Thrombin generation is associated with PlA1/A2 β_3 integrin polymorphism in aspirin-treated patients with coronary artery disease – the role of statins

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Abstract: Introduction. The PlA2 allele is present in about 20 to 30% of the European population. This allele has been associated with resistance to the antithrombotic action of aspirin in healthy PlA2 carriers. **Objective:** To evaluate the functional association of PlA2 polymorphism of β_3 intergrin with increased thrombin generation and platelet activation in patients with coronary artery disease (CAD), treated with low-dose aspirin and whether the effect of this polymorphism is modulated by statin administration. **Methods.** In 31 patients (25 M, 6 F) with CAD, aged 47 to 76 years, the thrombin-antithrombin complex generation (TAT) and the soluble form of CD40 ligand level (sCD40L) in blood collected every 60 seconds at sites of standardized microvascular injury were determined. **Results.** Coronary angiography revealed ≥1 major epicardial artery stenosis (≥50%) in all patients. Genotyping determined 18 P1A1 homozygous subjects and 13 PlA2 heterozygous carriers. Homozygous PlA1 subjects exhibited an increased fibrinogen level compared with PlA2 carriers (4.2 [IQ 2.39] g/l vs. 2.5 [0.73] g/l, $p<0.05$). Maximum TAT level observed 6 min after microvascular injury was higher in PlA2 carriers ($p=0.01$). Maximum sCD40L did not differ between PlA1A1 subjects and PlA2 carriers (p=NS). PlA2 allele did not alter the velocity of TAT production and sCD40L release. The analysis of the area under the concentration vs. time curve for TAT revealed that PlA2 carriers exhibited increased thrombin generation compared with PlA1 subjects (by 17.5%, $p<0.05$). Subjects treated with statins (n=12) had lower TAT generation and sCD40L release than non-treated (by 20%, $p<0.005$ and 23%, $p<0.005$, respectively). This effect was not altered by PlA2 presence. **Conclusions.** In a model of microvascular injury PlA1/A2 polymorphism influenced thrombin formation but not platelet activation in CAD patients treated with low-dose aspirin. The PlA2 allele did not alter the beneficial effect of statins on blood coagulation.

Key words: GPIIb/IIIa, PlA1/A2 polymorphism, platelet activation, statins, thrombin generation

INTRODUCTION

β_3 integrins are part of the vitronectin and the platelet surface-membrane glycoprotein GPIIb/IIIa receptor and play a key role in the cell adhesion, aggregation and other cell-to-cell interactions of platelets (see for review: [1]). Their biological function depends on β_3-integrin bounding to fibrinogen and to von Willebrand factor and is associated with intracellular signal transduction. This mechanism is regulated by cytoskeletal proteins (talin and, α-actinin) and protein kinese activation (Src and FAK family), which occurs during cell-adhesion [1].

Blockade of the GPIIb/IIIa / fibrinogen interaction with synthetic agonists (abciximab and integrelin) markedly reduces the risk of acute ischemic complications in patients undergoing percutaneous coronary revascularization [2] and combined with the intense thrombolitic treatment improves reperfusion in acute coronary syndromes [3]. It is well known that other anti-platelet drugs (aspirin, tienopiridins) reduce platelet activation due to decreased GPIIb/IIIa expression [4].

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Received: March 8, 2007. **Accepted in final form:** March 25, 2007.
Conflict of interest: none declared.
Pol Arch Med Wewn. 2007; 117 (1-2): 33-40

The polymorphism of the β_3 integrin gene (called PlA1/A2 polymorphism) is characterized by a thymidine to cytosine transition at nucleotide 1565, which results in Leu33 to Pro substitution, which defines the PlA1 and PlA2 alleles, respectively. The PlA2 allele is present in 20% to 30% of healthy [5] or coronary artery-diseased [6] Europeans.

