Lack of Elevation of $\beta$-Thromboglobulin and Platelet Factor 4 in Plasma during Exercise in Patients with Chronic Peripheral Arterial Occlusive Disease

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Summary. We investigated the behavior of $\beta$-thromboglobulin ($\beta$-TG) and platelet factor 4 (PF 4) during exercise – upright bicycle ergometry – in 30 patients (median age, 62.4 years) with arteriographically proven peripheral arterial occlusive disease (PAOD) in a chronic stable phase. In 15 patients the exercise study was done twice; the second time was concurrent with administration of acetylsalicylic acid (ASA) in a dosage of 1.0 g/day, while the first time was without ASA therapy. There were no significant differences in either the group of patients with or that without ASA with regard to the platelet-specific proteins at rest, immediately after, and 30 min after exercise. Blood collected simultaneously ($n=6$) from an arm vein and from a femoral artery and femoral vein also revealed no significant differences. Our findings support the conclusion that exercise-induced peripheral ischemia with severe symptoms of claudication does not produce platelet alpha-granule release.

Key words: $\beta$-Thromboglobulin – Platelet factor 4 – Peripheral arterial occlusive disease – Exercise

During the platelet-release reaction, $\beta$-TG and PF 4 are released from the platelets into plasma and assays of these proteins can be used to monitor in vivo platelet activation. Increased levels of $\beta$-TG and/or PF 4 in plasma have been reported in a number of clinical conditions [12]. Some authors reported elevation of these proteins during myocardial ischemia induced by exercise [6, 14]. In more recent studies, however, other investigators were unable to confirm such an elevation in patients with coronary artery disease during exercise-induced myocardial ischemia [11, 15].

We found only one study in the literature [2] concerning platelet activation during exercise – as indicated by increased plasma levels of $\beta$-TG and PF 4 – in patients with peripheral arterial occlusive disease (PAOD). In view of the inconclusive results of studies concerning platelet $\alpha$-granule release during exercise in patients with coronary artery disease, we made a study of the behavior of these proteins in plasma during exercise in patients with PAOD.

Since specimen collection is of primary importance in ensuring that in vitro release of PF 4 and $\beta$-TG is minimal, and since divergent results have been attributed to methodologic differences, we also studied the influence of different methods of specimen collection on plasma levels of $\beta$-TG and PF 4.

Patients and Methods

We studied 30 patients (20 male, 10 female; median age, 62.4 years; range, 45–70 years) who had arteriographically proven PAOD. Localization of the atherosclerotic disease was primarily in the femoral artery in 10 patients and in the iliac artery in 5, while 15 patients had severe changes in the iliac and femoropopliteal region. All the patients were in a chronic, stable phase of their disease, and they were symptomatic for at least 6 months with severe reduction of the pain-free walking distance. Hypertension was present in 16 patients, and 8 had manifest diabetes. Seven patients had symptoms of coronary artery disease, 4 of these had a history of prior myocardial infarction, while the other 3 were now stable with regard to angina.
Four patients were taking beta-blocking agents, and 4 were taking nifedipine. Serum creatinine did not exceed 1.3 mg/dl in any of the patients.

In 15 patients the exercise study was done twice within an interval of 3 weeks; the second time concurrently with the administration of acetylsalicylic acid (ASA) in a dosage of 1.0 g/day and the first time without ASA. In 15 patients the study was done only once. Of these 5 were taking no platelet-modifying drugs, while 10 were taking ASA 1.0 g/day. Upright bicycle ergometry was performed according to the standard protocol of the Austrian Society of Cardiology work load started with 25 W and was raised by increments of 25 W every 2 min until the patient reached the limit of his work tolerance or the test was interrupted prior to this limit because of lower-leg pain, angina, or ST depression. During the test ECG was recorded continuously and blood pressure readings were performed every 2 min, as well as 1, 3 and 5 min after finishing work.

In 28 patients the exercise testing had to be stopped because of severe symptoms of claudication. Chest pain was the predominant reason to interrupt the exercise in one patient, and in one patient the exercise was discontinued because of severe ST depression. The mean working capacity of the 30 patients (45 investigations) was 74% ± 20% compared to normals.

In 8 patients blood samples were taken simultaneously from an arm vein and from the femoral artery and femoral vein of the more affected leg at the groin 15 min before, immediately after, and 30 min after exercise, respectively. The blood samples were drawn with a 20-gauge needle into 5 ml-syringes, and 2.5 ml of blood was transferred immediately into precooled tubes provided with the Amersham kit (see below).

Our control group consisted of 58 healthy individuals (25 males, 33 females; median age, 32.5 years; range, 18–77 years) without clinical signs of atherosclerosis.

**Blood Sampling**

Venipuncture was performed by one physician for the entire period of the study. Blood specimens for plasma β-TG and PF 4 determinations were collected by separate venipunctures immediately before exercise, within 1 min after stopping exercise, and again 30 min after exercise. At each sampling time, duplicate blood samples were collected – after discarding the first 3 ml of blood – with an 18-gauge needle directly into Thrombotect tubes (Abbott Laboratories), that were precooled in a crushed-ice water bath. The samples were centrifuged within 1 h of collection for 45 min at 4°C and 3000 g. From the middle portion of the supernatant plasma, 0.5 ml was pipetted and all specimens were then frozen at −30°C before assay for β-TG (Amersham RIA test kit) and PF 4 (RIA kit, Abbott Laboratories). The mean of the duplicate plasma values was taken for further calculations.

In our laboratory the intraassay coefficient of variation (CV) for β-TG values > 30 ng/ml was 3.0% and for β-TG values < 30 ng/ml was 3.1%. The interassay CVs were 4.2% and 3.7%, respectively. When measuring PF 4, we found an intraassay CV of 10.2% for PF 4 < 10 ng/ml, and 4.2% for higher PF 4 values. The interassay CV for PF 4 was 21% and 5.5%, respectively.

We compared three different methods of specimen collection with regard to plasma levels of β-TG and PF 4. The blood was collected with an 18-gauge needle in Thrombotect tubes either directly without vacuum by removing the rubber stopper, or by means of a vacutainer system. Each of these methods was compared with another technique: using a 5-ml plastic syringe, 2.5 ml of blood was collected and immediately transferred into tubes provided with the Amersham kit. The precooled tubes were then also placed in a crushed-ice water bath. The further processing was done as described above. These studies for comparison of different methods of specimen collection were done by making repeated venipunctures – on different days – in 40 PAOD patients, including the 30 patients who were also in the exercise test.

**Analysis of Data**

The Wilcoxon signed-rank test was used for comparison of the values before and after exercise, and for comparison of the values obtained by different methods of specimen collection. The Wilcoxon rank-sum test was applied for comparison of the baseline values before the exercise test of the PAOD patients with the controls.

**Results**

The normal value for β-TG was 24.8 ng/ml (median; range, 12.0–44.2) and for PF 4 2.71 ng/ml (0.32–9.0); the blood was collected directly into Thrombotect tubes without vacuum.

The median levels of plasma β-TG were significantly (P<0.001) elevated in our patients with PAOD compared to healthy controls (32.4 vs 24.8 ng/ml) while the plasma levels of PF 4 were not significantly different (2.9 vs 2.71 ng/ml). The