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Circadian Fluctuations of Plasminogen Activator Inhibitor and Tissue Plasminogen Activator Levels in Plasma of Patients with Unstable Coronary Artery Disease and Acute Myocardial Infarction

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Key words

Circadian variation – Tissue plasminogen activator – Plasminogen activator inhibitor – Unstable coronary artery disease

Summary

A decrease in the fibrinolytic potential, mainly due to an elevation of plasminogen activator inhibitor (PAI), has been described in patients with stable coronary artery disease and a previous myocardial infarction. We investigated plasma levels of PAI and tissue plasminogen activator (t-PA) and their possible circadian variations in patients with unstable coronary artery disease (CAD). Sixty-three patients were studied for at least 2 consecutive days during their stay at the coronary care unit (CCU). Diurnal plasma fluctuations in PAI and t-PA and onset of further myocardial ischemic episodes were monitored. As controls we used 22 age-matched patients submitted to the clinic because of non cardiac chest pain or valvular disease who revealed no evidence of CAD. PAI levels were significantly elevated in patients with unstable CAD ($p < 0.0001$) but were not influenced by the extent of underlying CAD, history of previous myocardial infarction, known risk factors for CAD, or by extent of myocardial damage. The circadian variation of PAI levels with peak values between midnight and 6 A.M. found in controls was still present in patients but at a higher level. Preservation of circadian pattern in PAI plasma levels despite myocardial ischemic attacks indicates that elevation of PAI is rather not caused by a reactive phenomenon. On the other hand, elevated PAI levels and episodes of severe myocardial ischemia exhibiting a median time of onset at 10 A.M. seem to be closely related.

Introduction

In a recent study by Muller et al. (1) it was demonstrated that the time of onset of nonfatal myocardial infarction exhibits a marked circadian variation with the highest incidence between 6 A.M. and noon. These authors suggested that the timing of myocardial infarction might result from a variation in the tendency to thrombosis. Additional studies on the distribution of sudden cardiac death (2) and transient myocardial ischemia (3) also show a remarkable similar pattern with a low incidence during night and an increased incidence from 7 to 11 A.M. On the other hand, it is now clarified that myocardial infarction is caused mainly by coronary thrombi (4–13) and only in rare cases exclusively by vasospasm (14). Furthermore, a thrombotic tendency can be linked to impaired fibrinolysis and in fact it has been discussed in patients with stable coronary artery disease that reduced fibrinolytic capacity due to increased plasma levels of the fast acting plasminogen activator inhibitor (PAI) might be of pathogenetic importance for myocardial infarction (15–18). However, there are no sufficient data available on PAI and t-PA levels in patients with unstable CAD and acute myocardial infarction (AMI). It was the aim of this study to investigate PAI and t-PA levels and their possible circadian variation known to exist in healthy young volunteers (19), in patients with unstable coronary artery disease.

Subjects, Materials and Methods

Subjects

Sixty-three patients who had been admitted to the coronary care unit (CCU) because of severe myocardial ischemia of at least 15 min duration were studied. They were part of a series of 85 consecutive patients presenting with unstable angina at rest (New York Heart Association Class IV; 20) at our clinic between November 1985 and November 1986. Twenty-two patients underwent acute fibrinolytic therapy and/or acute percutaneous transluminal coronary angioplasty and were excluded from the study. Patients in the study were free of infectious or malignant diseases. None of the drugs given to the patients (routinely nitrates and the calcium-antagonist nifedipine) are known to interfere with values of fibrinolytic parameters; furthermore, we could not find any changes in PAI or t-PA levels upon initiation of treatment in 24 patients not treated with antianginous therapy before their admission to the CCU. Coronary artery disease (CAD) was verified in most patients (n = 52) by coronary arteriography which was not performed during days of blood collection but 1 to 6 weeks after admission to the clinic and in few cases (n = 11) by the history of a previous Q-wave infarction or clinical evidence of acute myocardial infarction. Characteristics of the patients including risk factors and results of coronary arteriography are summarized in Table 1. For control purposes we studied an age matched group of patients during their hospitalization for heart catheterization either because of non cardiac chest pain (n = 10) or because of evaluation of valvular disease (n = 12); in this group underlying CAD was excluded by a negative angiography which was performed the day after blood collection. Characteristics of controls are also given in Table 1. Since it could be shown previously that sex differences do not influence results of t-PA antigen and PAI activity, the significant differences in the sex ratio of controls and patients could be neglected. The study was approved by the Local Medical Ethics Committee.

