

## Platelet reactivity and its dependence on alpha-adrenergic receptor function in patients with ischaemic heart disease

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**SUMMARY** We studied 57 patients admitted to hospital with ischaemic heart disease, including nine patients with variant angina, to evaluate platelet reactivity and its dependence on alpha-adrenergic receptor function. The threshold concentration for biphasic platelet aggregation in response to adrenaline and adenosine diphosphate was measured in fresh platelet rich plasma. There were age related alterations in platelet responsiveness to adrenaline. In 27 age matched control subjects platelets showed adrenaline induced aggregation at a concentration higher than 0.1  $\mu\text{mol}$ . The threshold concentrations for adrenaline and adenosine diphosphate were 0.91  $\mu\text{mol}$  and 4.68  $\mu\text{mol}$ . In 16 patients with acute infarction, 14 with old infarction, nine with effort angina, and nine with rest angina, mean values of platelet aggregation threshold for both adrenaline and adenosine diphosphate were not altered significantly when compared with control subjects. In contrast, the values for adrenaline and adenosine diphosphate in nine patients with variant angina were 0.012  $\mu\text{mol}$  and 2.24  $\mu\text{mol}$  and seven of them showed obvious platelet hyperactivity to adrenaline at a concentration lower than 0.1  $\mu\text{mol}$ . The threshold concentration for adrenaline induced aggregation did not correlate with serum cholesterol and triglyceride levels.

Coronary spasm has been established as a cause of variant angina pectoris<sup>1,2</sup> and altered adrenergic activity has been suggested as being responsible for coronary spasm. Recent studies<sup>3-6</sup> have shown that the anginal attacks can be induced by the stimulation of alpha-adrenergic receptors of a large coronary artery, that is administration of adrenaline with or without propranolol, and can be suppressed by alpha-adrenergic blockade with phentolamine and phenoxylbenzamine.

Although increased platelet aggregation has been reported in patients with coronary artery disease,<sup>7-9</sup> the platelet alpha-adrenergic receptor function in patients with variant angina has not yet been examined. The present study was designed to determine whether platelet sensitivity to adrenaline was altered in patients with variant angina pectoris compared with age matched control subjects and patients with other ischaemic heart disease.

### Subjects and methods

We studied prospectively 57 patients with ischaemic heart disease who were admitted to Kobe University Hospital. Their ages ranged from 33 to 76 years, with an average of 58 years. The population of the study consisted of 27 patients with angina pectoris and 30 patients with transmural myocardial infarction. Patients with symptomatic angina pectoris were further subdivided into nine with effort angina, nine with rest angina, and nine with variant angina. In this study all of those with effort angina showed transient ST segment depression on exercise. Rest angina was characterised by episodes of chest pain accompanied by transient ST segment depression that occurred apparently unrelated to increased oxygen demand of the myocardium. Rest angina occurred frequently in conjunction with effort angina. Variant angina was categorised separately in this study and defined as transient ST segment elevation during chest pain that occurred at rest. The clinical diagnosis of acute myocardial infarction was made by both typical elec-

trocadiographic and enzymatic changes, and platelet function studies were performed within the first week after an attack of infarction. Remote myocardial infarction occurred at least two months before study and was confirmed in each instance by both electrocardiographic and enzymatic changes during an acute stage of myocardial infarction. Fifty one patients had undergone coronary angiography. In each case, except variant angina, narrowing of at least 70% of one or more of the major coronary arteries was seen. Three of six patients with variant angina had significant coronary narrowing demonstrated angiographically, whereas the remaining three patients had no or minor fixed coronary obstructive lesions.

The control group was composed of 27 age matched subjects with no evidence of ischaemic heart disease. These control subjects were aged from 31 to 82 years, with an average of 55 years. Five additional healthy male subjects, aged less than 30 years, volunteered for the examination of age related changes in platelet reactivity.

Patients with diabetes mellitus, hypertension, and evidence of acute cerebrovascular accident and peripheral obstructive arterial disease were excluded from this study. No subject received any medication including antianginal and platelet active drugs, except glyceryl trinitrate, for at least one week before the study.

Platelet reactivity was characterised by the aggregation method which was reported previously.<sup>10</sup> Venous blood from resting subjects, who fasted at least four hours and refrained from smoking for at least three hours, was collected in sodium citrate (0.11 mol/l). The physical activity was reduced to a minimum in all the subjects before blood was drawn. The blood specimens were then immediately processed for analysis. Platelet rich plasma was obtained by centrifugation of blood samples at 120 g for 5 min. The remaining blood was centrifuged at 2270 g for 15 min to obtain platelet poor plasma. Platelet aggregation studies were performed using the turbidometric method of Born.<sup>11</sup> The counts of the platelet rich plasma were adjusted to 300 000 to 400 000/mm<sup>3</sup> when necessary with the autologous platelet poor plasma.