A role of the PlA2 variant is not clear. It was observed that the PlA2 allele is associated with shorter bleeding time [7,8], greater surface-expressed P-selectin and GPIIb/IIIa-bound fibrinogen activation in platelets [9]. Clinical studies showed that PlA2-carriers have an increased risk of myocardial infarction [10] and restenosis after percutaneous coronary angioplasty [6]. Although epidemiological studies gave inconsistent results, questioning the role of the PlA2 polymorphism as a genetic coronary risk factor for acute myocardial infarction [11] and restenosis [12], the contribution of the PlA2 variant to acute ischemic events is still under investigation [13]. *In vitro* experiments showed that in the PlA2 positive cells the phosphorylation of pp125FAK kinase and the adhesiveness to fibrinogen were higher than in the PlA2 negative ones [14]. This observation correlates with *in vivo* conditions, where activated platelets adhere to the fibrin formed thrombus. Controversially, the Framingham Heart Study clinical trial showed that the association between fibrinogen and platelet aggregability was genotype specific, however, the increase in platelet aggregability with higher fibrinogen was present for the PlA1/A1 genotype [15].

The effect of the platelet β_3 integrin polymorphism was investigated several times as a modulator of anti-platelet therapy (aspirin) in healthy subjects [16,8] and in patients with advanced coronary artery disease [17,18]. It was observed that the presence of the PlA2 allele was associated with enhanced thrombin formation (thrombin B-chain release, thrombin-antithrombin complex formation) and impaired antithrombotic action of aspirin at the site of microvascular injury in PlA2 carriers [19]. Bleeding time in PlA2 carriers is more prolonged after 300 mg aspirin dose than in PlA1 homozygotes [8] and platelet aggregation is higher [7]. Recently, Dropiński et al. published results that after 14 days of aspirin administration (300 mg/day) the thrombin generation (F1+2 fragments concentration) in PlA1 homozygotes (n = 9), but not in PlA2 carriers (n = 19) was depressed. Bleeding time after aspirin was prolonged in PlA1 subjects only [17]. It is not clear whether in patients with advanced coronary artery disease (CAD) treated chronically with low-dose of aspirin (75 mg or 150 mg) the association between the PlA2 allele and thrombin generation is observed.

The present study was undertaken to determine whether in CAD patients treated with low-dose aspirin (75 mg) the PlA2 polymorphism of β_3 intergrins is associated with increased coagulant reactions and the activation of platelets at sites of microvascular injury. We also sought to evaluate the effect of simvastatin, reported to reduce thrombin generation [20] and platelet activation [21] in the cholesterol-reducing doses, in relation to the PlA2 allele.

PATIENTS AND METHODS

Thirty one consecutive patients with stable angina admitted to Interventional Cardiology Department of the Institute of Cardiology, Jagiellonian University Collegium Medicum, Cracow, Poland from May to June 2006 scheduled for coronary angiography were enrolled for the study. The diagnosis of CAD (class II or III according to CCS or class I with high risk of ischemia in other tests) was confirmed in ECG exercise stress test or heart imaging studies (coronary calcium scoring or nuclear cardiac imaging). Patients with sustained or recurrent angina after acute coronary syndrome (ACS) were also enrolled (at least 3 months after ACS). These patients were scheduled for coronary angiography due to unsatisfactory response to medical treatment. Troponin test at enrollment was <0.1 ng/ml. Exclusion criteria were: acute infection, use of vitamin K antagonists, heparin or thienopyridines, history of deep vein thrombosis or ACS within last 3 months, serious diseases affecting hemostasis: malignancies, autoimmunoe disorders, renal failure, heart failure (NYHA III/IV). The study obtained approval of the Jagiellonian University Bioethical Committee, and all patients gave informed consent.

Laboratory methods

Lipid profiles was assayed by routine laboratory techniques, fibrinogen was determined using the Clauss method, high-sensitivity C-reactive protein (CRP) was measured by immuno-turbidimetric method (Dimention Expand, Dade Behring, Marburg, Germany). Commercially available immunoenzymatic assays were used to determine plasma and supernatant soluble CD40 ligand – sCD40L (R&D, Wiesbaden-Nordenstadt, Germany) and thrombin-antithrombin complex – TAT (Enzygnost TAT, Dade-Behring, Germany). For measurement of the thrombin generation and the activation of platelets, blood oozing from two standardized bleeding-time wounds, performed with a Simplate R device (Organon Teknika) was collected into heparinized capillary tubes every 60 seconds until cessation of bleeding. Blood samples were then passed into an anticoagulant cocktail (NaCl 0,9%, EDTA 50 mmol/l, benzamidine 20 mmol/l, thrombin inhibitor D-Val-Leu-Lys-chloromethylketon [V3763 Sigma] 50 μM), centrifuged in 4°C (6000g by 20 min), and frozen (-70°C). As a quantitative measure of TAT and sCD40L we used the mean concentration at each time point (60s), the maximal concentration increase (usually between 2. and 3. min) and the area (S) under the concentration vs. time curve. Due to average bleeding time of about 6 min, the analysis was performed using samples collected during the first 6 min.