Methods

Blood Collection

Blood was drawn from patients during their stay at the CCU each day at four different time points (6 A.M., noon, 6 P.M., midnight) for at least

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Table 1 Characteristics of CAD patients and controls

	CAD patients	Controls
n	63	22
Sex (M/F)	49/14	10/12
Age (x ± S.D.)	59.9 ± 10.8	59.0 ± 8.8
Body weight index	26.0 ± 1.51	25.7 ± 1.1
Previous MI n (%)	31 (49.2)	–
Acute MI n (%)	36 (57.1)	–
Heparin n (%)	15 (23.8)	–
Number of vessels with >70% stenosis (n of patients)	0 (1)	–
	1 (18)	–
	2 (11)	–
	≧3 (23)	–
	n.d. (10)	
Risk factors		
Smoking n (%)	32 (50.8)	7 (31.8)
Hypertension n (%)	23 (36.5)	1 (4.5)
Hyperlipidemia (type II) n (%)	18 (28.6)	7 (31.8)
Diabetes (type II) n (%)	8 (12.7)	–

n.d. denotes not determined

two consecutive days from an antecubital vein using a 1.2 mm siliconized needle with minimal venous occlusion. After discarding the first ml, 7 ml of blood were drawn directly into plastic tubes prepared with EDTA (final concentration 5.0×10^{-2}M). Blood was centrifuged immediately (15° C, 3000 × g, 10 min) and plasma was stored at −70° C until used. From the control group blood was drawn at the same time points during one day in course of their stationary stay at the clinic.

Determination of t-PA Antigen and PAI Activity

Plasminogen activator inhibitor activity was determined according to the functional titration assay described by us (21). Tissue plasminogen activator antigen was measured by means of a sandwich ELISA technique described previously which detects t-PA and t-PA-PAI complexes (22). t-PA activity was not determined because t-PA activity cannot be detected in plasma samples obtained without stimulation e.g. by venous occlusion. Venous occlusion or another t-PA stimulation test was not performed in these severely ill patients because of ethical reasons.

Table 2 PAI activity and t-PA antigen concentration in the groups studied

	CAD patients	controls	p-value*
Number of patients	63	22	
PAI activity** IU/ml ($\bar{x}$ ± S.E.)			
6 A.M.	15.3 ± 0.7**	7.6 ± 0.4**	<0.0001
Noon	13.6 ± 0.8	5.7 ± 0.3	<0.0001
6 P.M.	12.6 ± 0.6	6.4 ± 0.5	<0.0001
Midnight	13.8 ± 1.3	6.5 ± 0.4	<0.0001
t-PA antigen ng/ml ($\bar{x}$ ± S.E.)			
6 A.M.	10.5 ± 0.6**	10.4 ± 1.0**	n.s.
Noon	8.9 ± 0.6	9.8 ± 0.6	n.s.
6 P.M.	9.9 ± 0.7	7.9 ± 0.7	n.s.
Midnight	9.4 ± 0.9	7.7 ± 0.8	n.s.

n.s. = not significant

* Significance between CAD patients and controls was calculated by means of the unpaired t-test. In both groups t-PA and PAI values could be shown to be normally distributed.

** The diurnal variation in PAI activity in CAD patients and controls was statistically significant at that time point as calculated by means of the ANOVA test.

Other Measurements

Whole serum cholesterol and triglycerides as well as creatine kinase (CK) and MB fraction of CK were measured enzymatically by a multianalyzer system (Hitachi Automatic Analyzer 705, Boehringer Mannheim, FRG).

Determination of Myocardial Ischemia

Severe ischemic episodes occurring during the stay of the patients at the CCU were recorded and verified by typical electrocardiographic changes indicating ischemia. These included either transient ST segment elevation or ST segment depression of more than 1.5 mm or T-wave inversion or pseudonormalization. Acute myocardial infarction was defined as an increase in the MB fraction of creatine kinase of 10% or more.

Statistical Analysis

To evaluate significant differences in t-PA and PAI levels at the different time points of blood collection, analysis of variance (ANOVA) was performed; in order to determine which time points differed significantly, we used the Duncan procedure on a posterior test. To test the significance of differences in t-PA and PAI levels between patients with or without acute myocardial infarction, or with and without previous myocardial infarction, we used the unpaired t-test. We also performed non-parametric tests to exclude differences in the distribution pattern of a variable in the different groups.

Possible changes of t-PA and PAI levels depending on the presence or absence of different risk factors (smoking, hypertension, hyperlipidemia, diabetes mellitus) were calculated using a descriptive frequency-counting procedure which also tested for homogeneity or independence of the 2-way table (Chi-square tests).

