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Coagulation and Platelet Activation after Retinal Vein Occlusions

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Abstract. The role played by coagulation and platelet activation in the pathogenesis of retinal vein occlusions (RVO) has been evaluated by measuring beta-thromboglobulin (B-TG), circulating platelet aggregates (CPA), thromboxane B_2 (TxB_2) and fibrinopeptide A (FPA) in 25 patients less than 40 years old, investigated after the acute phase of RVO. FPA and B-TG were significantly higher than in healthy subjects; CPA and TxB_2 were not different. These abnormalities, found in patients free from apparent generalized vascular disease, suggest that a thrombophilic state characterized by coagulation and platelet activation is present in a high proportion of young patients with RVO.

Introduction

Anatomic and hemodynamic changes in the retinal circulation and a defective control of intraocular pressure are thought to play an important role in the pathogenesis of retinal vein occlusions (RVO) [10]. There is, however, some evidence suggesting that generalized hemostatic alterations are also involved [1, 4, 8, 9]. The tests employed in these earlier studies were not very specific, sensitive or reproducible, thus making it difficult to interpret the results in the light of modern knowledge about the hemostatic processes.

We have reassessed the problem using more specific and sensitive techniques to investigate 25 patients less than 40 years old. We chose to measure beta-thromboglobulin (B-TG), a platelet-specific alpha-granule constituent secreted into plasma during the release reaction [6]; the presence of circulating platelet aggregates (CPA), considered to reflect in vivo platelet aggregation [11]; serum levels of thromboxane B_2 (TxB_2), the stable metabolite of the proaggregatory prostaglandin derivative thromboxane A_2 [5]; and plasma fibrinopeptide A (FPA), a fragment specifically cleaved off from fibrinogen by thrombin and considered to be the most sensitive indicator of increased activation of blood coagulation [7].

Material and Methods

We have investigated 25 patients (15 men and 10 women) less than 40 years old (mean age: 32; range: 14–39). The control group consisted of 30 healthy subjects matched for age, sex and smoking habits with the patient group. The diagnosis of RVO was confirmed by fluorescein angiography. 15 patients had occlusion of the central retinal vein, 10 of one of the branches; 11 patients had ischemia or new vessel formation, 14 retinal edema only. Hemostasis tests were carried out between 2 and 12 months after diagnosis in order to eliminate nonspecific alterations linked to the acute phase of the disease. Patients and controls were ostensibly healthy as judged by clinical and laboratory examinations carried out in the outpatient clinic of the Hemophilia and Thrombosis Center. In particular, none of them had signs of diabetes, hypertension, hyperlipidemia or other diseases related to atherosclerosis. None of the controls or patients had taken oral contraceptives or any drugs known to affect platelet function for at least 7 days prior to the study.

Blood Collection. Blood was taken from patients and controls after their informed consent had been obtained. Blood samples were drawn throughout the study by two experienced technicians, who were fully cognizant of the critical importance of good venipuncture for reliable assay results. Difficult blood sampling led to discarding the samples.

Fibrinopeptide A. Venous blood (4.5 ml) was collected into a syringe containing 500 U of heparin and 500 U of aprotinin (Trasylol®) in 0.5 ml physiological saline. The samples were transferred into plastic tubes and immediately centrifuged for 20 min at 5,000 *g* at 4 °C and platelet-poor plasma was stored at −30 °C. FPA was determined by the radioimmunoassay method of *Nossel* et al. [7] as modified by *Kockum* [3]. Standard FPA, anti-FPA antiserum and desaminotyrosyl-FPA were purchased from IMCO, Stockholm, Sweden. Desaminotyrosyl-FPA was iodinated by the chloramine-T method of *Hofmann and Straub* [2]. FPA values were expressed as nanograms per milliliter. The interassay and intraassay coefficients of variations were 11 and 10%.

Plasma B-TG [6]. 2.7 ml of venous blood were drawn into syringes kept at 4 °C in the refrigerator. The blood was immediately transferred into cooled plastic tubes and mixed with 0.3 ml of an antiplatelet anticoagulant freshly prepared at the time of use by adding 10 μg of prostaglandin E_1 and 54 mg of theophylline to 10 ml of 10% EDTA. The tubes were placed in crushed ice and then centrifuged within 30 min for 20 min at 4 °C and 2,000 rpm. The middle layer of platelet-poor plasma was removed, stored at −30 °C and assayed by radioimmunoassay using the reagents and following the instructions of a commercial kit (Radiochemical Centre, Amersham, UK). B-TG values were expressed as nanograms per milliliter. The interassay and intraassay coefficients of variations were 14 and 9%.

Circulating Platelet Aggregates. CPA were evaluated by minor modification of the method of *Wu and Hoak* [11]. We used two separate plastic tubes, one containing 2.5 ml of EDTA and the other of an EDTA/formalin solution. 2.5 ml of blood were directly drawn into each tube, after venipuncture with a 21-gauge butterfly needle and discarding of the first milliliter of blood. After incubation at 22 °C for 15 min, the samples were centrifuged at 220 *g* for 8 min to prepare platelet-rich plasma. Platelets were counted with a Thrombocounter C and Thrombofuge (Coulter Electronics, Herpenden, UK). Platelet aggregate ratios were calculated by dividing the platelet count in EDTA/formalin by the platelet count in EDTA. Blood samples with a large number of aggregates would have a ratio of less than 1 and samples with no aggregates would have a ratio of 1. The interassay and intraassay coefficients of variations were 16 and 15%.