Genotyping of the PlA1/A2 polymorphism

The Leu33 Pro polymorphism was determined by the polymerase chain reaction followed by the restriction fragments length polymorphism analysis – enzyme *MpsI* (ER0541, Fermantas, Vilno, Lithuania) as described [19].

Table 1. Characteristics of study participants

Variable	All patients; n = 31	Homozygotes PIA1; n = 18	PIA2 carriers; n = 13
male gender, n (%)	25 (81)	12 (67)*	13 (100)*
age, yrs	62 (47–76)	66 (48–76)	60 (47–75)
med	61 (47–76)		
women	69 (54–71)		
Fbg(g/l)	2.9 (1.99)	4.2 (2.39)*	2.5 (0.73)*
CRP (mg/l)	1.6 (1.43)	1.6 (1.39)	1.9 (1.32)
TC (mmol/l)	5.4 (1.89)	5.3 (2.07)	5.4 (1.53)
LDL-C (mmol/l)	3.4 (1.50)	3.5 (1.86)	3.3 (1.01)
HDL-C (mmol/l)	1.2 (0.23)	1.2 (0.32)	1.2 (0.15)
TG (mmol/l)	1.5 (0.76)	1.4 (0.84)	1.5 (0.53)
BT (s)	390 (170)	420 (155)	370 (160)
venous TAT (μg/l)	3.2 (0.81)	3.4 (0.8)	3.0 (0.60)
venous sCD40L (pg/ml)	228 (97)	230 (116)	228 (57)
current smokers, n (%)	12 (39)	6 (33)	6 (46)
diabetes, n (%)	2 (3)	2 (5.5)	0 (0)
hypertension, n (%)	12 (39)	6 (33)	6 (46)
aspirin 75 mg, n (%)	26 (84)	16 (89)	10 (77)
β-blockers, n (%)	20 (65)	12 (67)	8 (62)
ACE-I, n (%)	17 (55)	12 (67)	5 (38)
statins, min. 20 mg, n (%)	12 (39)	7 (39)	5 (38)

* $p < 0.01$

ACE-I – angiotensin-converting enzyme inhibitors, BT – bleeding time, CRP – C-reactive protein, Fbg – fibrinogen, HDL-C – high density lipoprotein cholesterol, LDL-C – low density lipoprotein cholesterol, sCD40L – soluble CD40 ligand, TAT – thrombin-antithrombin complex, TG – triglycerides, TC – total cholesterol

Table 2. Thrombin-antithrombin complex (TAT) and sCD40L in CAD patients the role of PIA1/A2 polymorphism

	Homozygotus PIA1; n = 18		PIA2 carriers; n = 13	
	TAT (nmol/l)	sCD40L (ng/ml)	TAT (nmol/l)	sCD40L (ng/ml)
maximal level	34.9 (4.8)*	8.9 (0.9)	42.3 (3.4)*	9.5 (1.1)
maximal velocity	9.2 (3.1)	2.7 (0.9)	8.7 (4.6)	2.8 (0.7)
$S_{total\ 180\ s}$	1438 (276)	546 (189)	1668 (624)	560 (77)
$S_{total\ 360\ s}$	6288 (1050)*	1975 (237)	7620 (792)*	2051 (252)

* $p < 0.05$

Statistical analysis

Data are expressed as medians and inter-quartile intervals (IQ) or otherwise stated. The Kolmogorov-Smirnov test was used to determine normal distribution. The Mann-Whitney U test was used to test differences between groups as appropriate. The main affects ANOVA was used to assess differences between parameters measured in the PlA1/A2 allele and the statin treated groups. Standard multiple linear regression analysis was used to determine predictors of the area under curve variables. Analysis were performed using Statistica 7.1 PL package (StatSoft, Inc. 2005). The level of significance was set at $p < 0.05$.

