Circadian variation of tissue plasminogen activator and its inhibitor, von Willebrand factor antigen, and prostacyclin stimulating factor in men with ischaemic heart disease

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Abstract

***Objectives*—To determine whether plasma concentrations of tissue plasminogen activator antigen, von Willebrand factor antigen, and prostacyclin stimulating factor and plasminogen activator inhibitor activity show circadian variation in men with ischaemic heart disease.**

***Design*—Blood samples were obtained every four hours for 24 hours from 10 men with ischaemic heart disease. The men were ambulant from 08:10 until 00:00 when they went to bed and they remained in bed until 08:00 the following morning.**

***Patients*—Ten men with positive diagnostic exercise tolerance tests with no significant past history, who were not regularly taking any medical treatment except for glyceryl trinitrate.**

***Results*—There was significant circadian variation in plasminogen activator inhibitor activity (p = 0·001) (peak value 04:00 and trough value 20:00), but not in plasma concentrations of tissue plasminogen activator antigen, von Willebrand factor, or prostacyclin stimulating factor.**

***Conclusion*—Men with ischaemic heart disease showed a significant circadian variation in fibrinolysis. The combination of peak values of plasminogen activator inhibitor activity and failure of plasma concentrations of tissue plasminogen activator antigen to increase in the early morning must predispose to thrombosis at this time. The circadian variation in fibrinolysis may contribute to the increased incidence of myocardial infarction in the morning.**

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The time of onset of acute myocardial infarction (MI) has a circadian pattern with a considerable morning increase in frequency.[1,2] Coronary angiograms in many patients after myocardial infarction show that the coronary artery stenoses caused by atherosclerosis are mild.[3] This suggests that thrombosis is responsible for most of the obstruction to coronary artery blood flow which results in myocardial infarction. Circadian variations in factors that promote thrombosis such as blood viscosity,[4] platelet aggregation[5,6] and fibrinolytic activity[7-9] have been reported in previous studies. The thrombotic tendency is greatest in the morning and this coincides with the circadian pattern of the time of onset of myocardial infarction.

Endothelial cell dysfunction has been implicated in the pathogenesis of thrombotic disorders and circadian variation of the endothelial cell function may contribute to the circadian variation in the time of onset of myocardial infarction. The fibrinolytic activity of blood is partly dependent upon the equilibrium between two endothelial cell products - tissue plasminogen activator (tPA) and its inhibitor plasminogen activator inhibitor (PAI). Patients who have sustained myocardial infarcts have impaired fibrinolysis[10,11] and this may be important pathogenetically. Furthermore, endothelial cells secrete factor VIII von Willebrand factor antigen (fVIII vWFAg), which is essential for platelet adhesion to the subendothelium, and also prostacyclin, which is a potent vasodilator and inhibitor of platelet aggregation. Prostacyclin release is controlled by prostacylin stimulating factor (PGI_2 SF) (fig 1).

Previous studies have described circadian variations in the endothelial cell release products tPA and PAI in normal volunteers[7-9] and in patients with ischaemic heart disease.[12,13] The patient studies investigated subjects who had myocardial infarcts or unstable angina. Some of the patients in these studies were confined to bed, being treated with drugs, and acutely ill. Furthermore, the results of

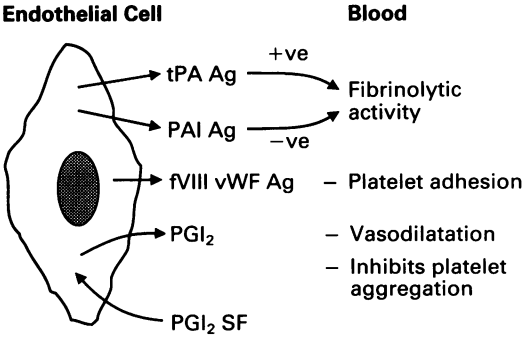

Figure 1 Actions of tPA, PAI, fVIII vWF Ag, and PGI_2 SF.

these studies were based on only two and four blood samples per 24 hour period.