To estimate a correlation between variables, Pearson's product-moment correlation coefficient and two nonparametric measures of association (Spearmen's rank-order correlation and Kendall's tau-b) were calculated. All analyses were performed using a computer program (SAS package, version 5.16).

Results

Assessment of PAI Activity in CAD Patients and Controls

PAI activity showed diurnal variations in CAD patients as well as in the control group. In CAD patients values at 6 A.M. were highest, decreased until noon, were lowest at 6 P.M. and increased slightly until midnight. Table 2 shows PAI values for CAD patients and controls. Comparing CAD patients with controls, PAI activities were significantly higher in CAD patients. The diurnal variation of PAI concentrations in CAD patients and age-matched controls was evaluated by demonstrating a significant variation of PAI values for the different collection times whereby the value at 6 A.M. reached a significance of $p < 0.0001$. The acrophase could be calculated to be between midnight and 6 A.M.

Assessment of t-PA Antigen in CAD Patients and Controls

As indicated before, t-PA antigen concentrations were measured only in samples of non-occluded plasma. t-PA antigen concentrations for CAD patients and controls are given also in Table 2. t-PA concentrations in CAD patients were not different to t-PA concentrations in age matched controls; there was a significant diurnal variation of t-PA antigen in CAD patients, and in controls with significantly elevated plasma levels of t-PA antigen in the morning ($p < 0.0001$) and a continuous decrease of the values until evening.

Table 3 PAI and t-PA plasma levels in relation to the extent of coronary artery stenosis in CAD patients

Number of vessels with stenosis of >70%	1	2	≧3
Number of patients	18	12	23
PAI activity IU/ml ($\bar{x} \pm$ S.E.)			
6 A.M.	13.6 ± 1.0	13.9 ± 1.7	17.1 ± 1.2
Noon	13.4 ± 1.1	12.1 ± 2.3	13.7 ± 1.0
6 P.M.	14.0 ± 1.6	10.6 ± 1.4	13.9 ± 0.9
Midnight	12.8 ± 1.3	7.6 ± 1.3	16.6 ± 3.2
t-PA antigen ng/ml ($\bar{x} \pm$ S.E.)			
6 A.M.	7.6 ± 0.5*	11.0 ± 1.5	12.0 ± 0.9*
Noon	7.2 ± 0.1	7.8 ± 0.8	10.3 ± 1.2
6 P.M.	6.8 ± 0.6*	8.5 ± 1.2	11.4 ± 1.2*
Midnight	7.1 ± 0.3	4.7 ± 0.6	9.9 ± 1.9

* Denotes significant differences ($p < 0.05$) as calculated by means of the unpaired t-test between the groups of CAD patients with 1, 2 or 3 vessels disease

Table 4 Influence of the history of previous myocardial infarction (PMI) on PAI and t-PA plasma levels

	no PMI	PMI	p-value*
Number of patients	32	31	
PAI activity IU/ml ($\bar{x} \pm$ S.E.)			
6 A.M.	15.3 ± 0.9	15.3 ± 1.1	n.s.
Noon	14.2 ± 1.2	13.2 ± 1.0	n.s.
6 P.M.	13.3 ± 0.8	11.7 ± 0.9	n.s.
Midnight	11.6 ± 0.8	16.3 ± 2.7	n.s.
t-PA antigen ng/ml ($\bar{x} \pm$ S.E.)			
6 A.M.	9.5 ± 0.8	11.8 ± 0.9	n.s.
Noon	7.8 ± 0.8	9.8 ± 0.9	n.s.
6 P.M.	9.7 ± 0.9	10.2 ± 1.2	n.s.
Midnight	7.1 ± 0.3	10.8 ± 1.7	n.s.

Table 5 PAI and t-PA plasma levels in patients with unstable CAD with or without development for acute myocardial infarction

	Unstable CAD without AMI	with AMI	p-value*
Number of patients	27	36	
PAI activity IU/ml ($\bar{x} \pm$ S.E.)			
6 A.M.	13.4 ± 0.9	16.5 ± 0.9	<0.02
Noon	11.8 ± 1.0	14.6 ± 1.1	n.s.
6 P.M.	11.6 ± 1.1	13.2 ± 0.7	n.s.
Midnight	7.7 ± 0.5	16.0 ± 1.6	<0.001
t-PA antigen ng/ml ($\bar{x} \pm$ S.E.)			
6 A.M.	11.4 ± 1.3	10.1 ± 0.6	n.s.
Noon	9.7 ± 1.3	8.5 ± 0.7	n.s.
6 P.M.	10.2 ± 1.4	9.7 ± 0.8	n.s.
Midnight	6.7 ± 0.9	10.4 ± 1.2	n.s.

n.s. = not significant.
* Significance was calculated by means of the unpaired t-test between values obtained for CAD patients with or without PMI or with or without AMI.

