**Brief Rapid Communications**

**Improvement of von Willebrand Factor Proteolysis After Prostacyclin Infusion in Severe Pulmonary Arterial Hypertension**

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**Background**—The presence of dysfunctional von Willebrand factor (vWF) in pulmonary arterial hypertension (PAH) was suggested to be related to increased proteolysis.

**Methods and Results**—In 10 patients with severe PAH, we studied the proteolysis of plasma vWF (vWF levels, multimeric distribution, proteolytic pattern, and cleaving protease activity) and hemodynamic variables (mean pulmonary artery pressure, cardiac index, and total pulmonary vascular resistance) at baseline and 30 days after initiation of continuous prostacyclin infusion. At baseline, vWF levels were significantly increased, vWF proteolysis was excessive, and vWF-cleaving protease activity remained normal. These biological abnormalities were reversible and paralleled the improvement of hemodynamics under vasodilator treatment with prostacyclin.

**Conclusions**—The excessive proteolysis of vWF in PAH is likely to be related to an increased susceptibility of vWF to proteases induced by high shear rates rather than to an enhanced release of enzymes. (*Circulation*. 2000;102:2460–2462.)

**Key Words:** hypertension, pulmonary • von Willebrand factor • proteins • prostaglandins

Pulmonary arterial hypertension (PAH) is characterized by an elevation in pulmonary arterial pressure and vascular resistance associated with pulmonary artery endothelial cell dysfunction. Plasma von Willebrand factor (vWF) is a large glycoprotein synthesized mainly in endothelial cells. It is the carrier protein for coagulation factor VIII, and it plays a crucial role in platelet adhesion and aggregation at sites of vascular injury. These functions are optimized by the multimeric structure of vWF, involving low-, intermediate-, and high-molecular-weight (LMW, IMW, and HMW) multimers built up from identical subunits of 270 kDa. The multimeric composition of vWF in plasma is physiologically regulated by a specific vWF-cleaving protease. In PAH, plasma vWF is increased but dysfunctional because of a loss of its HMW multimers, which may be related to several mechanisms: abnormal processing of vWF in endothelial cells, adsorption of the HMW multimers onto activated platelets, or increased proteolytic degradation of vWF by enzymes. Thus, vWF has been proposed as both a marker of endothelial perturbation and a predictor of prognosis in PAH. The behavior of plasma vWF after continuous infusion of prostacyclin, a vasodilator therapeutic agent commonly used in PAH, has been analyzed in only 1 study. The aims of the present work were to characterize vWF proteolysis in patients with severe PAH at baseline and after prostacyclin infusion and to evaluate the relevance of vWF-cleaving protease.

**Methods**

**Study Population**

Ten patients with severe PAH (1 man and 9 women, age 39.7 ± 10.4 years) were evaluated at baseline (D₀) and 30 days after initiation of continuous infusion of prostacyclin (D₃₀). Five patients had primary pulmonary hypertension and 5 had PAH secondary to fenfluramine derivatives (n = 3), a CREST (Calcinosis, Raynaud’s phenomenon, Esophageal dysfunction, Sclerodactyly, Telangiectasia) syndrome (n = 1), or Eisenmenger’s syndrome (n = 1). New York Heart Association functional class was III (n = 4) or IV (n = 5). Ten healthy volunteers (age 38.1 ± 10.2 years) were used as controls. All subjects were tested after appropriate consent had been obtained in accordance with the declaration of Helsinki.

**Biological Parameters**

Venous blood was collected onto 1/10 final volume of 3.8% sodium citrate and protease inhibitors (final concentrations: 5 mmol/L EDTA, 6 mmol/L Na-ethylmaleimide, and 1 mmol/L leupeptin), and platelet-poor plasma was obtained as described previously. Factor VIII clotting activity (VIII:C), vWF antigen (vWFAg), ristocetin cofactor activity (vWFRCo), and vWF-cleaving protease activity were measured as described previously. Multimeric composition of plasma vWF was estimated by SDS-1% agarose gel electrophoresis. The relative percentage of the LMW multimers (∼5mers) was determined by densitometric analysis of the autoradiographs ( Omni Media Scanner XRS, Bio Image program, Millipore Co). vWF subunit and proteolytic degradation fragments were analyzed by SDS-5% PAGE under reducing conditions followed by immunoblotting with a polyclonal anti-reduced vWF antibody; the results were

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expressed as the percent that each band contributed to the total in each lane. Each plasma sample was tested in triplicate.

Hemodynamic Variables
Mean pulmonary arterial pressure (mPAP), cardiac index (CI), and total pulmonary vascular resistance (TPVR) were obtained during right-side heart catheterization.

Statistical Analysis
Results from PAH patients (mean±SD) were compared with controls by a U-Mann Whitney test. The evolution within the PAH group was analyzed by Wilcoxon test. Significance was considered for $P<0.05$.

