Circadian variation in fibrinolytic activity in patients with variant angina

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Abstract
Background—Coronary artery spasm induces activation of the coagulation system. Turnover and maintenance of thrombus depend not only on formation but also on lysis. The relation between coronary spasm and fibrinolytic system has not been elucidated.
Objective—To examine whether there is impairment of or a circadian variation in fibrinolytic activity in patients with variant angina.
Methods—Plasminogen activator inhibitor (PAI) activity and concentrations of tissue plasminogen activator (t-PA) antigen were measured in venous plasma samples taken at 2200, 0600, and 1400 and 24 h Holter tapes were recorded in 15 patients with variant angina, 12 patients with stable exertional angina, and 12 controls.
Results—There were significant circadian variations in PAI activity and t-PA antigen with peak values at 0600 in all three groups. Mean (SEM) PAI activity (IU/ml) at 2200, 0600, and 1400 was 6·1 (1·1), 11·0 (1·3), and 4·4 (0·6) in the variant angina group; 1·8 (0·7), 5·6 (1·1), and 1·2 (0·3) in the stable exertional angina group; and 1·1 (0·5), 4·5 (0·8), and 0·7 (0·3) in the control group. Furthermore, both plasma PAI activity and t-PA antigen concentrations were significantly higher in the variant angina group than in the stable exertional angina group and the control group at each sampling time.
Conclusions—In patients with variant angina there was a circadian variation in fibrinolytic activity, which was lowest in the early morning, and impaired fibrinolytic activity particularly in the early morning, when attacks of angina occur most frequently.

(*Br Heart J* 1994;71:156–161)

Variant angina is caused by coronary artery spasm,[1 2] which has also been implicated in the pathogenesis of unstable angina and acute myocardial infarction.[3 4] Coronary thrombosis also plays an important part in the production of unstable angina or acute myocardial infarction.[5 6] The increased tendency to thrombosis can be explained by several mechanisms including increase in platelet aggregation,[7] activation of the coagulation system,[8 9] and a defective fibrinolytic system.[10]

Does coronary artery spasm trigger thrombus formation in the coronary artery? We found that coronary artery spasm induced activation of the coagulation system.[11–13] Turnover of thrombus depends not only on formation but also on lysis, however. The key components of the fibrinolytic system are tissue plasminogen activator (t-PA) and plasminogen activator inhibitor (PAI). t-PA promotes fibrinolysis,[14] whereas its specific inhibitor, PAI, rapidly inhibits t-PA by complexing with it.[15] PAI activity, mainly as PAI-1 activity, is a major determinant of overall fibrinolytic activity.[16 17] We showed that PAI activity increased in patients with acute myocardial infarction and unstable angina.[18 19] This confirmed the findings of other investigators.[20–22] In the present study we measured plasma PAI activity and t-PA antigen to see whether there is impairment or a circadian variation in the fibrinolytic system in patients with variant angina. We also examined the relation between the circadian variation in the fibrinolytic system and ischaemic attacks.

Patients and methods
PATIENTS
We studied 39 patients who underwent diagnostic cardiac catheterisation (32 men and seven women; mean (SEM) age 60·4(1·1), range 48–73). They were divided into three groups: the variant angina group, the stable exertional angina group and controls.

The variant angina group consisted of 15 consecutive patients with variant angina. All patients had attacks of chest pain at rest, usually occurring from midnight to early morning and associated with reversible ST segment elevation on electrocardiogram (ECG). We excluded patients with ST changes lasting more than 30 minutes, with new Q waves, or with raised plasma concentrations of creatine kinase or creatine kinase MB.

The stable exertional angina group consisted of 12 patients who had typical chest pain on exertion associated with horizontal or downsloping ST segment depression of greater than 1·0 mm at 80 ms after the J point. All patients in this group had experienced angina attacks at least once a month before the study. None had episodes of rest angina or showed ischaemia during a hyperventilation test.

