ANNALES
UNIVERSITATIS MARIAE CURIE-SKŁODOWSKA
LUBLIN-POLONIA
VOL. LXI, N1, 46 SECTIO D 2006

Chair and Department of Vascular Surgery and Angiology, Medical University of Lublin

JACEK WROŃSKI, JAN JAKUB KESIK, STANISŁAW PRZYWARA, MARCIN FELDO

The plasma level of Platelet Factor 4 in the patients with Buerger's disease

Platelet factor 4 (PF4) is protein with high biological activity, whose action is not completely known. It participates in the thrombotic process on few levels, having both prothrombotic and antithrombotic roles.

There is evidence that PF4 exerts a prothrombotic influence, by binding to heparin-like molecules and thereby preventing the activation of antithrombin (AT) (6). PF4 inhibits the activation of FXII, a plasma proenzyme which is activated by negatively charged agents such as exposed collagen on the damaged vascular wall (3). PF4 has been shown to inhibit the thrombin activatable fibrinolysis inhibitor (TAFI), what would promote local development of thrombus on the surface of endothelial cells (9). PF4 also plays an antithrombotic role via activation of activated protein C complex (aPC). PF4 binds to and stimulates the cofactor activity of thrombomodulin, an endothelialcell surface glycoprotein that binds thrombin and promotes the activation of protein C. PF4 stimulates aPC formation 25fold.

PF4 is one of the first antiangiogenic agents discovered, inhibiting endothelial cell multiplication *in vitro*. PF4 inhibits fibroblast growth factor-2 (FGF-2). PF4 also inhibits the proliferation and migration of endothelial cells induced by vascular endothelial cell growth factor (VEGF), the most potent angiogenic agonist. Both of the above mentioned angiogenesis inhibitory systems are heparin dependent and require high concentrations of PF4. The third antiangiogenic mechanism is different. This pathway involves a chemokine receptor CXCR3. It requires much lower PF4 concentration and it may play the physiological role in angiogenesis modulation (13). PF4 has also the pro-atherogenic activity. PF4 promotes atherosclerosis by: inhibiting LDL-mediated clearance of LDL, promoting the recruitment of peripheral blood monocytes to the lesion and facilitating their differentiation and estrification of ox-LDL in lesional macrophages. PF4 binds directly to ox-LDL, but not to native LDL, and thereby promotes the binding of ox-LDL to endothelial cells, smooth muscle cells, and macrophages, and causes a 10fold increase in the estrification of ox-LDL in the latter. PF4 and ox-LDL co-localize in atherosclerotic lesion, especially in macrophage-derived foam cells.

It has been known that activated platelets release mediators which promote smooth muscle cells proliferation (10).

PF4 as one of the chemokines participates also in the pathomechanism of inflammation, stimulating all its phases. PF4 induces highly purified human natural killer (NK) cells to produce interleukin (IL)-8 in the time-and dosage-dependent manner. This ability is retained even while PF4 is bound to heparin (7).

Many discussions about the primary mechanism of changes in Buerger disease have been conducted for years. The question has been raised whether vascular wall inflammation process induces thrombosis or whether the thrombosis induces perivascular inflammatory reaction. Only few authors investigated clotting disturbances and fibrinolysis in thromboangiitis obliterans (TAO).

The low number of reports on platelet function in contrast with the dynamic development of knowledge about the process of coagulation and fibrinolysis makes interesting to try to specify the PF4 role in pathogenesis of TAO.

MATERIAL AND METHODS

The blood samples of 30 TAO patients treated at the Department of Vascular Surgery and Angiology, Medical University of Lublin, were studied. All the patients had been informed about the procedure and the aim of the study. All of them had agreed to have their blood examined for this research. The agreement of the Board for Supervising Ethics in Medical Experiments at the Medical University of Lublin was obtained.

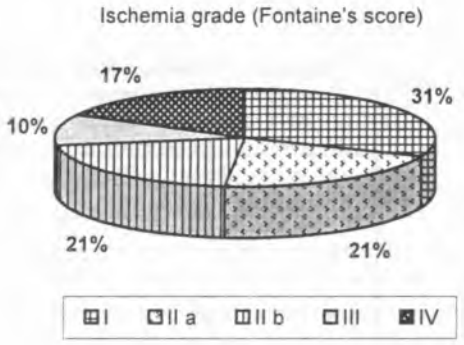

Fig. 1

The ischemia grade (in Fontaine's score) of the patients is presented in Figure 1. The PF4 concentration was determined by the immunoenzymatic method (ELISA) with Enzygnost ® PF4 set (Behring, Germany). Blood samples for analysis were drawn from the antecubital vein without venous stasis into plastic centrifugation tubes containing PF4 sampling medium in proportion 9:1. The samples were centrifuged for 10 min. at the speed of 1500 rpm.

