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Fibrinogen metabolism in the lungs

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Abstract

The aim of this study was to evaluate the role of the pulmonary vessel endothelium in the metabolism of fibrinogen (FBG), by measuring the FBG, D-dimer, and fibrin(ogen) degradation product levels in the blood from pulmonary and radial arteries from 99 patients undergoing aortocoronary bypass. For comparison, protein C, protein S, and factor VII, were also measured.

The results showed, with respect to the pulmonary arterial blood levels, significantly lower FBG levels (3.72 ± 0.83 vs 3.66 ± 0.81 g/L; $P < .001$) and higher fibrin(ogen) degradation product levels (7.36 ± 1.53 vs 8.15 ± 1.59 mg/L; $P < .000\,01$) in the radial arterial blood. No difference was found for D-dimer, protein C, protein S, and factor VII.

The study demonstrated that the pulmonary capillary endothelium contributes to the FBG catabolism for about a 0.02 fractional rate and support the view of an endothelial FBG catabolic pathway as the main catabolic pathway, owing to the fact that the pulmonary endothelial surface is about a 0.1 fraction of the peripheral vessel endothelial surface.

1. Introduction

Fibrinogen (FBG) is a large relatively abundant plasma protein exerting several functions, mainly in platelet aggregation, in fibrin clot formation, as an acute phase reactant in inflammation and infections; with a 340 kDa molecular weight, it also affects the blood rheology [1,2]. Therefore, the regulation of plasma FBG levels has been the subject of extensive investigations. However, despite the numerous studies dealing with the metabolism and distribution of iodine-labelled FBG in human beings, the site and the manner of normal catabolism of FBG is still uncertain [1,2]. Several pathways have been hypothesized in normal human FBG catabolism, but none of them has been recognized as a significant route.

Some studies suggest that about 1% to 6% of the normal FBG turnover is via conversion to fibrin, as a part of body vascular hemostasis in response to minute trauma or due to an escape from usual enzymatic inhibitors [1,3]. However, FBG turnover evaluated with and without heparin anticoagulation did not demonstrate any change in healthy subjects, and the intravascular fibrin formation was not found to be a quantitatively significant route in FBG metabolism in physiological conditions in human beings [4,5].

Another proposed pathway of FBG metabolism is the fibrinogenolytic catabolic pathway, but no change was observed in the half-life of labeled FBG during tranexamic acid inhibition of the fibrinolytic system [5], and the role and the degree of activity in vivo and in normal conditions of the nonplasmin fibrinolytic enzymes, such as the proteolytic enzymes released from leukocytes or monocytes, have not been ascertained [1].

Some studies based on a mathematical compartmental model support the view that FBG catabolism may occur in the vascular endothelium during the passage of FBG in the extravascular space, although there is no direct data for an endothelial pathway [5].

Therefore, neither the mechanism nor the site of FBG catabolism are known yet [1,2]. On the other hand, pulmonary circulation, in addition to bringing blood in

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contact with alveolar gas for gas exchange, has been recognized as having complex metabolic functions, including the activation or inactivation of some peptides.

The aim of the present study was, therefore, to evaluate the role of the pulmonary vessel endothelium in the metabolism of circulating FBG by measuring the levels of FBG and of its derivatives D-dimer (D-D) and fibrin(ogen) degradation products (FDP) in blood from pulmonary and radial arteries in human beings. For internal control, factor VII (F VII), as marker for possible tissue factor release in response to lung stress, and protein C (Pr C) and protein S (Pr S), as markers for thrombin generation, were measured [2].