The control group consisted of 12 patients with atypical chest pain not accompanied

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Table 1   Characteristics of the study groups (mean (SEM))

Characteristic	Variant angina	Stable exertional angina	Controls
No. of patients	15	12	12
Age (yr):			
Mean (SEM)	60·0 (1·6)	60·8 (2·2)	60·7 (2·0)
Range	48–69	51–72	49–73
Male/female	13/2	10/2	9/3
Previous myocardial infarction (n)	1	2	0
Blood pressure ≥150/90 mm Hg (n)	7	5	6
Smokers (n)	12	9	9
Diabetes mellitus (n)	3	2	1
Obesity (n)	2	1	1
Serum cholesterol (mg/dl)	200 (8)	217 (10)	194 (10)
Serum triglyceride (mg/dl)	176 (22)	143 (14)	144 (17)
Extent of coronary vessel organic stenosis ≥75% stenosis (m):			
0-vessel	12*	0	12
1-vessel	1	5	0
2-vessel	2	6	0
3-vessel	0	1	0
Coronary artery spasm (n):			
Single vessel spasm	7*	0	0
Multivessel spasm	8*	0	0
Ejection fraction <50% (n)	1	0	0
Medication used (n):			
β Blockers	0	1	0
Long acting nitrates	1	3	0
Calcium antagonists	3	3	0
Aspirin	1	1	0

*p < 0·01 v stable exertional angina.

with electrocardiographic changes of ischaemia on a treadmill exercise test and hyperventilation test.

The stable exertional angina group and the controls were selected from patients presenting for elective cardiac catheterisation during the same period and matched for age, sex, and other clinical variables with the group with variant angina. Table 1 shows the clinical characteristics of the three groups.

None of the patients was treated with thrombolytic agents such as urokinase, streptokinase, or alteplase, or anticoagulants or steroids. None of them had thromboembolism, collagen disease, disseminated intravascular coagulation, advanced liver disease, renal failure, malignant disease, septicaemia or other inflammatory disease. None of them had a prosthetic heart valve or a pacemaker.

STUDY PROTOCOL

Blood samples were obtained from patients every 8 h at 2200, 0600, and 1400. All patients underwent 24h Holter recording with a Del Mar Avionics (Irvine, California) model 447 two-channel recorder for at least 24 hours on the day of blood sampling. Furthermore, in seven patients with variant angina we obtained additional blood samples before (at 0600 or 1400) and within 15 minutes after attacks that occurred between 0600 and 0700 or between 1400 and 1500. An ischaemic attack was defined as more than 2·0 mm ST segment elevation or more than 1·0 mm horizontal or downsloping ST segment depression 80 ms after the J point that lasted for at least a minute.

The study protocol accorded with the guidelines of the ethics committee at our institution. Informed consent was obtained from each patient.

BLOOD SAMPLING

After admission blood samples were taken by venepuncture by specially trained physicians (TM and YM) who were evaluated by quality control procedures before and during the study.

The first 3 ml of blood was discarded, then 4·5 ml of blood was drawn directly into glass tube containing 0·5 ml of 3·8% buffered citrate solution and processed immediately. Samples were centrifuged immediately at 2000 × g for 15 minutes at 4°C to obtain platelet poor plasma and the plasma was stored at −80°C.

DETERMINATION OF t-PA ANTIGEN CONCENTRATION AND PAI ACTIVITY

We measured t-PA antigen by an enzyme linked immunosorbent assay (ELISA) using the reagent kit ASSERACHROM t-PA from Diagnostica Stago (Franconville, France).[23] Results were expressed as ng/ml. Intra-assay and inter-assay coefficients of variation were 2·4% and 4·7% respectively. The normal value for t-PA antigen at 0600 in our laboratory (n = 33) was 5·6(0·3) (mean(SEM)) ng/ml.

