. Five normal volunteers received a subcutaneous injection of DDAVP 0.3 μg/kg. vWf (open circles) and propeptide (solid squares) were measured by ELISA before and 1, 3 and 6 h after the injection. Values at 1, 3 and 6 h were significantly higher than the pre-injection values for both vWf and propeptide ($p < 0.01$ by the paired two-sided Student's t-test)

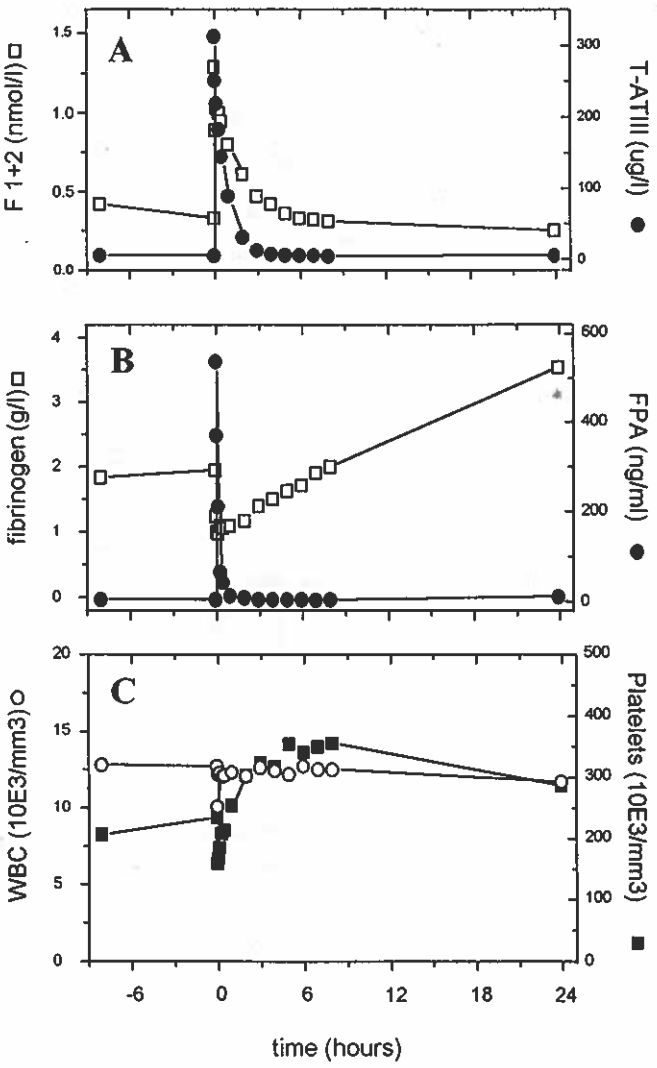

Fig. 4 Effect of experimental DIC (low dose group) on markers of activation of the coagulation system in baboons. Prothrombin fragments 1+2 (F1+2, panel A open squares), thrombin-antithrombin III complexes (T-ATIII, panel A closed circles), fibrinogen (Panel B, open squares) and fibrinopeptide A (FPA, panel B closed circles) were measured in the plasma from five baboons injected at time 0 with 0.45 U/kg FXa, together with phospholipid vesicles. Platelets (panel C, open circles) and white blood cells (panel C, solid squares) were also measured

peptide A levels, which return to baseline 30-60 min after the initial peak. Interestingly, the platelet levels were very little affected (Fig. 4C). We observed a small decrease from 318,000 to 252,000/mm^3 at 2 min, but the levels returned to 305,000/mm^3 after 5 min and remained stable at all later time points. The WBC counts also decreased abruptly by 33% at 2 min, but returned to pre-stimulation levels by 30 min and increased over the prestimulation values for the next 12 h (Fig. 4C).

The levels of vWf increased very rapidly, but the increases were remarkably small. The values increased by only 1.5 fold (Fig. 5). Such increases would easily escape detection were it not for the detailed time course feasible in this model: the increase in vWf was detected at 2-5 min in all animals studied. In marked contrast, the propeptide levels rose much more strikingly, with a maximum increase of 3.5 fold at 30 min. The values returned to near normal by 6 h, confirming that the propeptide has a rapid turnover. Since the FXa-induced activation of coagulation is very brief, as indicated by the rapid normalization of FPA, it is unlikely that the decrease in plasma propeptide is slowed down by ongoing release.