The platelet rich plasma was prewarmed for one minute by stirring in siliconised cuvettes placed in an aggregation module (PDK Rika Denki, NKK hema tracer 1. Model PAT-4M) before an aggregating agent was added. The light transmittance was recorded on a strip chart recorder. The blank for each study was a similarly treated sample of platelet poor plasma. Percentage aggregation was read as the percentage increase of light transmission observed from the graphic record after the different concentration of the aggregating agent was added. The platelet poor

plasma and platelet rich plasma were used as 100% and 0% light transmission. The sensitivity of platelets to adrenaline and adenosine diphosphate was defined as the lowest concentration of the aggregating agents to produce a complete second wave response and used as the threshold concentration. The methodological error of determining platelet reactivity was 5% obtained from duplicate estimations performed within one hour and processed separately. The aggregating agents tested were adrenaline (Sigma, Inc., St. Louis, Mo), prepared freshly before use, and adenosine diphosphate (Sigma, Inc.), maintained as a 1 mol frozen stock and diluted before used.

Platelet counts in whole blood were performed in an automated analyser counter. Triglyceride and total cholesterol concentrations in serum samples were measured in an automated analyser by enzymatic methods.

### Statistical analysis

Data except platelet reactivity were expressed as arithmetic mean values with the standard deviation of the mean. For the platelet response to various aggregating agents in these groups geometric means were presented. Differences between groups were statistically assessed by Student's *t* test for unpaired data. Because of the apparent log-normal distribution of platelet sensitivities in control subjects,<sup>12</sup> sensitivities were recorded as the log of the aggregating agent concentration, and the *t* test was performed on the logarithm of the individual values. A relation between two variables was tested by a coefficient of correlation. A probability (*p*) value of less than 0.05 was considered significant.

### Results

The clinical categories, numbers of individuals, average ages, and sexes are presented in Table 1. There were no significant differences in ages between the subjects as grouped in Table 1.

In all control subjects, the threshold concentration of adrenaline to produce a full biphasic response of platelet aggregation was at a concentration higher than 0.1  $\mu$ mol (Fig. 1). The response of platelets to adrenaline varied considerably from person to person ranging in concentration from 100 to 0.1  $\mu$ mol, in keeping with previous findings by others.<sup>13</sup> The intraindividual variations of platelet reactivity to this agent, which were evaluated by multiple determinations on the same subjects at intervals, were less than tenfold difference of concentration (Fig. 2). As the age and gender were known to influence platelet sensitivity to adrenaline,<sup>14</sup> the breakdown of the control subjects according to age and gender was undertaken.

Table 1 Comparison of groups undergoing platelet study

Diagnosis	No.	Sex		Age (y)	
		Male	Female		Male (female)
Control	27	17	10	55.8 ± 13.5	57.7 ± 12.2 (51.2 ± 15.3)
Acute myocardial infarction	16	12	4	59.1 ± 10.5	58.4 ± 11.6 (63.8 ± 4.0)
Remote myocardial infarction	14	14	0	54.4 ± 8.8	
Effort angina	9	8	1	59.9 ± 5.2	59.8 ± 5.5 (61)
Rest angina	9	8	1	56.3 ± 11.7	56.5 ± 12.3 (54)
Variant angina	9	8	1	59.4 ± 6.4	58.1 ± 5.5 (70)

Values are the mean ± standard deviation.

Increased sensitivity of platelets to adrenaline with advancing age in normal subjects was further documented in this study (Fig. 3). No sex dependence of platelet sensitivity in response to adrenaline was observed in a small number of our control subjects (Table 2).

The threshold concentrations to produce full biphasic aggregation for adrenaline and adenosine