We measured PAI activity by a chromogenic substrate assay using a reagent kit (Spectrolyse/PL) from Biopool (Umeå, Sweden).[16] Results were expressed as IU/ml. Intra-assay and inter-assay coefficients of variation were 9·4% and 11·4% respectively. The normal value for PAI activity at 0600 in our laboratory (n = 33) was 5·2(0·5) (mean(SEM)) IU/ml.

STATISTICAL ANALYSIS

We used one way analysis of variance to compare plasma t-PA antigen concentration and PAI activity at each sampling time, age, serum cholesterol, and serum triglyceride in the three groups. When the result was significant Duncan's multiple range test was performed. We evaluated significant differences in plasma t-PA antigen and PAI activity with time by analysis of variance. When this was significant we used Bonferroni's criterion for paired comparisons. Changes in plasma PAI activity and t-PA antigen concentrations before and after attacks were estimated by a paired t test. The clinical characteristics of the three groups in table 1, except age, serum cholesterol, and serum triglyceride, were compared by a χ^2 test. The distribution of the number of attacks occurring in the 8 hour intervals was first tested for differences among the three periods by a χ^2 test for goodness of fit. If this test showed significant differences, the period with the highest frequency was compared with the average of the other two periods combined. p Values of <0·05 were regarded as statistically significant. Data were expressed as mean (SEM).

Results

CHARACTERISTICS OF THE STUDY GROUPS

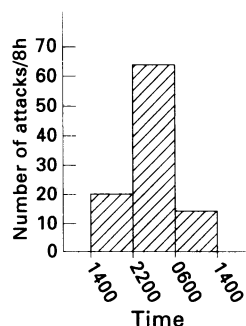Table 1 shows the clinical characteristics of patients with variant angina, of patients with

Figure 1   Circadian distribution of number of ischaemic attacks in patients with variant angina. There was a significant circadian variation in the frequency of the attacks, which reached a peak between 2200 and 0600 (p < 0·01).

stable exertional angina, and of controls. Plasma PAI activity and t-PA antigen concentrations are known to be influenced by age, sex, serum triglyceride, diabetes mellitus, hypertension, smoking, and obesity.[24-28] There were no significant differences in these variables among the three study groups. One patient with variant angina and two patients with stable exertional angina had a history of previous myocardial infarction at least 3 months before the study. In principle, all drugs except glyceryl trinitrate were stopped for at least 3 days before the study day. However, three patients with variant angina had severe attacks and were treated with antianginal drugs on the study day—two of them with calcium antagonist and the other with calcium antagonist and long acting nitrate. Three of the 12 patients with stable exertional angina were receiving medication on the study day—calcium antagonist and long acting nitrate in two and calcium antagonist, long acting nitrate, β blocker, and aspirin in the third—because they had the past histories of unstable angina and multivessel disease with severe organic stenosis. None of the controls was receiving medication on the study day. There was no difference in the number of patients receiving drugs (β blockers, long acting nitrates, calcium antagonists, aspirin and other drugs) between the variant angina group and the stable angina group.

CORONARY ARTERIOGRAPHY
Cardiac catheterisation including coronary arteriography was performed in all patients within a week after the study. Coronary arteriography showed coronary artery spasm during the attacks of angina provoked by intracoronary injection of acetylcholine[29] in 14 patients with variant angina during attack. In one patient intracoronary acetylcholine was not injected because he had severe organic coronary stenosis.

Three patients with variant angina had significant organic stenosis. Two had two vessel disease and the other had single vessel disease.