2. Materials and methods

The study was carried out, after obtaining informed consent, in patients undergoing aortocoronary bypass for atherosclerotic coronary disease. Patients with hematologic and coagulative disorders, endocrine and metabolic diseases, liver or renal impairment, or who were taking drugs affecting coagulation were excluded. All patients were operated on using the same anaesthetic and surgical techniques. After sternotomy and before heparin anticoagulation, hypothermia and extracorporeal circulation were performed, arterial blood specimens were drawn almost simultaneously into disposable sterile syringes. These were taken from the pulmonary and the radial arteries in 99 patients, 62.71 ± 8.41 years mean age, 81 men, and 18 women. The blood samples were transferred immediately after collection into citrated siliconized vacutainer glass tubes (Becton-Dickinson Vacutainer Systems, Belliver Ind Est, Plymouth, UK), exactly 4.5 mL of blood into 0.5 mL of 0.129-mol/L sodium citrate, for FBG, D-D, F VII, Pr C, and Pr S measurements, and in Dade FDP clotting tubes (Dade International Inc, Newark, DE), containing *Bothrops atrox* venom, to promote fibrin clot formation, and soybean trypsin inhibitor, to inhibit in vitro fibrinolysis, for FDP determinations. Fibrinogen was chronometrically measured by addition of excess human thrombin to dilute plasma (STA fibrinogen, Diagnostica Stago, Asnières, France); the intra- and interassay CVs were 1.5 and 2.1%, respectively. The Clauss method was used according to recent guidelines on FBG assays, because false estimate may be obtained with immunological methods where degraded forms of FBG are present [6]. D-Dimer and free Pr S were measured by monoclonal antibody-coated latex particle agglutination method (STA Liatest D-DI and STA Liatest free protein S, Diagnostica Stago); D-D assay was expressed in milligrams per liter of FBG equivalent units, and the intra- and interassay coefficients of variation (CVs) were 3.0% and 3.8%, respectively; Pr S assay was expressed as percent of a normal standard and the intra- and interassay CVs were 3.5% and 5.5%, respectively. Fibrin(ogen) degradation products were measured by a particle-enhanced turbidimetric inhibition immunoassay (Dade International Inc), whose intra- and interassay CVs were 2.5% and 3.5%, respectively. Protein C and factor VII were measured as percent of normal standard activity, by using as reagents, respectively, Pr C- and F VII-deficient plasmas (STA Pr C and STA F VII, Diagnostica Stago); intra- and interassay CVs were, respectively, 4.5% and 6.5% for both Pr C and F VII assays.

Pulmonary and radial artery samples from each patient were measured in the same assay run and each value was recorded as the mean of a duplicate assay. To avoid spurious results due to differences in the blood composition from the pulmonary and radial arteries, in each patient, the hematocrit (Hct) was measured in blood separately collected. Moreover, in 41 randomly selected subjects, additional blood was collected from the 2 arterial compartments to estimate by the routine method the creatinine (Cr) that is present in the plasma in a relatively constant concentration. Data variability were expressed as SD; Student t test for dependent samples and Pearson's coefficient of linear correlation were used for statistical analysis (Statistica software, StatSoft, Inc, Tulsa, OK, 1993); power and sample size calculation for paired studies was performed [7].

3. Results

Results, shown in Table 1, showed significantly lower FBG levels ($P < .001$) and higher FDP levels ($P < .000\,01$) in blood from the radial artery with respect to the pulmonary artery; with 0.05 type 1 error probability, the sample size calculation showed n = 67, the number of pairs of values needed to detect a true difference in the means, and the power calculation showed 0.93 probability to correctly reject the null hypothesis (n = 99 as the sample size). Moreover, the FBG concentrations in the pulmonary artery plasma exceeded that of the radial artery plasma in more than 80% of paired samples. No significant difference was found for D-D, F VII, Pr C, and Pr S in the 2 vascular compartments.

As reported [8], the Hct was slightly higher in the pulmonary blood with respect to the radial artery blood (0.362 ± 0.042 vs 0.361 ± 0.043, $P < .01$). With regard to Cr values, no significant difference was observed between blood from the pulmonary and radial arteries (106.81 ± 35.41 and 106.60 ± 33.61 μmol/L, respectively).

Table 1
The mean values ± SD of FBG and of the other investigated parameters in the blood from human pulmonary and radial arteries

	nr	Pulmonary artery	Radial artery	P
FBG	2.0-4.0 g/L	3.72 ± 0.83	3.66 ± 0.81	.000 78*
D-D	0.22-0.50 mg/L	0.46 ± 0.23	0.45 ± 0.21	.21
FDP	1.0-12.0 mg/L	7.36 ± 1.53	8.15 ± 1.59	.000 001**
Pr C	0.70-1.30	0.98 ± 0.18	0.99 ± 0.18	.68
Pr S	0.70-1.30	0.93 ± 0.17	0.93 ± 0.15	.81
F VII	0.70-1.30	0.79 ± 0.25	0.79 ± 0.26	.79

nr indicates normal ranges.
* $P < .001$.
** $P < .000\,01$.

The percentage difference between pulmonary and radial artery blood in FBG values was −0.0176 ± 0.037 (0.95 confidence limits: −0.011/−0.025; $P < .0001$); the percentage difference in FDP values was 0.117 ± 0.135 (0.95 confidence limits: 0.083-0.152; $P < .0001$).

