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Angiotensin converting enzyme DD genotype affects the changes of plasma plasminogen activator inhibitor-1 activity after primary percutaneous transluminal coronary angioplasty in acute myocardial infarction patients

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Abstract Angiotensin converting enzyme (ACE) DD genotype, and plasminogen activator inhibitor (PAI-1) 4G/4G genotype have been reported to affect PAI-1 activity in control subjects and atherosclerotic patients, but no data are available on the influence of angiotensin II type 1 receptor (AT1R) A1166C polymorphism on the inhibitor levels. The degree of fibrinolytic activation after percutaneous transluminal coronary angioplasty (PTCA) has been found to affect the risk of restenosis. The aim of this study was to investigate the possible influence of ACE I/D, AT1R A1166C, and PAI-1 4G/5G polymorphisms on the changes of PAI-1 activity after primary successful percutaneous transluminal angioplasty. In 29 consecutive acute myocardial infarction patients, undergoing primary successful angioplasty, genotyping of ACE I/D, AT1R A1166C, and PAI-1 4G/5G polymorphisms was performed by polymerase chain reaction and restriction fragment length polymorphism analysis, and PAI-1 plasma activity (chromogenic method) was assessed before and after angioplasty. Following angioplasty, PAI-1 activity increased in 10 of 29 patients and decreased or remained unchanged in 19 of 29. ACE DD genotype was significantly (P=0.04) associated with an increase of PAI-1 activity post angioplasty (OR DD/ID+II = 6.5, CI 95% 4.83-8.22). Whereas no effect of PAI-1 4G/5G and AT1R A1166C polymorphisms on PAI-1 response to angioplasty was demonstrated, these data suggest that renin-angiotensin system genes are involved in the regulation of the fibrinolytic response to balloon injury, possibly affecting angiotensin converting enzyme activity. This interaction between the renin-angiotensin system and hemostasis may be a mechanism by which ACE DD genotype affects the risk of restenosis after percutaneous transluminal angioplasty.

Key words Angiotensin converting enzyme · Plasminogen activator inhibitor-1 · Percutaneous transluminal angioplasty

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Introduction

The role of plasminogen activator inhibitor-1 (PAI-1) as a risk factor for both venous and arterial thrombotic disease has been demonstrated [1], whereas data on its involvement in the pathogenesis of restenosis after percutaneous transluminal angioplasty (PTCA) are less clear [2, 3]. In unselected patients undergoing elective PTCA, we recently demonstrated that post-procedural PAI-1 levels were significantly higher in patients with subsequent clinical recurrence owing to restenosis than in those without [4]. Namely, an earlier clinical recurrence due to restenosis was observed in those patients with an increase of PAI-1 levels within 1 h of the procedure. Several reports have suggested that the renin-angiotensin system (RAS) modulates plasminogen activation and fibrinolysis [5–11]. Recently, among subjects from a metabolic ward, individuals homozygous for deletion of two common polymorphisms (4G/5G and I/D), respectively of PAI-1 (4G/4G) and angiotensin converting enzyme (ACE) (DD) genotypes, were reported to exhibit higher levels of plasma PAI-1 antigen than those without deletions [12]. Moreover, variants of PAI-1 and ACE genes have been

demonstrated to account for a significant portion of the between-individual variability of circulating PAI-1 antigen concentrations in a general population without clinical evidence of atherosclerosis [13]. Although angiotensin II, which appears to be a major link between the RAS and fibrinolysis, has been demonstrated to modulate PAI-1 expression via an endothelial receptor specific for angiotensin IV and not via angiotensin II type 1 (AT1) or type 2 (AT2) receptors, to date no information is available about the influence of angiotensin II type 1 receptor (AT1R) A1166C polymorphism on PAI-1 levels. The aim of this study was to investigate the possible influence of ACE I/D, AT1R A1166C, and PAI-1 4G/5G polymorphisms on the changes of PAI-1 levels after primary successful PTCA.

Materials and methods

Patient selection

Twenty-nine consecutive patients (median age 60 years, range 46-75 years), with acute myocardial infarction (AMI), undergoing primary PTCA in our catheterization laboratory, were enrolled in this study. Criteria for inclusion were chest pain persisting over 30 min associated with electrocardiography demonstrating ST elevation ≥ 1 mm in two or more contiguous leads or left bundle branch block and successful primary PTCA. Exclusion criteria were inability to provide informed consent, cardiogenic shock, immunological disorders, and neoplastic disease. No patient was on treatment with ACE inhibitors or diuretics.