A second group of 6 animals was injected twice with a high dose of FXa (1.2 U/kg, together with phospholipids) at time 0 and 5 h. The results for FPA, thrombin fragments 1+2, and the thrombin-antithrombin complex were qualitatively very similar, with a second peak of each marker after the second stimulation. After each injection there was an almost complete depletion of fibrinogen (not shown). The first injection caused a rapid 5.5 fold increase in plasma propeptide at 30 min, followed by a rapid decline to near normal values within 5 h (Fig. 6A). vWf levels underwent a rapid 1.8 fold increase, and remained elevated for the next 5 h. A second injection of FXa induced a second peak of propeptide, although this was of lower magnitude than the first. The propeptide level returned to normal within 5 h after the second injection. vWf increased again to 234% over the baseline level, and decreased only very slowly over the subsequent 24 h. Again, these observations suggest that propeptide has a short half-life of approx. 1-2 h. In contrast, vWf remained elevated up to at least 24 h after the beginning of the study, in accordance with its longer half-life.

The high doses of FXa had a more pronounced effect on platelet counts (Fig. 6B). A decrease from 330,000/mm^3 to 169,000/mm^3 at 30 min was observed, followed by a partial restoration to 252,000/mm^3 at 5 h. The second injection of FXa caused a rapid drop to 128,000/mm^3 after 2 min (i.e. a 50% decrease), and a partial restoration to 199,000/mm^3 and 245,000/mm^3 10 min and 2 h later respectively. The transient drop in WBC count observed in the low dose group was not found after the first injection of the high dose of FXa, presumably because blood could not be drawn during the first 30 min, due to a transient venous collapse after FXa (Fig. 6B). After one hour, the WBC count had increased to 15,300 ± 1600/mm^3 and remained stable over

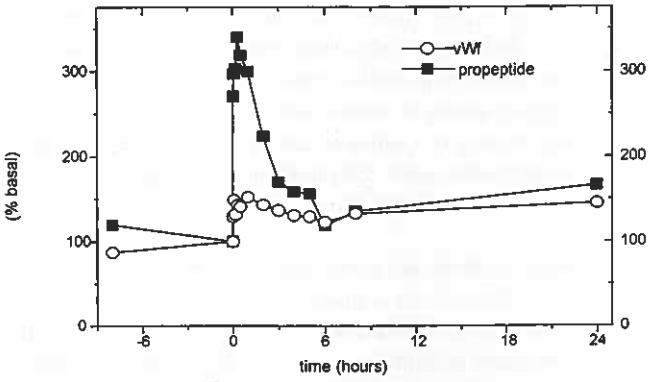

Fig. 5 Effect of experimental DIC (low dose group) on plasma vWf and propeptide. Five baboons injected at time 0 with 0.45 U/kg FXa, together with phospholipid vesicles (see Fig. 3). Results for vWf and propeptide are expressed as a percentage of the value at time 0. Error bars are omitted for the sake of clarity; standard errors were always less than 20%. All values from 2 min to 1 h were significantly higher than pre-injection control ($p < 0.05$) for both vWf and propeptide

the following 4 h. A sharp decrease to 10,100 ± 2300/mm^3 2 min after the second injection was again observed, followed by a return to pre-injection values 30 min later.

Discussion

This study describes an ELISA for the quantification of the propeptide. The assay is simple and reproducible, and can be applied to large numbers of samples. Since monoclonal antibodies are used, their availability is unlimited. Our assay is specific for the propeptide, as shown by the absence of a signal in patients with complete vWf deficiency (von Willebrand's disease type III). Both propeptide and vWf result from the cleavage of a common precursor, pro-vWf (1). Accordingly, the antibodies to propeptide also recognize pro-vWf. Although cultured endothelial cells release some uncleaved pro-vWf (32), this precursor is not found in normal plasma (26), suggesting that in vivo cleavage is completed either before or very rapidly after secretion. It is therefore most unlikely that pro-vWf contributes to the immunoreactivity detected in our propeptide assay.