The obtained results of the extinctions were processed using the curves given by the producer. For each sample the mean and standard deviation were set using Microsoft Excel ® 6.0 and Statsoft Statistica ® 4.0. Statistical significance was calculated using t-Student test in Fischer modification for measurable traits values and using Spearman test for unmeasurable traits values.

RESULTS

In the studied group of patients the average concentration of PF4 was: 75.08+/–47.06 μg/l. In one case (3.3%) the normal PF4 level was observed (1.4–6.1 μg/l). In 9 cases (30.0%)PF4 was between 6.2–40.0 μg/l, in 6 cases (20.0%) was between 40.1–100.0 μg/l, and in 14 cases (46.7%) was between 100,1–160 μg/l (Fig. 2).

After the statistic verification, different levels of concentration were noticed: the patients PF4 level was significantly higher than the norm ($p<0.05$). With the Spearman test, the correlations between unmeasurable traits (such as smoking, Raynaud syndrome, thrombophlebitis migrans, ischemia grade) and the measurable trait – the PF4 concentration were examined. The significant correlation between ischemia grade and the PF4 concentration was noticed with statistical significance $\alpha= 0.01$ ($R=0.53$).

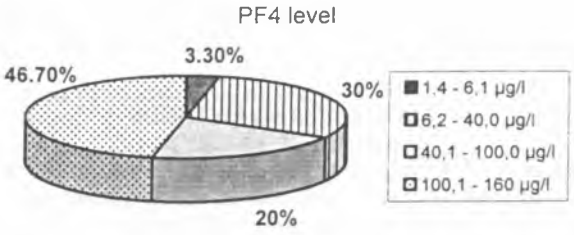

Fig. 2

Obtained results are presented in Table:

No	Age	Sex	PF4	Smoker ex- + actual ++	Raynaud Syndrome no –actual ++ previously+	Thrombophlebitis migrans no –actual ++ previously+	Ischemia grade (Fontaine's score)	Clinical progression within 3 months from the trial
1	39	M	135.17	+	-	+	IV	-
2	38	M	99.50	++	-	+	II a	+
3	47	M	67.93	++	+	-	I	-
4	35	K	6.75	++	+	-	I	-
5	41	M	14.59	+	-	+	I	-
6	36	M	100.28	++	-	+	IV	-
7	34	M	11.05	+	-	+	I	-
8	32	M	48.31	+	-	+	II a	-
9	44	M	131.24	+	-	++	II a	-
10	40	M	133.80	+	-	+	IV	-
11	46	M	111.86	+	+	-	II a	-
12	38	M	95.82	++	+	-	I	-
13	42	K	127.52	++	-	+	IV	-
14	45	M	31.65	++	-	+	II b	+
15	46	M	48.05	++	-	+	III	-
16	23	M	31.88	+	-	+	I	-
17	35	M	115.18	++	-	+	III	+
18	43	M	23.23	++	-	+	I	-
19	41	M	5.87	+	-	+	II a	-
20	29	M	94.31	++	-	+	III	+
21	34	M	6.63	++	-	+	II a	-
22	42	M	12.41	++	-	+	II b	-
23	40	M	107.38	+	-	+	I	-
24	38	M	108.73	+	-	++	II b	-
25	32	M	119.26	++	-	+	IV	+
26	30	M	114.76	++	-	+	I	+
27	43	M	123.14	++	-	+	III	+
28	31	M	64.15	++	-	+	II b	-
29	44	M	127.23	+	-	+	II b	-
30	43	M	35.14	+	-	+	II b	-

DISCUSSION

TAO is still an important challenge for science. Searching the causes of the disease is still not satisfactory. Despite the fast development of diagnostic techniques, pathogenesis of TAO is still unclear. Few trials of understanding the subclinical or manifested hypercoagulability in TAO and their satisfying initial results caused the taking of the present study whose results are presented in this paper. High PF4 concentration observed in 96.67% of the examined cases suggests not only the increased thrombocyte activation, but the significant role of PF4 as chemokine in inflammatory processes, which are an important factor in pathogenesis of Buerger disease. Statistically significant correlation between the ischemia grade and PF4 concentration makes the bases to state PF4 as the TAO intensification factor. What seems to be important, is the clinical progression (at least 1 grade in Fontaine score) in the patients with PF4 concentration higher than 100 μg/l, within 3 months from the trial.

