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Proteins C and S in Two Different Vaso-Occlusive Disorders

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In addition to congenital deficiencies, acquired defects of proteins C and S have been reported in clinical conditions associated with increased tendency to thrombosis. The present study was aimed at investigating plasma levels of these proteins in two vaso-occlusive disorders characterized by different mechanisms of endothelial damage. In the first one sickle cell disease, endothelial injury is mainly a consequence of anoxia [1] whereas in the second one, pulmonary hypertension, lesions are due to increased shear rate at the level of pulmonary microcirculation [2]. In both disorders, the pathogenesis of vaso-occlusion also involves alterations of blood coagulation and platelet function as well as defective fibrinolysis [3, 4]. Therefore, decreased plasma levels of protein C and protein S could represent an additional risk of thrombotic phenomena in these patients [5].

In our study, the first group of patients included 10 individuals (6 females and 4 males aged 15-32 years) with clinically homozygous sickle cell disease, who had not suffered from any form of crisis for at least 4 months prior to testing. The second group was represented by 9 patients (7 females and 2 males aged 8-30 years) with pulmonary hypertension associated with schistosomiasis (4 cases) or congenital heart disease (5 cases). Mean pulmonary arterial pressure in this group ranged from 39 to 87 mm Hg. Patients entered the study consecutively. None of them had had episodes of deep vein thrombosis or pulmonary embolism and none had used oral anticoagulants prior to the study. General laboratory tests showed no evidence for hepatic failure and no significant decrease of vitamin K-dependent clotting factors. Plasma levels of factor V were not significantly altered (80 ± 29 and $86 \pm 26\%$ activity in pulmonary hypertension and sickle cell disease, respectively). No women were using oral contraceptive agents. The control group consisted of 5 females and 3 males aged 26 to 41 years.

In both groups of patients peripheral venous blood was collected in steady-state condition. Samples were drawn into 0.1 vol of 3.8% sodium citrate. Plasma was separated by centrifugation at 2,000 g for 10 min and stored frozen at -70°C until assayed. Quantitation of protein C and free protein S antigen was performed by Laurell immunoelectrophoresis [6] using monospecific antisera (Diagnostica Stago, Asnières, France). Because 60% of plasmatic protein S is complexed with C4b-binding protein with no biological activity [7], free protein S instead of total protein S values are presented. Data are expressed as percentage of activity relative to pooled normal plasma. Levels of protein C and protein S in pulmonary hypertension, sickle cell disease and healthy controls were compared using oneway analysis of variance and Bonferroni t

test. The relationship between protein C and protein S levels in each group of patients was tested using the coefficient of correlation. A significance level of 0.05 was assumed. Results are reported as mean $\pm$ SD (table 1).

Total protein S levels were within the normal range in both groups (data not shown). In comparison with controls, patients with both vaso-occlusive disorders had a decrease in plasma levels of protein C as well as free protein S antigen, with no significant differences between groups (table 1). No correlation was observed between protein C and free protein S concentrations within groups ($r = 0.11$ for pulmonary hypertension and $r = \pm 0.42$ for sickle cell disease).

Since both protein C and protein S are mainly synthesized in the liver (vitamin K-dependent factors), we speculate that decreased plasma levels of these proteins in sickle cell disease might be explained in part by altered hepatic function as previously reported [8], even in the absence of overt hepatic failure. In contrast, in the absence of right ventricular failure, hepatic dysfunction is unlikely to occur in pulmonary hypertension. This could be a pos-

sible explanation for the trend toward lower levels of both proteins in sickle cell disease in comparison with pulmonary hypertension. Anyway, we have reasons to believe that impaired liver function as a solitary factor could not explain the decreased plasma levels of proteins C and S in our patients: (1) clinical and laboratory data compatible with overt liver disease were not present; (2) plasma levels of factor V were not significantly altered; (3) total protein S (which is decreased in liver disease) was within the normal range; (4) extensive endothelial damage, an important determinant of local consumption of coagulation factors has been described in both sickle cell disease and pulmonary hypertension [1, 2].

Plasma levels of protein C and protein S observed in our patients are higher than those reported in inherited deficiencies of these factors [9,10]. This is a possible explanation for the absence of recurring venous thrombotic episodes and thrombophlebitis in these patients. It should be remembered that in both pulmonary hypertension and sickle cell disease, microvascular obstruction possibly involving clotting factors seems to be more important from the pathophysiological point of view than thrombosis of great vessels such as veins of the legs [4,11].

In spite of different pathophysiological mechanisms, pulmonary hypertension and sickle cell disease share common features regarding microvascular lesions. Extensive endothelial cell damage is present in both conditions. As a result, chronic endogenous activation and consumption of proteins C and S with accelerated clearance from plasma, are possible explanations for decreased concentrations [12]. We have similarly observed decreased plasma levels

Table 1. Protein C and free protein S levels in vaso-occlusive disorders

of antithrombin III in patients with pulmonary hypertension [13]. By impairing fibrinolysis, decreased protein C levels may further contribute to microvascular accumulation of fibrin, a constant finding in patients with sickle cell disease [8]. Protein S is also synthesized by endothelial cells. Its release probably involves complex interactions between endothelial cell membrane, thrombomodulin, thrombin and protein C [14]. Thus, impairment of endothelial processing of protein S in the presence of extensive microvascular damage could also affect plasma levels.

Interestingly, in spite of the fact that free protein S is the cofactor for protein C, in the present study we observed a complete lack of correlation between plasma levels of these proteins in both groups of patients. Although we do not have a definite explanation for this finding, it likely reflects the complex nature of distinct mechanisms modulating the synthesis and consumption of these proteins in pathological conditions.

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