The aim of our study was to investigate in detail the circadian variation in tPA Ag, PAI activity, fVIII vWF Ag, and PGI_2 SF in men with stable ischaemic heart disease who were not taking medication and who were active during the day. This type of patient and pattern of activity is typical of many patients with ischaemic heart disease.

Patients and methods

Ten men (median age 59·5 years (range 48-69)) gave their informed consent to participate in the study which had been approved by the local ethics committee.

Four of the men were current smokers, four were ex-smokers (all had stopped smoking for at least two years) and two had never smoked. None was regularly taking medication or had any history of recent infection. All the subjects had been referred from outpatient clinics for diagnostic exercise tolerance tests that were subsequently reported as positive. A positive exercise test was defined as $\geqslant$ 1·5 mm ST segment depression occurring 80 ms after the J point that persisted for three consecutive beats. With the Bruce protocol the median (+ interquartile ranges) duration of exercise and time until $\geqslant$ 1·5 mm ST depression occurred were 6 minutes (4·5-7·5) and 3·7 minutes (3–7·5) respectively. Four patients subsequently had coronary angiography performed as part of their routine clinical management and all had angiographically proven ischaemic heart disease (> 50% stenosis of left main, left anterior descending, circumflex, or right coronary artery). On the day of the study all subjects were admitted to hospital, they were ambulant between 08:10 and 00:10 but refrained from vigorous exercise. The men ate a normal self selected diet and meal times were at 08:30, 12:30, 17:30, and 22:00. The subjects went to bed at 00:10 and remained in bed until 08:10. The men who smoked were asked to refrain from smoking for at least an hour before blood sample collection.

BLOOD SAMPLE COLLECTION

All blood samples were drawn from different sites in the antecubital fossae through a 19 gauge butterfly needle. Light tourniquet pressure was applied if required to assist venepuncture, the pressure being released for at least 10 seconds before the blood was drawn. The first sample from each man was obtained at 12:00: further samples were obtained from all patients at 16:00, 20:00, 00:00, 04:00, and 08:00. Six men also had another sample taken at 12:00 on the second day of the study.

METHODS

Tissue plasminogen activator (tPA Ag), plasminogen activator inhibitor (PAI) activity, and von Willebrand factor antigen (fVIII vWF Ag)

Five millilitres of blood was anticoagulated with 3·2% trisodium citrate (1:9v/v). The sample was immediately placed on ice and centrifuged at 2000 *g* for 15 min at 4°C. The plasma was then stored at −70°C for assay of tPA Ag by an ELISA technique (Kabi), PAI activity by a chromogenic substrate assay (Kabi), and fVIII vWF Ag by an ELISA technique (Dako).

Prostacyclin stimulating factor (PGI_2 SF)

The plasma samples for determination of PGI_2 SF were stored at −70°C. PGI_2 SF concentrations were measured by a bioassay.[14] Test plasma was added to umbilical artery rings that had been depleted of spontaneous PGI_2 production. If PGI_2 SF was present, the release of PGI_2 induced by PGI_2 SF was measured by a bioassay that detected inhibition of platelet aggregation (Bio Data Corporation platelet aggregometer). Inhibition caused by the test plasma is expressed as a percentage of that caused by normal pool plasma.

STATISTICAL ANALYSIS

Two way analysis of variance was performed on each index to evaluate the signficance of circadian variation while allowing for between subject differences. For all statistical analyses we used a statistical package *(SSPX)*.

Results

There was statistically significant circadian variation in PAI activity ($p = 0·001$), peak values occurred at 04:00 and trough values at 20:00. No statistically significant circadian variations were detected for tPA Ag ($p = 0·98$), fVIII vWFAg ($p = 0·125$), or PGI_2 SF ($p = 0·249$) (fig 2).