Coronary angioplasty

After initial coronary angiography with low-osmolar non-ionic contrast, all patients were treated with intravenous nitroglycerin, heparin (10,000 UI bolus plus 1,000 U/h) and acetylsalicylic acid (500 mg). PTCA, to treat the occluded infarct-related artery (IRA), was performed via the femoral approach with a steerable balloon catheter using the Judkins' technique [14, 15]. Coronary stenting was performed using standard techniques, including high-pressure dilatations after stent deployment. Intra-aortic balloon pumping, inserted via the opposite groin, was used in this study only for high-risk anatomy.

Blood sampling and laboratory methods

Blood was collected before and immediately after PTCA. Venipuncture was performed on the antecubital vein using a 19-G butterfly with minimal venous occlusion. The first 5 ml of venous blood was discarded. Blood was drawn directly into plastic tubes containing sodium citrate 0.129 M (1/10, v/v) for PAI-1 determination. Aliquots of citrated blood, placed in melting ice after sampling, were then centrifuged within 10 min at 1,500 *g* for 20 min at 4°C, and plasma was stored at –80°C. PAI-1 activity (control range 3-15 IU/ml) was determined according to Chmielewska et al. [16] using a commercial kit (Spectrolyse, Bio-Pool, Umea, Sweden).

Genomic DNA was isolated and purified from whole blood (from tubes containing EDTA) using QIAamp spin columns according to the manufacturer's instructions (QIAamp Blood Kit, QUIAGEN GmbH, Hilden, Germany). ACE genotypes were assessed by polymerase chain reaction (PCR) according to Rigat et al. [17]. DNA was amplified with 5% dimethylsulfoxide (DMSO) in the reaction mixture at an annealing temperature of 60°C, in order to reduce the incidence of mistyping ID as DD. Moreover, each DD genotype was subjected to a second PCR amplification without the 5% DMSO at an annealing temperature of 67°C and using a primer pair that recognizes an insertion-specific sequence. PCR products were separated by electrophoresis on a 2% agarose gel. The A1166C polymorphism at the AT1R locus was analysed using the primers and PCR conditions described by Katsuja et al. [18]. PCR products were then digested with 5 U *DdeI* restriction enzyme and separated by electrophoresis on a 2% agarose gel. The C1166 variant was identified if the *DdeI* restriction site was present.

The common guanine insertion/deletion polymorphism of the PAI-1 gene was identified by PCR amplification and digestion with the *BslI* restriction enzyme according to Margaglione et al. [12].

Angiographic analysis

The coronary flow was graded according to TIMI flow rate [19], and coronary occlusion was defined as TIMI 0 in the IRA. PTCA success was defined as ≤40% residual stenosis associated with TIMI grade 3 flow. Reocclusion was defined as greater than 90% restenosis with TIMI grade 0–1 flow. Angiograms were analyzed with a quantitative visual assessment according to a modification of the Brown-Dodge method, as previously reported [20, 21].

Follow-up

All patients were followed by clinical examination or phone call at 1 month, 6 months, and 1 year after PTCA. Ergometric testing (Bruce protocol) was performed in patients with one-vessel disease, and stress test with ^{201}Tl scintigraphy in those with two- or three-vessel disease at 1 month, 6 months, and 1 year. Reinfarction was defined as recurrent chest pain with new ST segment elevation associated with cardiac enzyme elevation. Recurrent ischemia was defined as effort or rest angina with documented ST segment or T wave changes. During the follow-up period, for ethical reasons, angiography was again performed only in patients with clinical recurrence or a positive treadmill test or a positive stress test with ^{201}Tl scintigraphy. Angiographic restenosis was defined as a decrease >50% of gain in luminal diameter achieved post PTCA.

Statistical analysis

Results are reported as median and range. The ACE, AT1R, and PAI-1 allele and genotype frequencies were obtained by direct count. The genotype distribution and allele frequencies between groups were compared by chi-squared test or Fisher's exact test. The Hardy-Weinberg equilibrium for genotype distribution was estimated by the chi-squared test. Plasma PAI-1 medians before and

after PTCA were compared using the Wilcoxon's signed rank test (continuous variables).

Logistic regression analysis was used to evaluate the influence of several variables [hypertension, body mass index (BMI), dyslipidemia, smoking habits, ACE, AT1R, and PAI-1 genotypes] on baseline and post-PTCA PAI-1 levels and on the occurrence or absence of a post-PTCA PAI-1 increase.