Results
In all PAH patients, hemodynamic variables (mPAP 75.0±12.8 mm Hg, CI 1.9±0.2 L/min$^{-1}$m$^{-2}$, TVPR 39.1±5.1 IU/m$^{-2}$ at $D_0$) were significantly improved at $D_{30}$ (mPAP 66.0±11.9 mm Hg, CI 2.1±0.3 L/min$^{-1}$m$^{-2}$, TVPR 31.5±4.4 IU/m$^{-2}$) ($P<0.04$).

At $D_0$, PAH patients exhibited a 2-fold increase of VIII:C and vWFAg levels ($P<0.005$), contrasting with a normal vWFRCo ($P<0.005$). A loss of the HMW multimers of vWF was observed concomitantly with a 3-fold increase of the LMW multimers (Figure 1A). In addition to the predominant 270-kDa subunit, 5 proteolytic fragments of vWF were present in both controls and PAH patients (Figure 2B), the latter demonstrating a similar 2-fold increase of all proteolytic fragments ($P<0.005$) (Figure 2C). In contrast, vWF-cleaving protease activity was similar to controls (Figure 2D).

At $D_{30}$, vWF-cleaving protease activity and vWFRCo remained normal, whereas VIII:C and vWFAg decreased to almost normal levels ($P<0.02$) (Figure 1A). The proportion of LMW multimers of vWF decreased concomitantly with a partial restoration of the HMW multimers (Figure 2A), and all proteolytic fragments decreased proportionally (Figure 2B).

Discussion
This study was focused on the features of vWF proteolysis in PAH and its evolution under prostacyclin infusion. Before treatment, enhanced vWF proteolysis was clearly demonstrated by both a rise in the proportion of its LMW multimers and an increase of its proteolytic fragments. In all patients, this excessive proteolysis was characterized by a 2-fold increase of the 5 proteolytic fragments present in controls, which suggests that it may be related to an enhanced physiological mechanism. This is at variance with the results of Lopes and Maeda,$^7$ who detected only 4 proteolytic fragments and a more significant increase in the 176-kDa fragment, with, however, a variable proteolytic pattern between patients. The reason for this difference may be methodological, because we used native plasma instead of immunoabsorbed vWF and different anti-vWF antibodies. This increased proteolysis of vWF prompted us to analyze the role of its physiological specific cleaving protease, the activity of which was recently demonstrated to be decreased in thrombotic thrombocytopenic purpura,$^4,12$ a thrombotic microangiopathy characterized by the presence of ultralarge multimers of vWF. Surprisingly, in PAH patients, despite enhanced proteolysis of vWF, we failed to demonstrate an increased activity of vWF-cleaving protease.

Thus, the mechanisms for the excessive proteolysis of vWF in PAH remain questionable. In the current study, the absence of relevance of vWF-cleaving protease and the presence of physiological proteolytic fragments make unlikely the involvement of an enhanced release of enzymes. Therefore, an increased susceptibility of vWF to proteases may be suggested. The latter is known to be induced by structural modifications of vWF as hyposialylation$^{13}$ and/or conformational changes induced by high shear stress.$^{14}$ A very recent study$^{15}$ showed that vWF was hyposialylated in PAH, but the evolution under prostacyclin was not analyzed. In the present...
In the present study, prostacyclin therapy failed to normalize completely all the abnormalities of vWF, but evaluation was performed only after a 1-month prostacyclin treatment.

Of course, all the mechanisms reported to explain vWF dysfunction in PAH are not exclusive but instead are probably associated to support the cellular, biochemical, and hemodynamic abnormalities observed in PAH. Among these mechanisms, the present study underlines the role of an excessive proteolysis of vWF and provides new clues to explain its pathophysiology. The improvement of vWF under prostacyclin emphasizes both the cellular and hemodynamic effects of this drug. However, the relationships between the pulmonary vascular disease involving high shear rates and the endothelial perturbation as an initiating and/or exacerbating factor need further investigation to elucidate the complex pathogenesis of PAH.

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**References**


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**Figure 2.** Typical multimeric distribution (A) and proteolytic pattern (B) of plasma vWF in control subject (C) and PAH patient at D_0_ and D_30_. A, Top, Multimeric composition of plasma vWF was estimated by SDS-1% agarose gel electrophoresis with diluted plasma in 10 mmol/L Tris-HCl buffer (pH 8), containing 2% SDS and 1 mmol/L EDTA (final vWFAg: 5 IU/dL). After heating for 15 minutes at 60°C, ~1 mIU of vWF was loaded in each well. Bottom, Scanning of above gel shows LMW multimers (≤5mers) and HMW multimers (>10mers). B, Top, Proteolytic degradation fragments were analyzed by SDS-5% PAGE using plasma samples diluted 5 times with buffer so that the final mixture contained 0.125 mol/L Tris-HCl (pH 6.8), 2% SDS, 5 mmol/L EDTA, 10% β-mercaptoethanol, and 8 mol/L urea. After heating for 15 minutes at 75°C, ~17 mIU was loaded in each lane. After vWF staining with [125I]-polyclonal antibodies, autoradiographs were performed at various times of exposure. Molecular weights of bands are indicated on right. Bottom, Patterns were quantified by scanning, showing 5 proteolytic fragments (smallest: 137 kDa) in addition to predominant subunit (270 kDa).
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