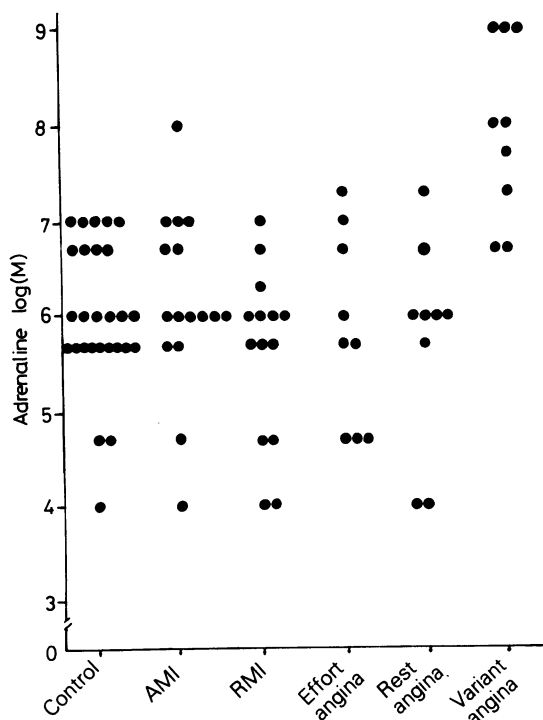


Fig. 1 Platelet aggregation responses to adrenaline in the study groups. The ordinate represents the threshold concentration to produce full biphasic aggregation for adrenaline. AMI, acute myocardial infarction; RMI, remote myocardial infarction.

diphosphate in control subjects and patients with ischaemic heart disease are listed in Table 2 and Fig. 1. Patients with angina pectoris except for those with variant angina were found to show similar platelet reactivity to adrenaline when compared with the con-

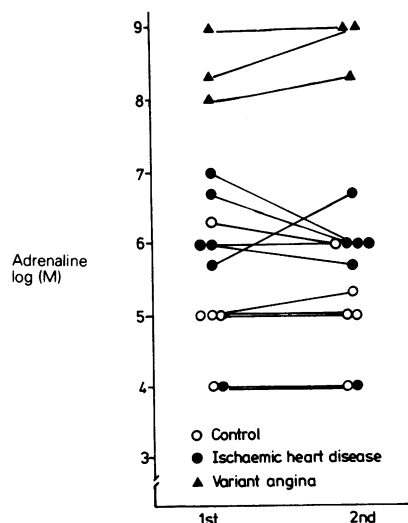


Fig. 2 The intraindividual variations in platelet reactivity to adrenaline in control subjects and patients with ischaemic heart disease.

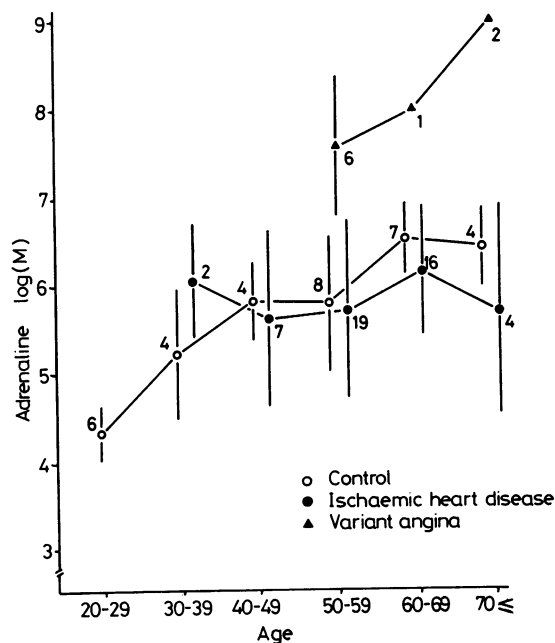


Fig. 3 Effect of age on platelet aggregation response to adrenaline in the study groups.

Table 2 Mean platelet threshold concentration of adrenaline and adenosine diphosphate induced aggregation in control subjects and patients with ischaemic heart disease

	No.	Adrenaline		Adenosine diphosphate		Thrombocyte ( $\times 10^4/\text{mm}^3$ )
		Threshold concentration Log (M)	Maximal aggregation (%)	Threshold concentration Log (M)	Maximal aggregation (%)	
Control	27	6.04 $\pm$ 0.76	55.7 $\pm$ 15.9	5.33 $\pm$ 0.45	56.5 $\pm$ 15.7	19.4 $\pm$ 7.0
Male	17	6.00 $\pm$ 0.88	58.0 $\pm$ 18.1	5.28 $\pm$ 0.53	59.1 $\pm$ 18.1	18.6 $\pm$ 7.0
Female	10	6.08 $\pm$ 0.41	52.4 $\pm$ 8.9	5.49 $\pm$ 0.14	51.9 $\pm$ 8.5	21.7 $\pm$ 6.6
Acute myocardial infarction	16	6.22 $\pm$ 0.85	55.5 $\pm$ 10.1	5.55 $\pm$ 0.28	53.4 $\pm$ 10.6	14.3 $\pm$ 6.1
Remote myocardial infarction	14	5.58 $\pm$ 0.88	59.8 $\pm$ 10.2	5.47 $\pm$ 0.56	59.2 $\pm$ 12.2	19.5 $\pm$ 5.5
Effort angina	9	5.83 $\pm$ 0.95	62.3 $\pm$ 10.5	5.35 $\pm$ 0.38	60.3 $\pm$ 14.9	16.1 $\pm$ 3.9
Rest angina	9	5.68 $\pm$ 1.15	63.3 $\pm$ 11.2	5.45 $\pm$ 0.59	64.7 $\pm$ 11.4	18.7 $\pm$ 2.1
Variant angina	9	7.93 $\pm$ 0.88 ( $p < 0.001$ )	55.7 $\pm$ 16.5	5.65 $\pm$ 0.83	57.2 $\pm$ 9.2	18.8 $\pm$ 5.7