Results

The baseline clinical and angiographic characteristics of patients investigated are shown in Tables 1 and 2. All patients were treated 12 hours after the onset of symptoms, and 24 of 29 (82.7%) within 6 h. Primary PTCA was successfully performed in all patients enrolled in the study. Stents were delivered to the lesion and deployed in 24 (83%) of 29 patients; 1 stent was used in 19 of 24 (79%) and 2 stents in 5 of 24 (21%). In 5 patients (17%) stenting was not used due to optimal angiographic results after PTCA or vessel diameter less than 3 mm. Two patients (6.89%) underwent abrupt closure (reocclusion within 24 h) after PTCA. One needed multiple stent deployment. One pericardial tamponade, due to myocardial perforation treated with surgical repair, occurred 12 h after PTCA. No patient died during the procedure or needed emergency aortocoronary bypass surgery. After the procedure all patients underwent a noninvasive follow-up. Time between pre- and post-PTCA blood collection ranged from 45 to 65 min.

Table 1 Clinical characteristics (*CAD* coronary artery disease, *AMI* acute myocardial infarction, *HDL* high-density lipoprotein)

Age (years)	60 (range 46–75)
Family history of CAD (*n*)	17 (58.6%)
Body mass index	25.7 (range 19.1–35.1)
Smokers	18 (62%)
Hypertension	14 (48%)
Mean systolic blood pressure (mmHg)	150 (range 90–180)
Mean diastolic blood pressure (mmHg)	70 (range 70–100)
Diabetes mellitus	2 (7%)
Previous AMI	2 (7%)
Total cholesterol (mg/dl)	220 (range 126–280)
HDL cholesterol (mg/dl)	39 (range 21–71)
Triglycerides (mg/dl)	164 (range 74–900)

Table 2 Angiographic characteristics and outcomes (PTCA percutaneous transluminal coronary angioplasty)

Diseased vessels (≥70% stenosis)		
One	11	(38%)
Two	14	(48%)
Three	4	(14%)
Infarct-related artery		
Left anterior descending	13	(45%)
Circumflex artery	11	(38%)
Right artery	5	(17%)
Post-PTCA residual stenosis (%)		
<10%	21	(72.5%)
10–25%	8	(27.5%)

Follow-up

During follow-up (median 12 months, range 8–14 months) 7 patients who had experienced recurrent ischemia repeated coronary angiography. In 6 (20%) restenosis in the IRA was demonstrated: 4 patients were treated with PTCA and 2 patients with aortocoronary bypass surgery; all these patients had been treated with stenting during primary PTCA and 1 had also had abrupt reocclusion of the IRA. No patient had reinfarction.

Genetic analysis and PAI-1 plasma levels

The ACE, AT1R, and PAI-1 genotype distribution was compatible with the Hardy-Weinberg equilibrium. The genotype frequencies of the ACE, AT1R, and PAI-1 gene polymorphisms are reported in Table 3. No significant difference was found between baseline and post-PTCA PAI-1 plasma levels [baseline 8.1 IU/ml (range 3–37.1) vs. post PTCA 7.5 IU/ml (range 2.9–37.8), P=0.52)]. After PTCA, PAI-1 levels were higher than baseline in 10 of 29 patients (34%) [3.9 IU/ml (range 1.0–10.2)] and decreased or unchanged in 19 of 29 (66%) [-2.3 IU/ml (range -9.7 to 0)]. PAI-1 plasma levels before and after the procedure according to ACE DD genotype are reported in Table 4.

Univariate analysis revealed no association between baseline PAI-1 plasma levels and classical cardiovascular

Table 3 Genotype and allele frequencies of ACE, AT1R, and PAI-1 polymorphisms

ACE genotype		ACE allele		AT1R genotype		AT1R allele		PAI-1 genotype		PAI-1 allele	
DD	0.41	D	0.64	CC	0.07	C	0.33	4G/4G	0.41	4G	0.60
ID	0.45			AC	0.52			4G/5G	0.38		
II	0.14	I	0.36	AA	0.41	A	0.67	5G/5G	0.21	5G	0.40

Table 4 Plasminogen activator inhibitor-1 (*PAI-1*) plasma levels before and after PTCA according to ACE DD genotype

ACE genotype	Baseline PAI-1 levels (IU/ml)	*P*	Post PTCA PAI-1 levels (IU/ml)	*P*
DD	8.4 3-21.9		12.1 3.4–22.4	
		0.69		0.09
ID+II	8.1 3.4–37.1		4.9 2.9–37.8	

risk factors (hypertension, BMI, smoking habits, dyslipidemia) (Table 5). Moreover, baseline PAI-1 plasma levels were not associated with ACE, AT1R, and PAI-1 genotypes. The occurrence of post-PTCA plasma PAI-1 levels above median level (7.5 IU/ml) was significantly associated with ACE DD genotype [OR DD/ID+II=5.49 (CI 95% 3.85–7.13) P=0.05], but not with the other risk factors nor genotypes (Table 5).