Very few studies concerning the platelet function in Buerger disease have been published. Carr (1) reported 2 cases of TAO patients in whom evaluations for hypercoagulable states and of platelet function were performed. Platelet contractile force (PCF) was found to be 82% higher than normal in 1 TAO patient and 340% higher than normal in the second patient. This was true despite the fact that platelet aggregations confirmed suppression of aggregation by antiplatelet medications. Elevated PCF has been noticed in a variety of conditions, such as coronary artery disease and diabetes mellitus, in which endothelial function is abnormal.

PF4 concentration was found in many diseases with clinical features of coagulation system disorders both as an antiheparin substance and as a blood platelets activity marker. PF4 concentration is significantly elevated in myocardial infarction (MI) (48.4 +/−15.16 ng/ml) and unstable angina patients (44.7 +/−15.9 ng/ml) (15).

The plasma level of a PF4 was evaluated, before and after exercise in coronary artery disease. The tests showed that the significant increase in PF4 levels after exercise is associated with clinically significant coronary artery disease (angina, ST segment depression during the stress test): 29.9 +/−15.5 ng/ml, after stress test 67.7 +/−26.1 ng/ml; in healthy subjects: 4.1 +/−2.5 ng / ml, after stress test 5.3 +/−2.6 ng/ml (12).

It was shown that the greater than normal PF4 level measured in the coronary sinus and the coronary artery, immediately after percutaneous transluminal coronary angioplasty tended to decline (5). Sadayasu compared the plasma levels of PF4 5 min after intravenous heparin injection in patients with coronary artery disease (CAD) and normal control subjects. There was a significant difference in plasma PF4 levels at 5 min after heparin injection between the CAD group (100.1 +/−38.1) and the control group (61.0 +/−24.0) (p less than 0.01). It confirms PF4's antiheparine activity and also shows that there is a correlation between the PF4 ratio after heparin and the Gensini CAD score, which defines the severity of coronary atherosclerosis (14).

Platelet activation has an independent role in the pathogenesis of atherosclerosis. The relationship between platelet activation and arterial atherosclerosis was established by measuring the plasma concentrations of two specific platelet activation markers, beta-thromboglobulin (beta TG) and platelet factor 4. The concentration of PF4 unlike beta TG did not show any correlation with the growth of artery lesions (4). Plasma level of PF4 in patients with arteriographically proven peripheral arterial occlusive disease in degree I and IIa according to Fontaine's score after the low-dose (100 mg daily) acetylsalicylic acid (ASA) remained unchanged (3.6–3.9 ng/ml) (8).

It was found that PF4 does not seem to be influenced by sympathetic stimuli in normal subjects and in patients with peripheral vascular disease and to evaluate any correlations among plasma levels of catecholamines, beta-thromboglobulin (beta-TG) and PF 4 (2).

In the study we conducted on the group of peripheral arterial occlusive disease patients in an advanced phase (degree III and IV according to Fontaine score) we found the PF4 concentration higher than normal with 70% of patients (between 6.2–100.0 μg/l, mean 14.5 μg/l). There was no correlation between plasma level of PF4 and progression of disease.

Pitsilos (11) examined atherosclerotic plaques taken during carotid thrombendarteriectomy. She detected PF4 in the cytoplasm of luminal and neovascular endothelium, in macrophages and in

regions of plaque calcification. The presence of PF4 in macrophages and neovascular endothelium correlated with the grade of lesion. In early lesions, PF4 was commonly found in macrophages of early lesions. She concludes that the correlation between PF4 deposition, lesion severity and symptomatic atherosclerosis suggests that persistent platelet activation may contribute to the evolution of atherosclerotic plaques.

CONCLUSIONS

1. The correlation between the ischemia grade in Buerger disease patients and PF4 level proves the significant role of blood platelets and chemokine in the progression of the disease.

2. Obtained results show the presence of subclinical or manifested hypercoagulability in TAO independently of progression of the disease.