All values are mean  $\pm$  standard deviation. Probability (p) value compared with control (n=27).

trol subjects. In contrast, the platelets of patients with variant angina showed pronounced hypersensitivity to adrenaline. The threshold concentration for adrenaline was 0.012  $\mu\text{mol}$  and was significantly lower in these patients than in control subjects ( $p < 0.001$ ). All patients except two in this group showed distinct platelet hyperactivity to adrenaline at a concentration lower than 0.1  $\mu\text{mol}$  (Fig. 1). Repeated examination of platelet response to adrenaline in some of these patients disclosed that increases in sensitivity to adrenaline were reproducible (Fig. 2). This hyperactivity was not influenced by age or gender (Fig. 3). No significant differences in platelet reactivity were

apparent when patients with variant angina were considered in two groups, the group depending upon whether or not they had coronary angiographic fixed coronary obstruction (0.021  $\mu\text{M}$  versus 0.013  $\mu\text{M}$ ,  $p > 0.05$ ). Average platelet threshold concentration of adrenaline induced aggregation was not altered in patients with acute and remote myocardial infarction. Analysis of aggregation data relative to the angiographically determined coronary obstructive lesions in those patients who were not classified as variant angina showed no apparent relation. No significant differences among the groups of patients with ischaemic heart disease and control subjects studied

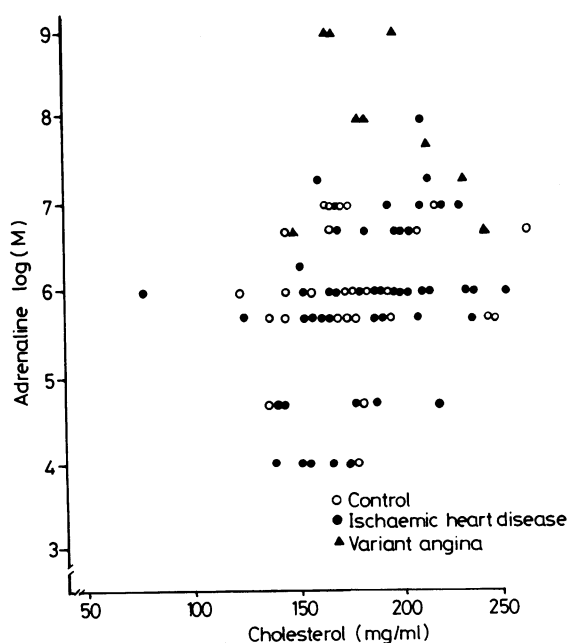


Fig. 4 Relation between platelet aggregation response to adrenaline and serum cholesterol level in the study groups.

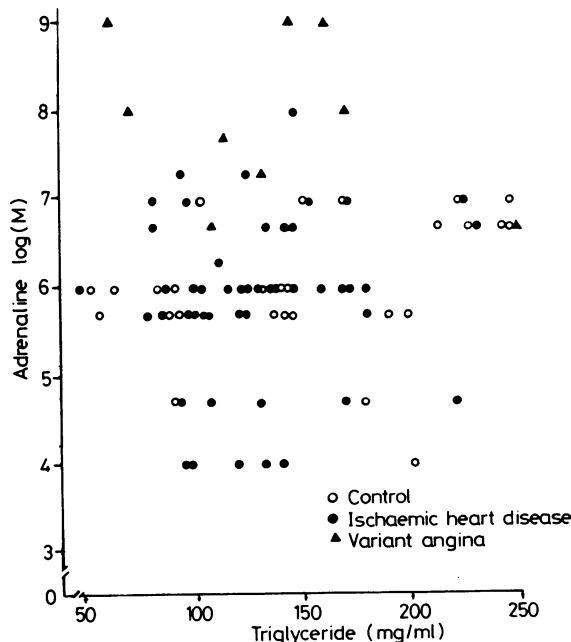


Fig. 5 Relation between platelet aggregation response to adrenaline and serum triglyceride level in the study groups.

were found in platelet counts or platelet aggregation with adenosine diphosphate (Table 2). In patients with variant angina, the threshold concentration for adenosine diphosphate was not significantly different from that of control subjects ( $p>0.05$ ).