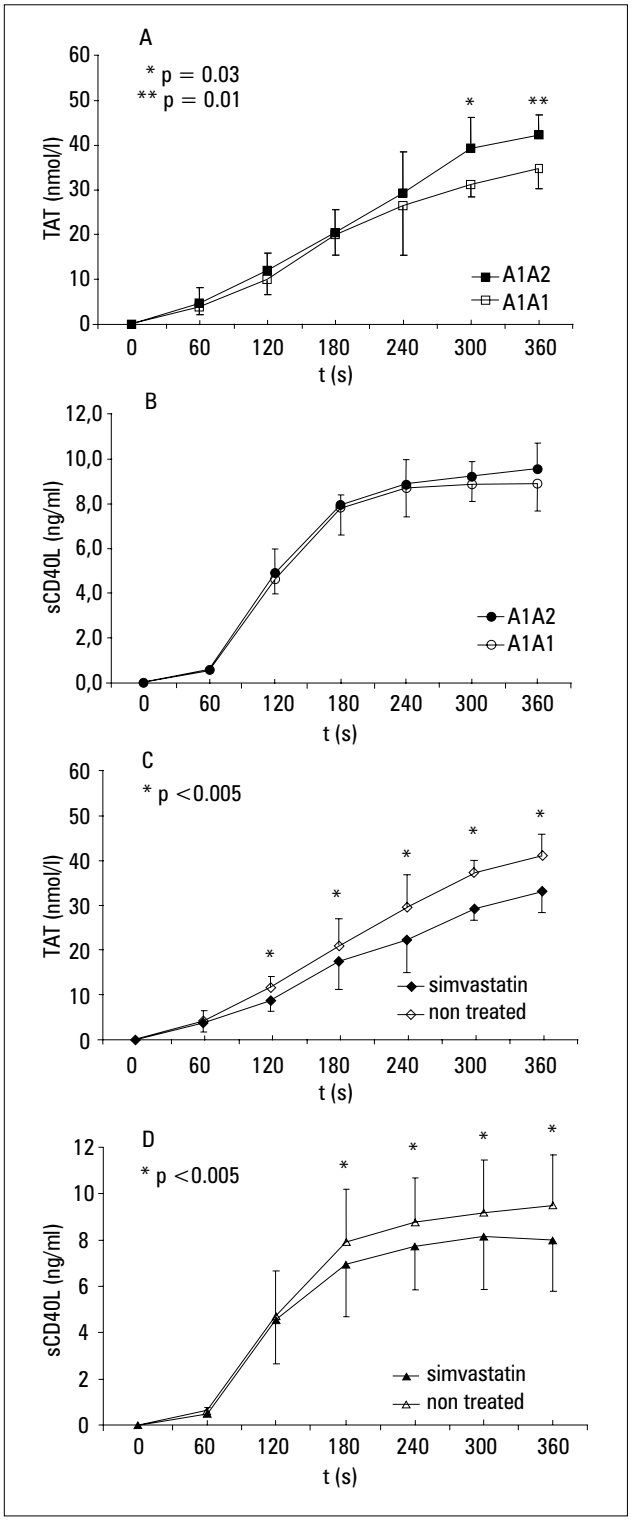

Figure 1. The time-course of the generation of the release of soluble CD40 ligand (sCD40L) at the site of microvascular injury and its modulation by the PlA1/A2 polymorphism and simvastatin (20 mg/day). Oozing blood was collected into heparinized capillary tubes every 60 seconds until cessation of bleeding. Results are shown for the PlA1/A2 group (A, B) and the simvastatin group (C, D). Data are presented as mean±SD.