In vitro experiments have shown that vWf is stored in Weibel-Palade bodies along with equimolar amounts of its propeptide (25, 33). Stimulation of exocytosis is therefore expected to cause the release of equimolar amounts of the two moieties. Our in vivo findings support this observation. One hour after the injection of DDAVP to normal individuals, the increases in plasma levels of propeptide and vWf (expressed in molar units) are quite similar. We determined the propeptide plasma level to be approx. 0.7 μg/ml, which is at least 10 times lower than most estimates for plasma vWf (16, 23). Even after conversion to molar units, the propeptide level is approximately 5 times lower than vWf. This lower basal level is due to the rapid turnover of the propeptide. Our observations in both humans and baboons suggest that the circulating half-life is less than 3 h. This is probably an overestimate, given the continuing effect of DDAVP (which has itself a half-life of approx. 1 h) on endothelial secretion. Thus when equimolar amounts of vWf and propeptide are secreted, the increase in plasma propeptide – expressed in relative units – is much more apparent due to its low pre-stimulation value. Given these kinetic properties, the propeptide is well suited for the assessment of acute endothelial secretion.

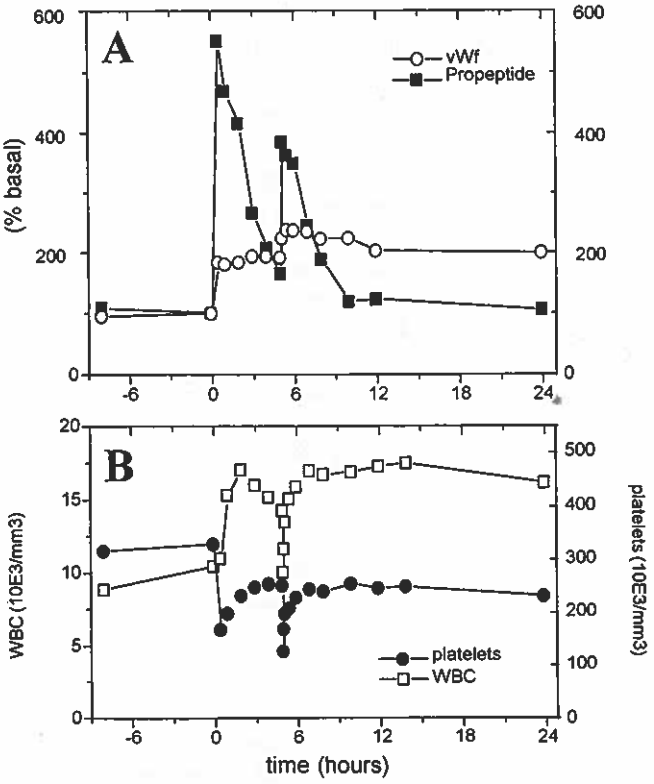

Fig. 6 Effect of experimental DIC (high dose group) on plasma vWf and propeptide, and on WBC and platelets. Six baboons were injected with FXa 1.2 U/kg together with phospholipids at times 0 and 5 h. Panel A: Values for vWf and propeptide are expressed as a percentage of the values at time 0. All vWf values following the first injection up to 24 h were significantly elevated compared to time 0 ($p < 0.001$). Propeptide values were elevated from 30 min to 7 h ($p < 0.05$), but were not significantly different from pre-injection levels at later time points. Panel B: platelets and WBC from the same animals are shown

Exocytosis occurs in a polarized fashion to the baso-lateral side of the cells (17). A part of the vWf released may remain trapped in the subendothelium and fail to reach the plasma. Although the propeptide binds to bovine type I collagen in vitro, there is no evidence for propeptide adhesion to the subendothelium in cell culture experiments (25). Thus, the molar amounts of vWf entering the bloodstream should be smaller than those of propeptide. The increases in plasma vWf and propeptide after DDAVP (in molar units) are very similar. Although we certainly cannot rule out the possibility that some vWf is retained in a biologically active form in the subendothelium, these observations suggest that retained vWf represents only a small proportion of the vWf released from the endothelial cells.

Since vWf is stored not only in the endothelium, but also in megakaryocytes and platelets (7), the interpretation of plasma propeptide levels needs to take the possible contribution from platelets into account. In vivo release of propeptide from platelets is very unlikely in the case of DDAVP, which has minimal effects on platelet activation. In a previous study, we found only minimal increases in the surface expression of granule membrane proteins (31). The propeptide level in serum, which would be expected to contain all releasable platelet propeptide, is 2.8 fold higher than in plasma, whereas this ratio is 1.3 in the case of vWf (not shown). In the DIC studies, platelet activation occurring in the process of microthrombi formation could induce propeptide release. In the low-dose group, where the propeptide level increased more than 3 fold, we only observed a small decrease of platelet counts

after 2 min, followed by normalization already after 5 min. In the high dose FXa group in which more obvious platelet activation (as reflected by a 50% lowering of platelet counts) occured, platelet-derived propeptide could account in part for the increase in plasma propeptide. Although platelet-derived propeptide is unlikely to account entirely for the >5 fold increase in plasma propeptide, these considerations underscore our conclusion that DIC is a weak stimulus for endothelial vWf release (see below).