All the patients with stable exertional angina had major coronary arteries with ≥90% diameter stenosis. None of the controls had significant organic stenosis in their coronary arteries and no coronary artery spasm was induced by intracoronary injection of acetylcholine in any of control subjects. Table 1 shows the number of diseased vessel in the three groups.
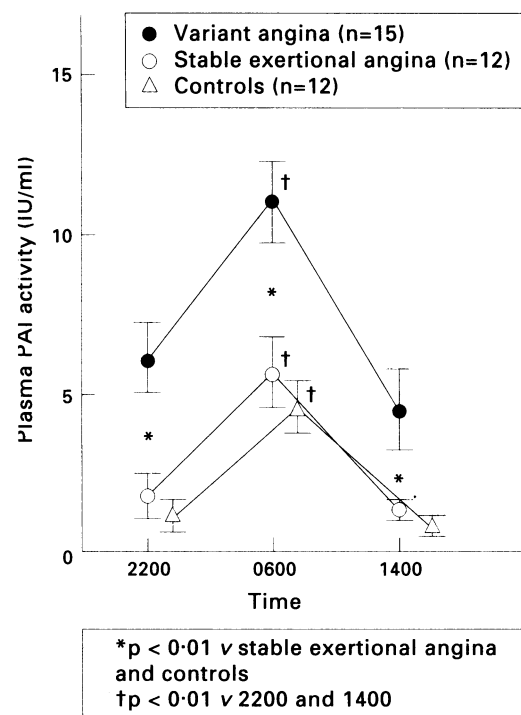

Figure 2   Plasma PAI activity in patients with variant angina, with stable exertional angina, and in controls. Data are expressed as mean (SEM). Plasma PAI activity showed a significant circadian variation with a peak at 0600 in patients with variant angina, in those with stable exertional angina, and in controls (p < 0·01). PAI activity was significantly higher in patients with variant angina than in those with stable exertional angina and in controls at each sampling time (p < 0·01).
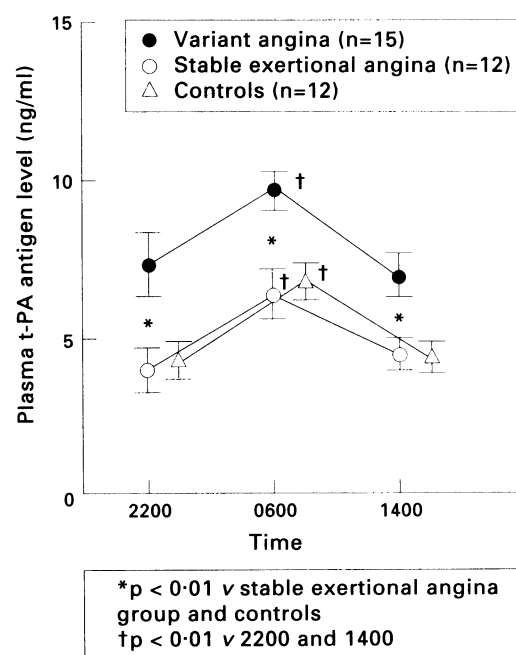

Figure 3   t-PA antigen concentration in patients with variant angina, in patients with stable exertional angina, and in controls. Data are expressed as mean (SEM). Plasma t-PA antigen concentrations showed a significant circadian variation with a peak at 0600 in patients with variant angina, in those with stable exertional angina, and in controls (p < 0·01). t-PA concentrations were significantly higher in patients with variant angina than in those with stable exertional angina and controls at each sampling time (p < 0·01).

Table 2   Plasma PAI activity and t-PA antigen concentration (mean (SEM))

	2200	0600	1400
PAI activity (IU/ml):			
Variant angina	6·1 (1·1)*	11·0 (1·3)*†	4·4 (0·6)*
Stable exertional angina	1·8 (0·7)	5·6 (1·1)†	1·2 (0·3)
Controls	1·1 (0·5)	4·5 (0·8)†	0·7 (0·3)
t-PA antigen (ng/ml):			
Variant angina	7·4 (0·9)*	9·6 (0·6)*†	7·0 (0·6)*
Stable exertional angina	4·0 (0·5)	6·4 (0·8)†	4·5 (0·5)
Controls	4·3 (0·6)	6·8 (0·6)†	4·4 (0·5)

*p < 0·01 v stable exertional angina and controls; †p < 0·01 v 2200 and 1400.