RESULTS

There were 25 men aged 61 (46 to 76 years) and 6 women aged 69 (54 to 71 years) in the study group. Genotyping determined 18 PlA1 homozygous subjects and 13 PlA2 heterozygous carriers. The characteristic of the PlA2 positive and PlA1 homozygotes is shown in tab. 1. The groups did not differ with respect to age, cholesterol, CRP levels, coronary risk factors and treatment, except for sex distribution (in PlA2 group were male subjects only) and fibrinogen: 4.2 (2.39) g/l in homozygous for PlA1 vs. 2.5 (0.73) g/l in PlA2 carriers, p <0.05. Coronary angiography revealed et least one major epicardial artery stenosis (≥50%) in all patients. All these patients regularly took aspirin: 26 patients (84%) in a dose of 75 mg/d, the other 3 patients – 150 mg/d and 2 patients – 300 mg/d, moreover 12 (39%) were treated with simvastatin usually in dose 20 mg/d, two of them in a dose of 40 mg/d. The effect of simvastatin intake was observed as significant reductions in total cholesterol and LDL-cholesterol levels: 4.3 (1.25) mmol/l vs. 6.2 (1.30) mmol/l in non-treated with statin (p <0,0001) and 2.4 (1.32) mmol/l *vs* 4.0 (1.04) mmol/l (p <0,0001), respectively.

Levels of TAT and sCD40L in shed blood rose gradually, the maximum rate of increase was observed between 2. and 3. min after injury (fig. 1) and did not differ between PlA1 homozygous and PlA2 carriers. Maximum TAT levels were detected in 6. min after injury and in PlA2 carriers and were significantly higher than in PlA1 subjects: 42.3 (3.4) nmol/l vs. 34.9 (4.8) nmol/l, p = 0,01 (Fig. 1A, Table 2), respectively. In the PlA2 group, maximum sCD40 levels did not differ significantly corresponding to the PlA1: 8.9 (0.9) ng/ml *vs* 9.5 (1.1) ng/ml, NS (fig. 1B, tab. 2). Comparing the area (S) under the concentration vs. time curve for TAT reveled that PlA2 carriers exhibited an increased thrombin generation than than PlA1 subjects: 7620 (792) nmol/l·s *vs* 6288 (1050) nmol/l·s, p <0.05 (tab. 2). S values for the sCD40L curve were not significantly different.

Interestingly, subjects treated with statin had lower TAT generation and sCD40L release than non-treated at every time point. Concentrations of TAT were decreased by 20% (p <0.005) in the statin group: 29.6 (2.7) nmol/l vs. 37.6 (9.5) nmol/l and 33.2 (3.8) nmol/l vs. 41,3 (8,1) nmol/l, respectively in 5. ad 6. min (fig. 1C, tab. 3). Similarly the sCD40L levels were decreased by 23% and (p <0.005) starting from 3 min after injury: 8.5 (2.4) ng/ml vs. 9.2 (0.9) ng/ml in 5 min and 8.8 (2.3) ng/ml vs. 9.5 (1.0) ng/ml in 6 min, respectively (fig. 1D, tab. 3). This effect was not altered by the PlA2 presence. S values for the TAT and the sCD40L curves were also significantly lower for the statin-treated patients, respectively: 5760 (1188) nmol/l·s *vs* 7509 (1971) nmol/l·s and 1961 (504) ng/ml·s *vs* 2093 (260) ng/ml·s, p <0.005 (tab. 3). ANOVA analysis showed differences in thrombin generation between the statin-treated patients and the non-treated (fig. 2).

In a standard multiple linear regression analysis model incorporating all patients, the independent predictors of variable

Table 3. Thrombin-antithrombin complex (TAT) and sCD40L in CAD patients the role of simvastatin treatment

	Statin-treated; n=12		Non-treated; n=19	
	TAT (nmol/l)	**sCD40L (ng/ml)**	**TAT (nmol/l)**	**sCD40L (ng/ml)**
maximal level	33.2 (3.8)*	8.8 (2.3)**	41.3 (8.1)*	9.5 (1.0)**
maximal velocity	8.6 (4.9)	2.4 (0.4)*	9.2 (3.6)	3.1 (0.6)*
$S_{total\ 180\ s}$	1260 (327)*	394 (246)	1548 (369)*	554 (61)
$S_{total\ 360\ s}$	5760 (1188)*	1961 (504)*	7509 (1971)*	2093 (260)*

* $p < 0.005$. ** $p < 0.05$

S for the TAT were being a PlA2 carrier and a statin-treated patient. Regression parameters for the PlA1/A2 polymorphism and the statin treatment were respectively: 0.43 and -0.76 ($R = 0.73$, $R2 = 0.53$, $0 < 0.005$).