Influence of Severity of Coronary Artery Disease or History of Previous Myocardial Infarction on PAI and t-PA Values

Table 3 shows PAI values in CAD patients with high degree stenosis (>70%) of one, two or three and more coronary arteries. No significant difference between one-, two- or three vessels disease could be calculated but there was a tendency for PAI values to be increased with the number of vessels involved. t-PA antigen values were only significantly elevated in CAD patients with three vessels disease as compared to one vessel disease and only for the 6 A.M. and 6 P.M. blood samples ($p < 0.05$). A history of previous myocardial infarction (PMI) had no significant influence on PAI activities or t-PA antigen concentrations (Table 4).

Influence of Known Risk Factors for Coronary Artery Disease on Fibrinolytic Parameters

In CAD patients the risk factors smoking, hypertension, diabetes mellitus, and hyperlipidemia showed no significant correlation with t-PA antigen or PAI activity values.

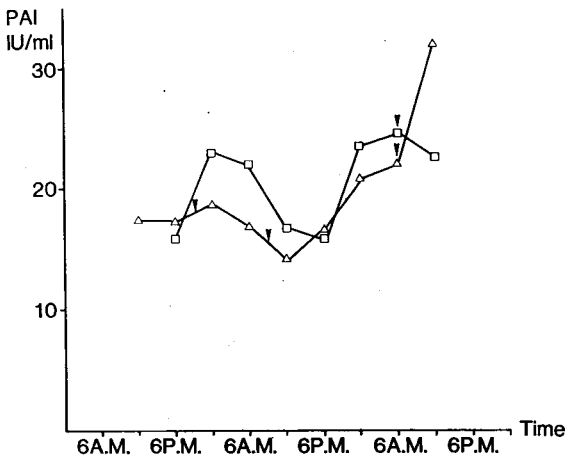

Fig. 1 PAI plasma levels in 2 patients with acute myocardial infarction. During observation period patients exhibited further ischemic attacks indicated by arrows (↓)

PAI and t-PA Values in CAD Patients with and without Acute Myocardial Infarction

PAI activities in CAD patients who developed acute myocardial infarction (AMI) (n = 36) were significantly elevated at the midnight ($p < 0.001$) and the 6 A.M. values ($p < 0.02$) as compared to CAD patients without signs of AMI (n = 27, Table 5). However, in CAD patients with signs of AMI a significant diurnal variation was still conserved. In addition, there was no correlation between maximal plasma levels of creatine kinase and the corresponding PAI values ($r = 0.0585$, $p = 0.7162$) (data not shown); t-PA values were also in those patients unchanged.

Onset of Myocardial Ischemia and PAI Levels

As shown for two individual patients who developed further myocardischemic attacks during the observation period, three out of the four ischemic attacks observed were not followed by a significant increase in PAI levels; the diurnal fluctuation of PAI levels remained – despite being on a higher level – obviously unaltered by the ischemic attacks (Fig. 1). However, myocardischemic attacks themselves followed a circadian pattern. In total, 39 ischemic attacks could be monitored excluding the initial attack

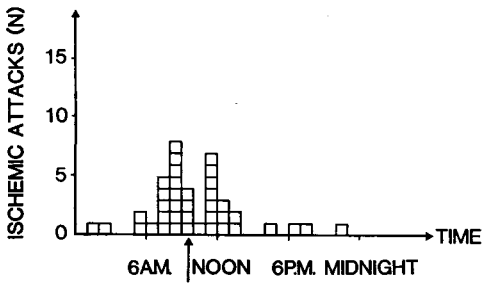

Fig. 2 Circadian variation of the onset of myocardial ischemic attacks. □ denotes a single ischemic attack. The arrow (↑) indicates the time median of ischemic attack

having led to the admission of the patients to the CCU. 26 (66.6%) of the attacks started between 6 A.M. and noon, 4 (10.2%) between midnight and 6 A.M., 7 (17.9%) between noon and midnight. The time median of attacks in all patients was at 10 A.M. (Fig. 2).