Figure 4 Line graph showing plasma PAI activity (A) and t-PA antigen concentrations (B) before and after eight ischaemic attacks in seven patients with variant angina. Plasma PAI activity and t-PA antigen were significantly higher after the attacks than before the attacks (p < 0·05).
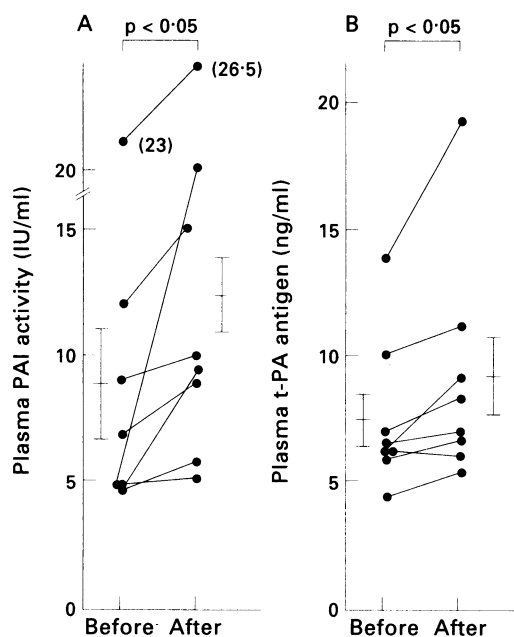
RESULTS OF 24 HOUR HOLTER ECG

Thirteen patients with variant angina had between one and 14 ischaemic attacks and 96 of these episodes occurred on the study day. Sixty four (67%), 12 (13%), and 20 (20%) of 96 attacks occurred between 2200 and 0600, between 0600 and 1400, and between 1400 and 0600, respectively. There was a significant circadian variation in the frequency of attacks with the peak incidence occurring from 2200 to 0600 (p < 0·01) (fig 1). None of the patients with stable exertional angina had attacks during the study.

PLASMA PAI ACTIVITY

Table 2 and fig 2 show mean plasma PAI activity (IU/ml) in the three study groups at 2200, 0600, and 1400. Plasma PAI activity showed considerable circadian variation with a peak level at 0600 in all of the three study groups (p < 0·01) and was significantly higher at all of sampling times in the variant angina group than in the stable exertional angina group and in the controls, particularly at 0600 (p < 0·01) (fig 2). There were no significant differences in plasma PAI activity at any of the sampling times between the stable exertional angina group and the control group (fig 2).

PLASMA t-PA ANTIGEN CONCENTRATION

Table 2 and fig 3 show the mean plasma t-PA antigen concentrations (ng/ml) in the three study groups at 2200, 0600, and 1400. The plasma t-PA antigen concentrations showed the same significant circadian variation, with a peak at 0600, as plasma PAI activity in all of the three study groups (p < 0·01) and the concentration was significantly higher at all sampling times in the variant angina group than in the stable exertional angina group and the controls, particularly at 0600 (p < 0·01, fig 3). There were no significant differences in plasma t-PA antigen concentration at any

of the sampling times between the stable exertional angina group and control group (fig 3).

PLASMA PAI ACTIVITY AND t-PA ANTIGEN CONCENTRATIONS AFTER ISCHAEMIC ATTACKS

In seven patients we took blood samples before and within 15 minutes after eight ischaemic attacks in addition to routine sampling every eight hours. Plasma PAI activity and t-PA antigen concentration were significantly higher after the attacks (8·8 (2·2) v 12·3 (2·5) IU/ml, p < 0·05 for PAI activity and 7·4 (1·0) v 9·1 (1·6) ng/ml, p < 0·05 for t-PA antigen) (fig 4).