DISCUSSION

A major finding of this study is that carriers of the PlA2 allele with coronary artery disease chronically taking low-dose aspirin exhibit increased thrombin generation at microvascular injury and that the PlA2 variant does not influence the rate of this process. The PlA2 allele dos not differentiate patiens in respect to platelet activation observed as sCD40L release (fig. 1). Moreover, the present study has been confirmed that thrombin generation (TAT) is decreased in all statin-treated patients despite many cardiovascular risk factors e.g. hypercholesterolemia. However, this antithrombotic nature of statins does not depend on the PlA1/A2 polymorphism – the antithrombotic effect is observed in PlA2 carriers and non-carriers (fig. 2).

Our study contributes to a debate about the role of the PlA1/A3 β_3 integrin polymorphism in blood clotting at the site of microvascular injury. Previous studies carried on healthy male volunteers demonstrated that the PlA2 allele is associated with enhanced thrombin generation: activation of thrombin enzyme and thrombin-antithrombin complex formation [16,19].

Our present study shows that in CAD patients the maximal TAT levels are significantly higher in PlA2 carriers but dynamics of TAT generation are not different. During the first 3 minutes after injury (microvascular injury model) the concentration of TAT increases in PlA2 carriers and non-carriers at the same velocity. A recent study by Dropiński et al. [17] showed that in survivors of myocardial infarction (MI) the thrombin generation assessed by the rate of F1+2 prothrombin fragment concentration did not differ between the PlA2 heterozygous and the PlA1 homozygous cases. In that study, thrombin generation was analyzed in group not receiving aspirin at the beginning of the study and after 14 days of aspirin administration (300 mg/d). The authors showed that the aspirin treatment resulted in a decrease of F1+2 concentration only in PlA1 homozygous subjects and no change in the F1+2 levels was observed between PlA2 carriers and non-carriers during the first 3 minutes at the site of microvascular injury.

Our present study demonstrates the difference in the maximal TAT levels in 5 and 6 min after injury. Probably this discrepancy between reported study and our data is due to: methods of thrombin generation assessment and studied groups. In our current study analysis of TAT profile was performed for longer period (6 min) and our group had 31 patients (mostly men), without MI, and some of them had hypercholesterolemia (non-treated with simvastatin). Dropiński et al. does not refer exact data concerning their sample characteristics.

Our results demonstrate that the PlA1/A2 polymorphism has no effect on the rate of thrombin generation and CAD patients treated with low-dose of aspirin. In healthy men [19] such relation was observed within first 3 min after injury (the same model).

We might speculate that the coronary artery disease and the constant aspirin treatment influence dynamics of thrombin formation. The variance of aspirin dose (3 patients took 150 mg/d, 2 – 300 mg/d) should not have an effect on TAT, as well 75 mg and 300 mg of aspirin lowers thrombin generation (F1+2) to a similar extent [22].

The present study provides the evidence that simvastatin in a hypolipolipemize dose (20 mg/d) attenuates TAT formation independent of the PlA1/A2 polymorphism. Our data show that at the site of microvascular injury the levels of TAT were lower in statin-treated patients compared to non-treated beginning from 2 min after injury. This results are consisted with to the previous finding of Undas et al. that simvastatin administration significantly retarded prothrombin activation in hypercholesterolemic men after 3 days [20] and 3 months [19, 23] of treatment in a dose 20–40 mg/d. Our finding confirms this observation and shows that antithrombotic impact of statin is not disturbed by the PlA2 allele presence in patients with CAD receiving an effective hypolipemic treatment. A possible mechanism by which simvastatin affects coagulation is through the inhibition of post-translational modification of major endothelial proteins (e.g. tissue factor) [23].

Anti-platelet effect of statin was observed as reduction of sCD40L levels at the site of microvascular injury in the group