Exocytosis from Weibel-Palade bodies also results in translocation of P-selectin from the granule membrane to the cell surface, facilitating leukocyte rolling, which mediates adhesion to the endothelium and subsequent extravasation (34, 35). In vitro, following histamine-induced exocytosis, the surface expression of P-selectin is very transient: after a peak at 2 min, it is internalized by endocytosis within 30 min (35). Interestingly, the injection of FXa causes a rapid and transient decrease in WBC count, with a time course which is strikingly similar to these in vitro observations. The decrease also correlates closely with the increase in propeptide. The observed decrease is compatible with leukocyte adhesion to P-selectin newly exposed after exocytosis of the Weibel-Palade bodies. This interpretation provides further support for the notion that propeptide is a marker of endothelial exocytosis.

DDAVP is used in the clinical setting to increase plasma vWf levels and reduce bleeding time in patients with von Willebrand disease and other bleeding disorders (11). Although it has no effect on vWf release from cultured endothelial cells, DDAVP is assumed to induce vWf release from Weibel-Palade bodies in vivo. DDAVP has no direct secretory effect on platelets, the only alternative source of cellular vWf (31). Our results confirm the observations of McCarroll et al. showing that DDAVP induces a rapid increase in plasma propeptide as well as vWf (36), and strengthen the view that DDAVP induces exocytosis from WP bodies. The effect of DDAVP on vWf levels could conceivably be due to recirculation of extracellular vWf sequestered from the bloodstream (e.g. in the spleen). However, the parallel increase of propeptide and vWf in equivalent amounts argues against this hypothesis, given the distinct structures and adhesive properties of these two proteins.

The DIC model in baboons used in the present report makes it possible to examine the activation of coagulation and fibrinolytic responses with a detailed time course. This model is therefore well suited to study the effect of DIC on vascular function, in particular on endothelial secretion. The low dose FXa injection caused a modest, 50% increase in plasma vWf. This would have escaped detection were it not for early collection of samples, showing that the increase occured within 2-5 min. Indeed, most post-stimulation values overlap with the range of normal values as defined for clinical use (16). In contrast, propeptide increased more than 3 fold, with an equally rapid time course. A higher dose of FXa induced a larger, 5.5 fold increase in propeptide levels, indicating a dose-related response to FXa. A second injection of FXa 5 h later was still capable of inducing a second peak of propeptide, which was only 30% lower than the first peak. The rapid, simultaneous increase in vWf and propeptide strongly suggest that FXa-induced DIC induces secretion from pre-formed endothelial stores. However, our results indicate that vWf release in this DIC model is surprisingly modest. Although the FXa injected generated enough thrombin to decrease fibrinogen levels by 50% (group A) or 100% (group B), vWf levels went up by only 50% or 90% respectively. Increases in propeptide were more obvious, and in the low dose group were helpful in determining that vWf release occured at all. Hence, thrombin and fibrin are quite inefficient at raising plasma vWf. These observations stand in contrast with their strong effect on vWf release in cultured cells, when compared to other secretagogues such as epinephrine (9, 10, Vischer and Wollheim, submitted). This discrepancy could be explained in part by rapid inactivation of thrombin at the endothelial surface, e.g. by cell-associated antithrombin III.

In conclusion our new assay for plasma propeptide could provide a valuable marker of acute secretion from endothelial cells in vivo, essentially due to the rapid turnover of the protein. Although some plasma propeptide could derive from platelets, this is unlikely to be of major significance except in situations of massive platelet activation such as vasculitis or acute respiratory distress syndrome. Propeptide levels may be useful to determine weak secretory responses, as in our DIC model or in other situations such as physical activity, and differentiate them from non-specific increases due to hemoconcentration and/or dehydration. Future work should also determine whether propeptide, given its simpler structure and kinetic properties, could be a more reliable marker of endothelial secretion than vWf in chronic vascular disorders.

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