Discussion

Coronary spasm is important in the production of acute myocardial infarction in some patients.[3 4] None the less it is not clear whether coronary spasm causes intracoronary thrombus formation. We found that plasma fibrinopeptide A (FPA), a specific marker of thrombin generation and activation of coagulation system,[30] was increased during or after episodes of angina in patients with coronary spastic angina.[11 12] We also showed that a circadian variation in plasma FPA concentrations paralleled the episodes of angina in patients with variant angina and showed that this circadian variation resulted from the attacks.[11] Furthermore, we showed that FPA was released into the coronary circulation after coronary spasm.[13] These findings indicate that coronary spasm activates the coagulation system and may initiate intracoronary thrombus formation. However, the maintenance of thrombus depends not only on its formation but also on its lysis. We and others have reported an increase in plasma PAI activity in patients with acute myocardial infarction and unstable angina.[18-22] Others have reported circadian variation in plasma PAI in these patients.[31]

Our study showed that there were significant circadian variations in plasma PAI activity and t-PA antigen, with a peak level at 0600, in patients with variant angina, in those with stable exertional angina, and in controls. We also showed that the circadian variation in these variables in patients with variant angina paralleled the ischaemic attacks. Furthermore, the present study showed that both plasma PAI activity and t-PA antigen concentrations were significantly higher at each sampling time in patients with variant angina than in those with stable exertional angina and in controls. These results indicate circadian variation in fibrinolytic activity in patients with variant angina with the lowest level in the morning when the incidence of ischaemia is highest. The results also suggest that fibrinolytic activity is impaired in these patients, particularly in the early morning when angina attacks are most common.

Thus the present study together with our previous studies[11-13] suggests that coronary spasm may trigger thrombus formation in

coronary arteries and that the reduction of fibrinolytic activity may slow the removal of thrombus, ultimately leading to acute myocardial infarction in some patients, particularly in the early morning.

The present study cannot clearly show whether the increase in plasma PAI activity and t-PA antigen concentration is the result or the cause of attacks in patients with variant angina. We showed that plasma PAI activity and t-PA antigen concentration increased significantly after episodes of angina in patients with variant angina in this study, which indicates that the increase in plasma PAI activity and t-PA antigen concentration may be the result of the ischaemic attacks.

In vivo PAI inhibits t-PA activity by complexing with it,[14] and PAI activity represents free PAI not combined with t-PA antigen and thus antifibrinolytic activity. In the present study t-PA antigen was also increased in patients with variant angina. However, because PAI activity was increased in these patients the degree of increase in t-PA antigen was less in relation to that of PAI.

The mechanism responsible for the increase in PAI and t-PA in patients with variant angina is still unknown. There are reports that stimulation of human endothelial cells and hepatic cells by thrombin resulted in the release of both t-PA and PAI-1.[32 33] Our previous studies in patients with variant angina showed that plasma FPA concentrations were increased and showed a circadian pattern with a peak in the early morning. In the present study we found circadian variation of plasma PAI activity and t-PA antigen in patients with variant angina that resembled that of plasma FPA. Thus the increase in plasma PAI activity and t-PA antigen in these patients may result from the reaction of thrombin on endothelial cells and/or hepatic cells. It has been reported that thrombin activates platelets,[34] which contain PAI-1[35] and transforming growth factor beta (TGF-β) and epidermal growth factor (EGF). These growth factors stimulate synthesis of PAI-1 in human hepatic cells and endothelial cells in culture and in vivo.[33 36-38] Thus PAI-1 may be released from activated platelets or TGF-β and EGF may be released from activated platelets to stimulate synthesis of PAI-1. PAI is also an acute phase reactant.[15] The increase in plasma PAI activity may in part reflect the acute phase reaction.

We found that the plasma PAI activity in patients with variant angina showed a dynamic circadian variation with a peak in the early morning which paralleled the distribution of attacks. PAI activity at each sampling time was higher in patients with variant angina than in patients with stable exertional angina or controls, particularly in the early morning. This antifibrinolytic tendency in the early morning may be an important factor in the higher incidence of cardiovascular events in the morning.[39]

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