Discussion

More than 90% of myocardial infarctions are caused by coronarthrombotic occlusion (4–12). Furthermore, in recent studies determination of fibrin degradation products (13) or fibrin and fibrinogen-related antigens (23) have shown evidence for the presence of acute thrombosis in unstable coronary artery disease and acute myocardial infarction. An increased tendency to thrombosis can be explained by several mechanisms including increase in platelet aggregation (24, 25) and activation of the clotting system (25–30). However, the main cause of thrombus formation is thought to be due to a defective fibrinolytic system (15, 31–34). Reduction of the fibrinolytic potential can be caused by two major mechanisms, either by an impaired potency of endothelial cells to release t-PA or by an increase in the plasma concentrations of PAI (31). The development of specific and sensitive assay system for t-PA and PAI has made it possible to investigate the role of these major determinants of the fibrinolytic system in diseases associated with thrombus formation. For example, an impaired t-PA release could be shown to be responsible for the defective fibrinolysis in about 10% of patients with recurrent deep vein thrombosis (33). On the other hand, chronic CAD has been shown to correlate more with elevated plasma PAI-levels (16–18).

We investigated whether PAI levels are also increased in patients with acute CAD. In fact, it could be shown that PAI levels were significantly increased in patients as compared to age matched controls who were also hospitalized and under comparable stress conditions. In contrast to studies on patients with chronic CAD (16–18), we investigated exclusively patients with unstable angina during their stay at the CCU. Therefore, the group of patients studied should relatively be unaffected by influences outpatients are usually affected with. In addition, we used an age matched control group of patients who were also hospitalized at the same clinic; these control patients also underwent coronary angiography but were found to show no evidence of CAD. Therefore, the elevation of PAI levels in CAD patients should rather correlate with the CAD than with other factors. To further exclude that the increase in PAI levels is caused by an unspecific phenomenon in these severely ill patients, we investigated the diurnal variation of PAI levels over 48 hours. Diurnal fluctuations of PAI levels had up to now only been studied between 9 A.M. and 3 P.M. in healthy individuals (19) while our study extends available data on PAI levels to the whole day. Thereby a diurnal variation of PAI values was found in controls and in CAD patients with peak PAI values in the early morning. A conserved diurnal variation of PAI levels in patients, however, makes it unlikely that elevation of PAI is a non specific acute phase phenomenon.

Similarly to data published for patients with chronic CAD (16–18) we could also not demonstrate significant differences in PAI activity between patients with one, two or three vessels disease nor did we find such differences between patients with or without previous myocardial infarction. In the CAD patients elevation of PAI levels or alteration in t-PA antigen concentration did furthermore not correlate to known risk factors for CAD: we were neither able to demonstrate a correlation between low t-PA antigen levels and chronic smoking as described by Allen et al. (36) nor did we find significant correlation between serum triglycerides and PAI levels as did others (16, 37). This failure to demonstrate a correlation between triglycerides and PAI in both hospitalized groups might be explained by the fact that the majority of triglyceride levels was within the normal range, likely due to the low fat diet given at the hospital. We did not prove such a correlation in outpatients of our clinic with known CAD and therefore cannot exclude it.

The mechanism for the elevation of PAI in patients with unstable coronary artery disease is not known. First, the possibility of an acute phase reaction as one could expect due to the emotional stress occurring in patients suffering from recurrent chest pain and which has been described to exist in different other diseases (38), and also in acute myocardial infarction (39) has to be discussed. However, an acute phase reaction would have induced alterations of PAI levels depending on the time of onset of chest pain and therefore would have altered the typical circadian variation of PAI levels as could be shown in this study and by others (19). In contrast, elevation of PAI levels in unstable CAD and acute myocardial infarction was independent on the onset of the ischemic attack, because fluctuation of PAI levels remained unchanged during and after a single myocardischemic event (Fig. 1). Furthermore, control patients with non cardiac chest pain exhibited normal PAI activities in plasma. There was also no significant effect of the size of acute myocardial infarction as judged by maximal creatine kinase levels and the corresponding PAI levels making it unlikely that PAI elevation might have originated from myocardial damage in patients with AMI. It is, however, unclear why at one occasion PAI levels increased after an ischemic attack. This ischemic attack was not different from the other two observed in the same patient which were not followed by a PAI level increase.

From our data it is therefore not likely that elevation of PAI levels in acute CAD is predominantly a reaction of the organism to the ischemic event. In contrast, in our patients the median time of onset of ischemic episodes was at 10 A.M. (Fig. 2) and therefore within the same time range found in other studies (1, 2). The peak levels of PAI therefore seem to precede the median time of onset of ischemic episodes by several hours. However, at that time the coronary thrombus formation might have been initiated. Although this study does not prove such a causal relationship, a temporarily increased thrombotic tendency based on PAI elevation can be discussed as an important mechanism contributing to coronary thrombus formation.

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