Discussion

It is now accepted that over 90% of myocardial infarcts result from thrombotic occlusion of a coronary artery.[15] Factors that promote thrombosis are more prevalent in the morning and this is believed to contribute to the increased morning incidence in onset of myocardial infarction and other thrombotic events.

Fibrinolysis is a complex enzyme cascade system that results in the conversion of plasminogen to plasmin. Plasmin then degrades fibrin, which is responsible for the structural integrity of a thrombus. Fibrinolytic activity is dependent upon the interaction of a series of activators and inhibitors. In human plasma the major activator is tissue plasminogen activator (tPA) and its inhibitor is plasminogen activator inhibitor (PAI). The total quantities of tPA and PAI in plasma can be measured by antigen (Ag) methods and tPA and PAI activity by chromogenic substrate assays. There is a strong positive correlation between tPA Ag and PAI Ag and a strong negative correlation between PAI activity and tPA activity.[9 13] Alterations in PAI dominate fibrinolytic activity, thus an increase in PAI Ag and activity results in a decrease in tPA activity despite an increase in tPA Ag concentrations. Raised concentrations of PAI, which

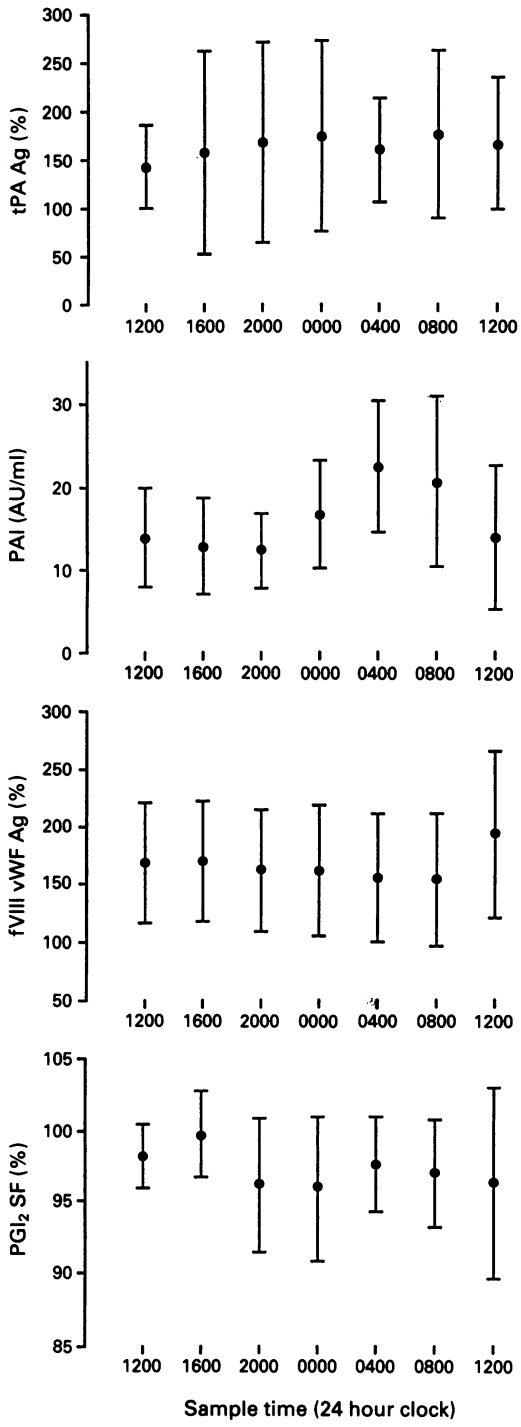

Figure 2 Circadian variations in tissue plasminogen activator antigen, plasminogen activator inhibitor activity, factor VIII von Willebrand factor antigen, and prostacyclin stimulating factor.

may be important pathogenetically, have been detected in patients with coronary artery disease.[10 11]