Serum TxB_2 [5]. Venous blood was added to 0.1 vol of EDTA (5 mmol/l). After platelet counting, addition of thrombin (10 U/ml) and incubation at 37 °C for 30 min, the clotted blood was centrifuged at 5,000 *g* for 30 min and TxB_2 in the supernatant serum was measured by radioimmunoassay, using a specific antiserum kindly provided by Dr. *J.B. Smith.* The TxB_2 serum values were expressed as pmol/10^8 platelets. The interassay and intraassay coefficients of variation were 15 and 12%.

Statistical Analysis. The chi square test was used for exact testing of the normality of the distribution. Since the data were not normally distributed, values are given as median and ranges, and the significance of the differences between groups was assessed with a nonparametric test (Mann-Whitney U test).

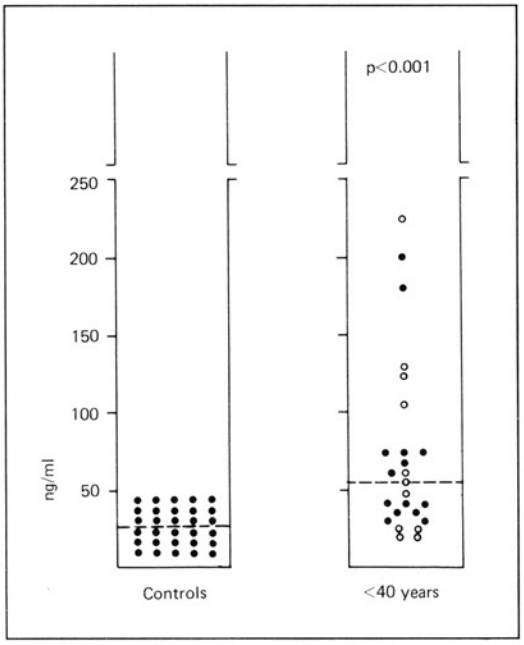

Fig. 1. Plasma B-TG in healthy controls and patients after retinal vein thrombosis. The broken line indicates the median values. ○ = Patients with retinal ischemia; ● = patients with retinal edema.

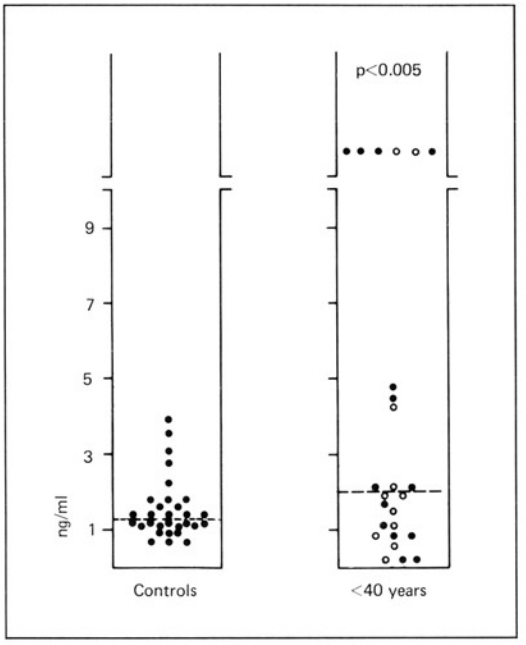

Fig. 2. Plasma FPA in healthy controls and patients after retinal vein thrombosis. The broken line indicates the median values. ○ = Patients with retinal ischemia; ● = patients with retinal edema.

Results

In patients with RVO, the values for CPA and TxB_2 were not different from those of controls (median CPA ratio 0.82, range 0.56–1.04 in patients vs. 0.92, range 0.68–1.06, in controls; median TxB_2 [in pmol] 340, range 145–806, vs. 380, range 156–971). Even though there was some overlap between the B-TG and FPA values of patients and controls (fig. 1, 2), the differences between the medians were highly significant ($p < 0.001$ and $p < 0.005$). In 9 patients the FPA values were beyond the upper range for controls, and the B-TG values were beyond the upper range for controls in 12 patients; abnormal values of both parameters were found in 7 patients (fig. 1, 2). When patients were subdivided according to the site of occlusion (central retinal vein vs. branch) and the angiographic pattern (retinal ischemia vs. edema), the values of FPA and B-TG were not significantly different (fig. 1, 2).

Discussion

The present study provides evidence for elevated plasma FPA and B-TG levels (or both) in a number of young patients with RVO. They were present not only in patients with ischemic complications and in those with occlusion of the central retinal vein, but also in less severe cases characterized by

branch occlusion or retinal edema. The abnormalities of FPA and B-TG are thought to be indicative of increased circulating thrombin [7] and platelet activation [6], with release of intracellular platelet constituents. Since the patients were young and apparently free from underlying disease, it is unlikely that the abnormalities observed are secondary to the concomitant presence of atherosclerosis and related vascular disease (diabetes, hyperlipidemia, hypertension). They are also unlikely to reflect the local thrombotic process, because they were observed well after the acute phase of the disease. In addition, the thrombi which form in the retinal veins are usually too small to justify the systemic signs of alteration of hemostasis. Notwithstanding the limits inherent in its nonprospective design, this study therefore suggests that a primary thrombophilic state, characterized by increased circulating thrombin and platelet activation, is present in a high proportion of young patients with RVO. Perhaps anticoagulants and/or agents that affect platelet behavior should be submitted to more accurate clinical trials in these conditions.

Addendum to the Proofs

Peduzzi et al., Thromb. Res. *24:* 105–118 (1981) have found a significant increase of factor VIII, decrease in antithrombin III and prolongation of euglobulin lysis time in patients with RVO.

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