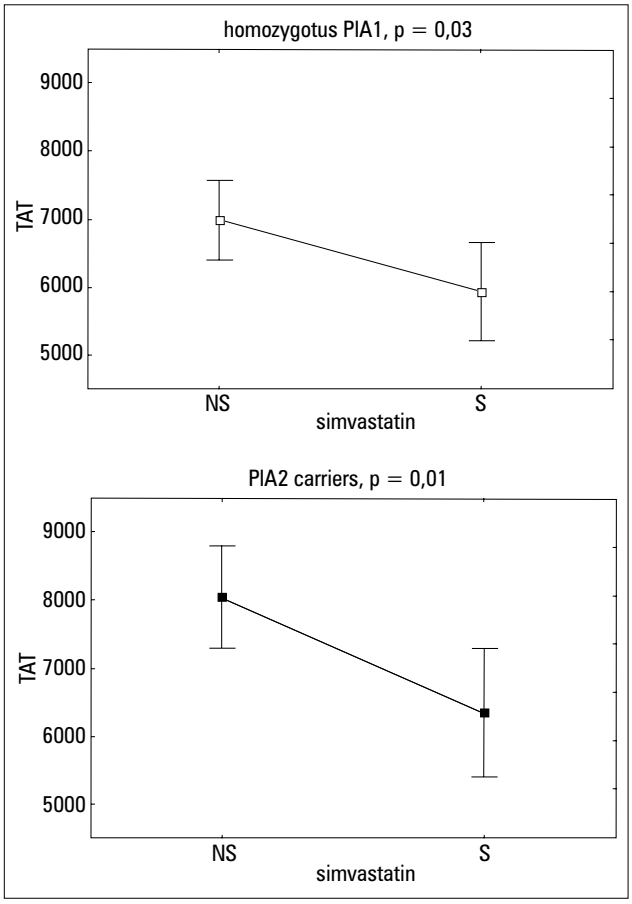

Figure 2. Affect of simvastatin treatment (S) in patients with CAD carriers of the PlA2 allele and homozygous for PlA1. The main affects ANOVA was used.

of simvastatin-taking patients. CD40 protein ligand is released mostly by activated platelets in that model [24] and the highest rate of sCD40L release is detected between 1 and 3 min after injury, attaining a plateau in 5–6 min [21]. Simvastatin decreased sCD40L levels at the site of microvascular injury and in circulating blood (not showed). Similar impact of statins on sCD40L levels in hypercholesterolemia was observed in patients treated with pravastatin (40 mg/d) and cerivastatin (0.2 mg/d) [25]. Our previous study concerning a microvascular injury model showed that simvastatin at a dose 40 mg/d inhibits sCD40L release after a 3- and a 28-day treatment in hypercholesterolemic subjects [21]. In the present study we confirm these data for patients with coronary artery disease.

Our novel finding is that the the PlA1/A2 polymorphism does not disturb the antithrombotic and antiplatelet properties of statins. This means that genotyping of the platelet surface-membrane glycoprotein GPIIb/IIIa receptors is not beneficial in predicting low antithrombotic effectiveness of aspirin+statin therapy. We cannot expect that only one mutation (even very frequent) may affect blood coagulation processes in coronary artery disease.

ACKNOWLEDGEMENTS

We thank Prof. Anetta Undas for critical review of this manuscript.