Moreover, ACE DD genotype was significantly associated with the occurrence of a PAI-1 increase after PTCA [OR DD/ID+II=6.53 (CI 95% 4.83–8.22) P=0.04] (Table 6). No significant association was found between either AT1R or PAI-1 genotypes and post-PTCA PAI-1 change [OR CC/AC+AA=2.1 (CI 95% 0.5–3.71) P=0.38; OR 4G/4G vs. 4G/5G+5G/5G=0.5 (CI 95% -1.14–2.10) P=0.38]. Neither were plasma PAI-1 variations post PTCA related to other risk factors (Table 6). The presence of ACE DD genotype and AT1R C allele did not significantly influence the post-PTCA PAI-1 level increase [OR DD/AC+CC vs. ID+II/AC+CC 3.33 (CI 95% 0.45–24.45) P=0.35]. Multivariate analysis adjusted for BMI, hypertension, smoking habits, dyslipidemia, AT1R (CC+AC versus AA), and PAI-1 (4G/4G versus 4G/5G+5G/5G) genotypes confirmed ACE DD genotype as an independent predictor of post-PTCA PAI-1 increase [OR 10.78 (CI 95% 8.5–13.06) P=0.05].

During follow-up, 6 of 29 (20%) patients had clinical recurrence with confirmed angiographic restenosis; 4 of them were ACE DD homozygotes and had a PAI-1 plasma level increase post PTCA.

Table 5 Association of clinical and genetic factors with PAI-1 levels (IU/ml) (above vs. below value) (*OR* odds ratio, *CI* confidence interval)

	Baseline PAI-1 levels (IU/ml)		Post PTCA PAI-1 levels (IU/ml)	
Variable	OR (CI 95%)	*P*	OR (CI 95%)	*P*
BMI>25	0.35 -1.19 to 1.89	0.19	0.35 -1.19 to 3.5	0.19
Hypertension	0.80 -1.58 to 2.38	0.78	0.80 -0.78 to 2.38	0.78
Smoking habits	3.66 2.10 –5.21	0.11	1.12 -0.36 to 1.6	0.87
Dyslipidemia	1.09 -0.71 to 2.88	0.92	2.60 0.72–4.48	0.33
ACE (DD vs. ID+II)	2.86 1.32–4.40	0.19	5.49 3.85–7.13	0.05
AT1R (CC+AC vs. AA)	1.99 0.49–3.49	0.37	3.66 2.14–5.18	0.11
PAI-1 (4G/4G vs. 4G/5G+5G/5G)	0.89 -0.59 to 2.37	0.88	0.88 -0.6 to 2.36	0.87

Table 6 Association of clinical and genetic factors with the occurrence or absence of a post-PTCA PAI-1 increase (univariate analysis)

Variable	OR (CI 95%)	*P*
BMI >25	0.58 -0.97 to 2.13	0.50
Hypertension	0.28 -1.38 to 1.93	0.13
Smoking habits	1.09 -0.47 to 1.88	0.91
Dyslipidemia	1.07 -0.83 to 2.97	0.94
ACE (DD vs. ID+II)	6.53 4.83–8.22	0.04
AT1R (CC+AC vs. AA)	2.10 0.48–3.71	0.38
PAI-1 (4G/4G vs. 4G/5G+5G/5G)	0.48 -1.14 to 2.10	0.38
ACE DD/AT1R AC+CC combined genotype vs. ID+II/AT1R AC+CC combined genotype	3.33 0.45–24.45	0.35

Discussion

The results of this study suggest that RAS genes are involved in the regulation of the fibrinolytic response to balloon injury, possibly influencing ACE activity. ACE I/D polymorphism has been found to be associated with several ischemic cardiovascular disorders [22–25] and in this study, in keeping with previous results, the prevalence of the ACE D allele was higher than observed in a healthy population from the same region [22]. Otherwise, AT1R A1166C genotype and allele frequencies were similar to those observed in a healthy population from the same region [22].