Previous studies of circadian variations in fibrinolysis in healthy volunteers showed increased PAI Ag, PAI activity, and tPA Ag with low tPA activity in the morning.[7-9] In two studies patients with ischaemic heart disease were studied. Huber *et al* studied 63 patients (49 men) admitted to a coronary care unit with severe myocardial ischaemia. These patients were treated with antianginal therapy and bed rest: 49% had had previous myocardial infarction. Blood samples were obtained for at least two consecutive days at 06:00, 12:00, 18:00, and 00:00. Significant circadian variations similar in timing to those found in healthy volunteers were detected for PAI activity and tPA Ag. The only other study in patients with ischaemic heart disease was that by Angleton *et al* who investigated 15 men with angiographically confirmed ischaemic heart disease and a past history of myocardial infarction or unstable angina.[13] Only two blood samples were obtained, the first at 08:20 (±60) minutes and the second at 19:30 (±70) minutes. Like Huber *et al*, Angleton *et al* reported that PAI activity and tPA Ag concentrations were significantly higher in the morning sample.[13] Thus our study extends previous work on PAI activity in healthy volunteers and patients with myocardial infarction or unstable angina on drug treatment to another group of patients—men with stable ischaemic heart disease on no treatment except for glyceryl trinitrate who followed a normal daily routine. The highest values of PAI activity detected in this group were at 04:00. So the thrombotic tendency was enhanced in the early morning.

In our study we detected no significant circadian variation in tPA Ag concentrations in contrast to the results in healthy volunteers.[9] Although Angleton *et al* did show a significant morning increase in tPA Ag, 47% of their patients with ischaemic heart disease had no change in tPA Ag concentrations over the time course of the study. We found that tPA Ag did not vary significantly during the study period while the mean PAI activity doubled from trough to peak values. These results suggest that PAI is the major influence on circadian fluctuation in fibrinolysis.

FVIII vWFAg, which is produced and released by endothelial cells and platelets, is essential for platelet adhesion to the subendothelium. A role for fVIII vWFAg in atherogenesis has been suggested by animal studies which showed resistance to the development of atherosclerosis in pigs with homozygous von Willebrand's disease[16] and also by the increased amounts of fVIII vWF detected by immunocytochemical staining in the coronary arteries of patients with ischaemic heart disease compared with controls.[17] Furthermore, significantly increased fVIII vWFAg concentrations have been detected in patients with acute myocardial infarction[18] and are associated with an increased thrombotic tendency. However, the circadian variation in thrombotic tendency seems to be independent of changes in fVIII vWFAg concentrations. No significant changes were detected in the study patients or earlier in healthy controls.[8]

Prostacyclin is produced by endothelial cells and is a potent vasodilator and inhibitor of platelet aggregation: its production is stimulated by PGI_2 SF. Patients with atherosclerosis may have reduced prostacyclin production.[19] However, previous studies which investigated patients with various manifestations of atherosclerosis, namely angina and peripheral vascular disease, showed no significant differences in PGI_2 SF

concentrations compared with controls.[20 21] Despite this, circadian changes in platelet function[5 6] and vessel tone are well recognised[22] and we believed that it was of interest to determine whether there was circadian variation in PGI_2 SF. No significant circadian variation was detected in our patients with ischaemic heart disease nor in healthy volunteers.[8]

We showed circadian variation in fibrinolysis, with the lowest activity being detected in the morning, in a group of patients not formerly investigated. Our subjects were representative of a large number of patients with ischaemic heart disease in that they were stable on no regular drug therapy and were active during the day. Many such patients present for the first time with an acute myocardial infarct or sudden cardiac death and thus have not previously been on drug treatment. We found PAI activity varied significantly over the 24 hour study period whereas tPA Ag, fVIII vWFAg, and PGI_2 SF concentrations did not. Increased PAI activity has been reported in patients with ischaemic heart disease and the circadian changes detected in PAI activity may predispose to thrombosis in the morning.

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