REFERENCES

1. Bennett JS. Structure and function of the platelet integrin αIIβ3. J Clin Invest. 2005; 115: 3363-3369.
2. The EPILOG Investigators. Platelet glycoprotein IIb/IIIa receptor blockade and low-dose heparin during percutaneous coronary revascularization N Engl J Med. 1997; 336: 1689-1697.
3. Magnus Ohman E, Kleiman NS, Gacioch G, et al. Combined accelerated tissue-plasminogen activator and platelet glycoprotein IIb/IIIa integrin receptor blockade with integrilin in acute myocardial infarction. Results of a randomized, placebo-controlled, dose-ranging trial. Circulation 1997; 95: 846-854.
4. Schneider DJ, Baumann PQ, Holmes MB, et al. Time and dose dependent augmentation of inhibitory effects of abciximab by aspirin. Thromb Haemost. 2001; 85: 309-313.
5. Sperr WR, Huber K, Roden M, et al. Inherited platelet glycoprotein polymorphisms and a risk for coronary heart disease in young Central Europeans. Thromb Res. 1998; 90: 117-123.
6. Kastrati A, Schöming A, Seufarth M, et al. PIA polymorphism of platelet glycoprotein IIIa and risk of restenosis after coronary stent replacement. Circulation 1999; 99: 1005-1010.
7. Feng D, Lindpaintner K, Larson MG, et al. Increased platelet aggregability associated with platelet GPIIIa PIA2 polymorphism: the Framingham Offspring Study. Arterioscler Thromb Vasc Biol. 1999; 19: 1142-1147.
8. Szczeklik A, Undas A, Sanak M, et al. Relationship between bleeding time, aspiryn and the PIA1/A2 polymorphism of platelet glycoprotein IIIa. Br J Haematol. 2000; 110: 965-967.
9. Michelson AD, Furman MI, Goldschmit-Clermont P, et al. Platelet GPIIIa PIA polymorphisms display different sensitivities to agonists. Circulation 2000; 101: 1013-1018.
10. Ardissino D, Mannucci PM, Merlini PA, et al. Prothrombotic genetic risk factors in young survivors of myocardial infarction. Blood 1999; 94: 46-71.
11. Ridker PM, Hennekens CH, Schmitz C, et al. PIA1/A2 polymorphism of platelet glycoprotein IIIa and risks of myocardial infarction, stroke, and venous thrombosis. Lancet 1997; 349: 385-388.
12. Mamotte CD, van Bockxmeer FM, Taylor RR. PIA1/A2 polymorphism of glycoprotein IIIa and risk of coronary artery disease and restenosis following coronary angioplasty. Am J Cardiol. 1998; 82: 13-16.
13. Oksala NK, Heikinnen M, Mikkelson J, et al. Smoking and the platelet fibrinogen receptor glycoprotein IIb/IIIa PIA1/A2 polymorphism interaction the risk of lacunar stroke and midterm survival. Stroke 2007; 38: 50-55.
14. Vijayan KV, Goldschmidt-Clermont PJ, Christine Roos, et al. The PIA2 polymorphism of integrin ß3 enhances outside-in signaling and adhesive functions J Clin Invest. 2000; 105: 793-802.
15. Feng D, Lindpaintner K, Larson MG, et al. Platelet glycoprotein IIIa PI (a) polymorphism, fibrinogen, and platelet aggregability: The Framingham Heart Study. Circulation 2001; 104: 140-144.
16. Undas A, Sanak M, Musiał J, et al. Platelet glycoprotein IIIa polymorphism, aspirin and thrombin generation. Lancet 1999; 353: 982-983.
17. Dropiński J, Musiał J, Sanak M, et al. Antithrombotic effects of aspirin based on PIA1/A2 glycoprotein IIIa polymorphism in patients with coronary artery disease. Thromb Res. 2007; 119: 301-303.
18. Cook GE, Liu-Straton Y, Moeschberger ML, et al. Effect of platelet antigen polymorphism on platelet inhibition by aspirin, clopidogrel, or their combination. J Am Coll Cardiol. 2006; 47: 541-546.
19. Undas A, Brummel K, Musiał J, et al. PIA2 polymorphism of β_3 integrins is associated with enhanced thrombin generation and impaired antithrombic action of aspiryn at the site of microvascular injury. Circulation 2001; 104: 2666-2672.
20. Undas A, Celinska-Löwenhoff M, Brummel-Ziedins KE, et al. Simvastatin given for 3 days can inhibit thrombin generation and activation of factor V, and enhance factor Va inactivation in hypercholesterolemic patients. Arterioscler Thromb Vasc Biol. 2005; 25: 1524-1525.
21. Undas A, Celińska-Lowenhoff M, Stępień E, et al. Effects of simvastatin on angiogenic growth factors released at the site of microvascular injury. Thromb Haemost 2006; 95: 1045-1047.
22. Undas A, Undas R, Musiał J, et al. A low dose of aspirin (75 mg/day) lowers thrombin generation to a similar extent as a high dose of aspirin (300 mg/day). Blood Coagul Fibrynolysis. 2000: 11: 231-234.
23. Undas A, Brummel K, Musiał J. Simvastatin depresses blood clotting by inhibiting activation of prothrombin, factor V, and factor XIII and by enhancing factor Va inactivation. Circulation 2001; 103: 2248-2253.
24. Henn V, Slupsky JR, Grafe M, et al. CD40 ligand on activated platelets triggers an inflammatory reaction of endothelial cells. Nature 1998; 391: 591-594.
25. Cipollone F, Mezzetti A, Porreca E, et al. Association between enhanced soluble CD40L and prothrombotic state in hypercholesterolemia: effects of statin therapy. Circulation 2002; 106: 399-402.