Balloon injury is followed by a rapid activation of the fibrinolytic system, documented by euglobulin lysis time shortening, PAI-1 activity decrease, and D-dimer increase [4]. However, some patients showed an increase in PAI-1 activity shortly after PTCA, and among these patients restenosis-related clinical recurrence was significantly more frequent [4]. Among the possible determinants of this behavior, in this preliminary study, we observed that the regulation of PAI-1 activity after PTCA is influenced by genetic factors. In particular, DD genotype of I/D ACE gene polymorphism is significantly and independently related to an increase in PAI-1 activity after PTCA. RAS plays an important role in the regulation of the fibrinolytic system. Namely, angiotensin II stimulates PAI-1 production [7, 26] and ACE, via bradykinin, may influence tissue-type plasminogen activator (t-PA) secretion from the vascular endothelium [27, 28]. Clinical studies have confirmed the link between ACE and fibrinolysis: angiotensin II infusion causes an increase in PAI-1 plasma levels [8] and bradykinin infusion selectively increases plasma t-PA levels in a dose-dependent manner [29]. Captopril treatment is found to reduce both ACE activity and PAI-1 plasma levels in patients with recent myocardial infarction [10]. Interestingly, short-term ACE inhibition by enalapril was found to influence the fibrinolytic response to stimulation such as physical exercise, with a post-exercise decrease in PAI-1 levels in healthy subjects [30]. It is noteworthy that no significant change in baseline PAI-1 activity was induced by enalapril, as recently confirmed in healthy subjects treated with captopril [31], but differences were clear-cut after physical exercise. In our study, the lack of PAI-1 increase in 10 of 29 patients cannot be attributed to ACE inhibitor administration, as no patient was on treatment.

ACE DD genotype has been found to be associated with increased PAI-1 levels [13, 32] in healthy subjects. In our small group of AMI patients, the genotype and allele frequencies of PAI-1 polymorphism resembled those reported in other Caucasian populations; 4G/5G polymorphism of the PAI-1 gene was not significantly associated with baseline PAI-1 activity, whereas this polymorphism was found to be independently related to PAI-1 antigen levels in healthy subjects [13]. However, due to differences in the subjects inves-

tigated and PAI-1 assay, the two studies cannot be compared. At variance with the ACE I/D polymorphism, in the present study, the PAI-1 4G/5G polymorphism was not related to fibrinolytic response after balloon injury. However, the activation of inflammatory processes in AMI patients undergoing PTCA may have influenced the fibrinolytic response via different mechanisms that could mask the effect of 4G/5G polymorphism. No effect of AT1R A1166C polymorphism on PAI-1 response to PTCA has been demonstrated. PTCA injury in humans results in the upregulation of ACE at sites of active repair [33], and studies on experimental animals have demonstrated that high-dose ACE suppression with benazepril can inhibit intimal hyperplasia, which plays a relevant role in restenosis [34]. Moreover, in humans, the ACE content of atherosclerotic plaques has been shown to be associated with restenosis after PTCA or directional atherectomy [35].

Our data are consistent with those of Ribichini et al. [36] who, in a selected cohort of patients with ischemic heart disease, found a significant association of both the DD genotype and high plasma activity of ACE with in-stent restenosis, whereas ACE polymorphism seems to have no effect on restenosis after balloon PTCA [37, 38]. Interestingly, in our study 4 of 6 patients with clinical recurrence and angiographically proven restenosis had a PAI-1 increase immediately after PTCA and were ACE DD homozygotes.

A number of limitations of the present study should be recognized. First, it included a relatively small number of patients, so these results need to be confirmed by more-extensive studies. In particular, the lack of association between either PAI-1 4G/5G or AT1R A 1166C (especially the latter) and PAI-1 activity might be due to the limited number of patients.

Moreover, only PAI-1 activity (and not PAI-1 antigen, which gives a better estimate of the total inhibitor present in plasma) was measured. However, we were interested in PAI-1 activity because in a previous investigation [4] its behavior after elective balloon PTCA was demonstrated to be predictive of clinical restenosis.

In conclusion, these data suggest that RAS genes are involved in the regulation of the fibrinolytic response to balloon injury, possibly by influencing ACE activity. In view of the role of PAI-1 in restenosis after PTCA [4], knowledge of the genetic control of PAI-1 increase may be of importance to identify new therapeutic targets and new preventive strategies [39].

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