3. The PF4 level can be considered as a marker of Buerger disease progression.

REFERENCES

1. Carr M.E. Jr. et al.: Enhanced platelet force development despite drug-induced inhibition of platelet aggregation in patients with thromboangiitis obliterans – two case reports. Vasc. Endovascular Surg., 36, 6, 473, 2002.
2. Catalano M. et al.: Beta-TG and plasma catecholamines levels after sympathetic stimuli in hypertensives and patients with peripheral vascular disease. Thromb. Haemost., 28, 63, 3, 383, 1990.
3. Dumenco L.L. et al.: Inhibition of activation of Hageman factor (factor XII) by platelet factor 4. J. Lab. Clin. Med., 112, 394, 1998.
4. Ghaddar H.B. et al.: Correlation of specific platelet activation markers with carotid arterial wall thickness. Thromb. Haemost., 74, 3, 943, 1995.
5. Korovesis S. et al.: Release of platelet activation markers during coronary angioplasty. Coron. Artery Dis., 11, 5, 391, 2000.
6. Lane D.A. et al.: Anticoagulant activities of heparin oligosaccharides and their neutralization of platelet factor 4. Biochem J., 218, 725, 1984.
7. Marti F. et al.: Platelet factor 4 induces human natural killer cells to synthesize and release interleukin-8. J. Leukoc. Biol., 72, 3, 590, 2002.
8. Minar E. et al.: Lack of influence of low-dose acetylsalicylic acid (100 mg daily) on platelet survival time, beta-thromboglobulin and platelet factor 4 in patients with peripheral arterial occlusive disease. Thromb. Res., 1, 52, 3, 219, 1988.
9. Mosnier L., Bouma et al.: Platelet Factor 4 upregulates protein C activation relative to TAFI activation. In: XIX Congress of The International Society on Thrombosis and Haemostasis. Birmingham, UK, OC376, 2003.
10. Nassar T. et al.: Platelet factor 4 enhances the binding of oxidized low-density lipoprotein to vascular wall cells. J. Biol. Chem., 21, 278, 8, 6187, 2003.
11. Pitsilos S. et al.: Platelet factor 4 localization in carotid atherosclerotic plaques: correlation with clinical parameters. Thromb. Haemost., 90, 6, 971, 2003.
12. Placanica G. et al.: Short-term effect of exercise on platelet factor 4 in normal subjects and in patients with coronary artery disease. Cardiologia, 44, 11, 993, 1999
13. Sachais B.S. et al.: Interactions of platelet factor 4 with the vessel wall. Semin. Thromb. Hemost., 30, 3, 351, 2004.
14. Sadayasu T. et al.: Heparin-releasable platelet factor 4 in patients with coronary artery disease. Clin. Cardiol., 14, 9, 725, 1991.
15. Saxena R. et al.: Evaluation of factors predisposing to arterial thrombosis in coronary artery disease. Indian Heart J., 42, 6, 433, 1990.

SUMMARY

Platelet factor 4 (PF4) is protein with high biological activity, whose action is not completely known. It participates in the thrombotic process on few levels, having both prothrombotic and antithrombotic roles. The aim of the study was to specify the PF4 role in pathogenesis of TAO. The study involved the blood sample of 30 TAO patients treated at the Department of Vascular Surgery and Angiology, Medical University of Lublin. In the studied group of patients the average concentration of PF4 was: 75.08+/−47.06 μg/l. In one case (3.3%) the normal PF4 level was observed (1.4–6.1 μg/l). In 9 cases (30.0%)PF4 was between 6.2–40.0 μg/l, in 6 cases (20.0%) was between 40.1–100.0 μg/l, and in 14 cases (46.7%) was between 100.1–160 μg/l. The significant correlation between ischemia grade and the PF4 concentration was noticed, which proves the significant role of blood platelets and chemokine in the progression of the disease. Obtained results show the presence of subclinical or manifested hypercoagulability in TAO independently of progression of the disease.

Stężenie czynnika płytkowego 4 w osoczu chorych z rozpoznaniem zakrzepowo-zarostowego zapalenia tętnic

Czynnik płytkowy 4 jest jednołańcuchowym białkiem o dużej aktywności biologicznej, którego mechanizm działania nie jest do końca poznany. Uczestniczy w procesie krzepnięcia na kilku poziomach, wykazując właściwości zarówno pro-, jak też przeciwzakrzepowe. Celem badania było określenie roli czynnika płytkowego 4 w mechanizmach patogenezy zakrzepowo-zarostowego zapalenia naczyń. Badaniu poddano osocze trzydziestu chorych z rozpoznaniem zakrzepowo-zarostowego zapalenia naczyń, hospitalizowanych w Klinice Chirurgii Naczyń i Angiologii Akademii Medycznej w Lublinie. W badanej grupie chorych uzyskano średnie stężenie czynnika płytkowego czwartego 75,08+/−47,06 μg/l. W jednym przypadku (3,3%) stwierdzono prawidłowy poziom czynnika (1,4–6,1 μg/l). U 9 chorych (30,0%) poziom PF4 mieścił się w przedziale 6,2–40,0 μg/l, u 6 chorych (20,0%) w przedziale 40,1–100,0 μg/l, u 14 chorych (46,7%) w przedziale 100,1–160 μg/l. Stwierdzono również istotną korelację między stopniem niedokrwienia a wysokim stężeniem czynnika płytkowego 4, co dowodzi znaczącego udziału płytek krwi oraz roli chemokin w rozwoju choroby. Uzyskane wyniki wskazują na stan utajonej lub jawnej nadkrzepliwości w zakrzepowo-zarostowym zapaleniu naczyń, niezależnie od stopnia klinicznego zaawansowania.