It has been suggested that platelets from individuals with hypercholesterolaemia show increased sensitivity to the aggregating agents.<sup>12</sup> Accordingly, analyses were performed to determine whether the serum lipid alterations were responsible for the observed platelet hypersensitivity. The mean values for serum cholesterol and triglyceride of each group with ischaemic heart disease were not significantly different when compared with control subjects. In addition, it was shown in Fig. 4 and Fig. 5 that the threshold concentration for adrenaline induced aggregation did not correlate with serum cholesterol and triglyceride levels.

## Discussion

Human platelets aggregate and undergo release reactions when incubated with catecholamines. Activation of an alpha-adrenergic receptor in human platelets is responsible for the initiation of platelet aggregation by adrenaline and noradrenaline.<sup>15-17</sup> We have shown that platelets of patients with variant angina showed pronounced hyperactivity to adrenaline while the platelet response to adenosine diphosphate was not increased significantly. There were no discernible differences, however, in platelet reactivity to adrenaline or adenosine diphosphate among the clinical subgroups of patients with ischaemic heart disease except for those with variant angina.

Clinical studies of human platelet function have shown a large incidence of abnormally reactive platelets in patients with ischaemic heart disease.<sup>7-9</sup> Other investigators have noted no changes in platelet adhesiveness or aggregation in patients with coronary artery disease.<sup>18</sup> For patients with variant angina, very few data on platelet function are available. Robertson *et al.*<sup>19</sup> measured the circulating platelet aggregates in patients with vasotonic angina, showing that the numbers of platelet aggregates in coronary sinus blood were increased during episodes of ischaemia, but not during non-ischaemic periods. In our study an attempt was made to minimise variability of factors known to influence platelet function and to characterise the reliability and reproducibility of platelet aggregation analyses employed.

In control subjects similar in age to patients with ischaemic heart disease, platelet sensitivity to aggregating stimuli increased with age, confirming the previously reported studies.<sup>13</sup> The data presented here appear to show that platelet reactivity to adrenaline was quantitatively different for variant

angina. Platelet sensitivity to adrenaline was not related to the extent of coronary arterial lesions and the time elapsing from the episode of angina pectoris before the blood sample was collected. In some patients with variant angina pectoris a pathological alteration of the alpha-adrenergic system is thought to be responsible for coronary spasm. Yasue *et al.*<sup>3,4</sup> have shown that the alpha-adrenergic receptor stimulation with adrenaline and propranolol induced anginal attacks associated with the spasm of a large coronary artery. Levene and Freeman<sup>5</sup> have successfully used phentolamine to reverse the spasm during coronary angiography and phenoxybenzamine to improve clinical symptoms. Recent observation<sup>20</sup> showed the high prevalence of migraine and Raynaud's phenomenon in patients with variant angina. These considerations raise the possibility that variant angina is a coronary manifestation of a generalised vasospastic disorder, and a common underlying defect or mechanism may be responsible for all three conditions. Whether platelet alpha-adrenergic receptor activity reflects alpha-adrenergic receptor activity in the cardiovascular system or elsewhere has yet to be established.

The mechanism responsible for the observed platelet hypersensitivity to adrenaline in variant angina is unknown. Since catecholamines are known to alter platelet function,<sup>21</sup> an increase in plasma catecholamine levels in these patients could have influenced our findings. This possibility is unlikely, since Robertson *et al.*<sup>22</sup> have shown that plasma and urinary catecholamines and their metabolites in a pain-free interval and during an anginal attack were not raised, suggesting that episodes of variant angina were not associated with a generalised increase in sympathetic outflow. It has been shown that subjects with familial hyperbetalipoproteinaemia have abnormal platelet function including decreased threshold concentration for stimulation of aggregation by adenosine diphosphate, adrenaline, and collagen.<sup>12</sup> Shattil and his associates<sup>23</sup> have shown that the acquisition of cholesterol by platelets *in vitro* is associated with increased sensitivity to aggregating agents and increased serotonin release after stimulation. Therefore, we have considered the possibility that platelet hypersensitivity reflected the changes in serum cholesterol and triglyceride levels. There was no correlation, however, between platelet reactivity and serum cholesterol and triglyceride levels. Whether the alterations in platelet function may be related to the common underlying metabolic abnormalities shared with vascular smooth muscles in these patients awaits further investigations.

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