A significant positive linear correlation was found both in pulmonary and in radial artery blood between FBG and D-D values ($r = 0.40$, $P < .0001$ and r = 0.38, $P < .001$, respectively), whereas no significant correlation was found between FBG and FDP and between FDP and D-D values in both the vascular compartments.

4. Discussion

The study showed significantly lower FBG levels and higher FDP levels in the radial artery blood with respect to the pulmonary artery blood, whereas no difference was found in D-D, F VII, Pr C, and Pr S between the 2 arterial compartments. Because the Cr values were unmodified after the pulmonary pass, in the same way, the very slight respiratory change of Hct did not significantly affect the results. These results show that the pulmonary vessel endothelium exerts a significant role in the metabolism of FBG, showing a FBG fractional catabolic rate of about 0.02.

The vascular endothelial cell layer forms a diffusion barrier between blood and tissues, particularly in the pulmonary circulation, where there are no target tissues to justify an appreciable hormone and protein diffusion, because only the basal membrane and the pulmonary epithelial cell layer are interposed [9]. For this reason, the difference observed in concentrations between pulmonary and radial arteries seems to be an expression of the catabolism of FBG, because no such difference was found for D-D, F VII, Pr C, and Pr S. Moreover, a mere FBG uptake or diffusion without catabolism would not result in a concentration difference in the steady state. This is confirmed, on the other hand, by the significantly increased levels of FDP in the radial artery blood with respect to the pulmonary artery blood, with about a 0.12% difference in concentrations.

Each molecule of human FBG contains 3 domains, which, when cleaved, give rise as terminal digestion products to 1 fragment E, corresponding to the central region of the FBG molecule, and 2 fragment D's. Moreover, 2 intermediate products were identified: in sequence, the fragment X (D-E-D), after release of the fibrinopeptides A and B, and the fragment Y (D-E), after release of one fragment D. This fragmentation of the FBG molecule explains the higher percentage difference found in FDP values with respect to the percentage difference in FBG values, but it also unequivocally confirms that the difference observed in FBG concentrations are due to FBG catabolism in the lungs. On the other hand, endothelial cells contain enzymes metabolizing various circulating peptides [10]. Even though their concentration in the pulmonary vessel endothelium does not seem to be higher than in other tissue vessels, here, several metabolic activities occur because of the large extent of the pulmonary vascular bed [11-13].

Because the FDP assay cannot distinguish between proteolytic action on FBG or on soluble fibrin, direct evidence whether FBG is broken down directly or after fibrin formation does not seem to be provided. However, the unmodified concentration of D-D after the pulmonary pass indicates that no stabilized fibrin is generated in the pulmonary circulation, but it also indicates, in the steady state, that no fibrin is formed during the pulmonary pass, owing to the very rapid cross-linking of soluble polymers to form stabilized fibrin, of which D-D is a derivative. Furthermore, F VII, Pr C, and Pr S values were unchanged after the pulmonary pass.

Although the study was performed in cardiac patients undergoing bypass surgery, it has been reported that FBG catabolism takes place at a constant fractional rate, independent of the synthetic rate [5,14]. This seems to agree with the existence of a fixed step in FBG removal, as a constant uptake and catabolic rate in the endothelial cells in the context of the FBG transcapillary efflux, reported to involve about 0.60 of the FBG plasma pool per day [5], without a down-regulation mechanism. In this regard, although we found a significant positive correlation between FBG and D-D and unmodified D-D levels in the 2 vascular compartments, no correlation was found between FBG and FDP, and between FDP and D-D. This seems to be in favor of a catabolic mechanism of FBG in the pulmonary circulation, independent of FBG levels and of fibrin formation.

Another remark is that the pulmonary endothelial surface is evaluated as about 70 m^2 [9], whereas the peripheral vessel endothelial surface as 700 m^2 [15]. Therefore, the pulmonary fractional catabolic rate near to 0.02 agrees well with the reported about 0.24 total fractional catabolic rate of the FBG plasma pool per day, proposed to take place during the passage of FBG in the extravascular space [5]. In turn, the products of FBG degradation are actively cleared by the liver and the kidneys [16].

In conclusion, although FBG catabolism has been reported to proceed by several pathways, our study demonstrates that the lungs, with about 0.1 of the total endothelial surface, contribute to FBG catabolism for about a 0.1 fraction of the reported total catabolic rate of FBG. Being 3 to 5 days the FBG half-life [2], it supports the view of an endothelial FBG catabolic pathway as the main catabolic pathway.

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