

Platelet-release reaction in myocardial infarction

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Summary and conclusions

A study was made of the platelet-release reaction in heparinised platelet-rich plasma taken from 26 patients, of whom 22 had sustained a definite and four a possible myocardial infarction, and from 54 age-matched controls. No significant differences in reaction were observed between the two groups. Significant differences were seen, however, between eight patients who died within a year after infarction and the controls ($P < 0.01$) and the remaining 18 patients who survived ($P = 0.02$). These differences were abolished when sodium citrate was included in the experimental procedure.

Poor prognosis was thus related to an increased platelet-release reaction after infarction.

Introduction

Many studies of platelet aggregation in patients with myocardial infarction have been undertaken in an attempt to show that platelet hyperactivity is associated with thrombotic disease. Perhaps because of the inherent variability of platelet aggregation¹ it is only when groups of patients with infarction have been compared with normal controls that significant differences have been reported.²⁻¹¹ Another aspect of platelet behaviour that may relate to thromboembolism is the platelet-release reaction,¹² the secretory process whereby aggregating agents are liberated from intraplatelet stores. In this investigation we have determined the extent of the platelet-release reaction in patients with myocardial infarction and controls. Since sodium citrate, the anticoagulant that is usually used for platelet studies, augments the platelet-release reaction,¹³⁻¹⁵ we used platelet-rich plasma (PRP) prepared from blood collected into heparin; nevertheless, we also studied the influence of citrate on the results obtained. We also investigated whether a relation existed between the extent of the release reaction and prognosis in the patients.

Patients and methods

Blood samples were taken from 26 patients, of whom 22 had sustained a definite myocardial infarction and four a possible myocardial infarction as judged by symptoms, electrocardiographic changes, and concentrations of circulating cardiac enzymes. Blood samples were also taken from 54 age-matched ambulant volunteers (hospital staff, outpatients attending a dermatology clinic with minor complaints, and surgical inpatients awaiting elective operations). The table gives details of the patients and volunteers. In the group with myocardial infarction the blood samples were taken within 24 hours (19 patients), 48 hours (three), 72 hours (one), 96 hours (one), or 120 hours (two) after the onset of symptoms. In view of the inhibitory effect of non-steroidal anti-inflammatory agents on the platelet-release reaction, studies were carried out only on patients and volunteers who asserted that they had not taken aspirin or other anti-inflammatory agents during the previous week.

Venous blood samples were collected mid-morning into heparin (final concentration 10 U/ml blood). PRP and platelet-poor plasma

(PPP) was prepared by centrifugation. When the platelet count was over $300 \times 10^9/l$ PRP autologous PPP was used to bring the count down to that value; when the count was between 150×10^9 and $300 \times 10^9/l$ the PRP was studied undiluted. The platelets were first labelled by incubating the PRP with tritium-labelled serotonin (3H -serotonin) for one hour at 25°C, and the release reaction was then induced by two separate procedures, the percentage of the accumulated 3H -serotonin released serving to indicate the extent of the reaction. In the first procedure samples of the labelled PRP were stirred with 10 μmol adenosine diphosphate (ADP)/l; in the second procedure 13 mmol sodium citrate/l (final concentration) was added to samples of the PRP and the samples were then stirred with 10 μmol ADP/l. Full details of the preparation, labelling, and subsequent study of PRP in the absence and presence of citrate have been reported.¹⁵

The results obtained in the patients with myocardial infarction were divided according to whether the patients survived up to one year after the event or died within one year. The table shows clinical details for both these subgroups. Of the eight patients who died, four died in hospital and four after discharge.

The Wilcoxon rank sum test was used for all group comparisons.

Results

HEPARINISED PRP

Figure 1 shows the extent to which ADP induced the release of 3H -serotonin from platelets in heparinised PRP from the patients and controls. It also shows the results broken down into those for the 18 patients who survived to one year after the event and those for the eight patients who died. No significant differences were observed in the results between the patients as a whole and the age-matched controls. Significant differences were observed, however, in the results between the patients who died and the controls ($P < 0.01$) and between the patients who died and those who survived ($P = 0.02$). Platelets from the patients who subsequently died released more 3H -serotonin than did platelets from the other patients and controls.

To ensure that the platelet hyperactivity was not a simple reflection of other, more obvious, prognostic factors we compared the clinical details of the groups (table). As we could find no association in the control group between age or sex and the extent of the release reaction, the minor differences in mean age and the different sex ratios between the groups cannot account for the differences in release reaction. The non-survivors might have had more severe infarcts, which in turn might have exerted a greater effect on the release reaction, but the

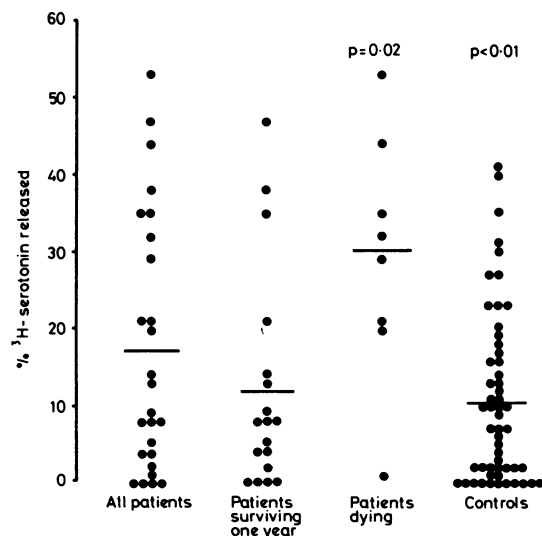


FIG 1—Extent to which adenosine diphosphate induced release of 3H -serotonin from platelets in heparinised platelet-rich plasma. Horizontal line represents mean percentage of 3H -serotonin released.

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Details of subjects studied. Results are shown according to whether patients survived up to one year after infarction

	Patients with myocardial infarction			Controls
	All patients	Patients surviving	Patients dying	
No in group	26	18	8	54
Mean (\pm SE of mean) age	57.2 \pm 2.3	54.3 \pm 2.7	63.6 \pm 3.2	56.0 \pm 2.0
Sex	20M, 6F	15M, 3F	5M, 3F	32M, 22F
Mean (\pm SE of mean) highest SHBD (U/l)	619 \pm 68	602 \pm 68	658 \pm 169	
No with:				
Previous myocardial infarction	6	2	4	
Diabetes	3	2	1	
No receiving drugs on admission:				
Diuretics	6	2	4	
Digoxin	4	1	3	
Beta-blockers	4	2	2	
No given defibrillation during admission	1	1		

SHBD = Serum α -hydroxybutyrate dehydrogenase.

table shows that there was no significant difference in mean cardiac enzyme concentrations between the survivors and the non-survivors. The non-survivors did, however, differ in respect of their history before the admitting infarct: more of them had had previous infarcts and had been treated with digoxin and diuretics. Further studies are therefore needed to clarify the relation between previous health and the extent of the enhanced release reaction after infarction.

EFFECT OF CITRATE

Figure 2 shows the extent to which ADP induced the release of 3 H-serotonin from platelets in heparinised PRP to which citrate had been added. In some patients not enough PRP was obtained for studies in both the absence and presence of citrate, so the results are shown for 24 patients (17 who survived and seven who died) and 49 controls. The addition of citrate abolished the significant differences that had been observed between the groups of patients in the heparinised system.

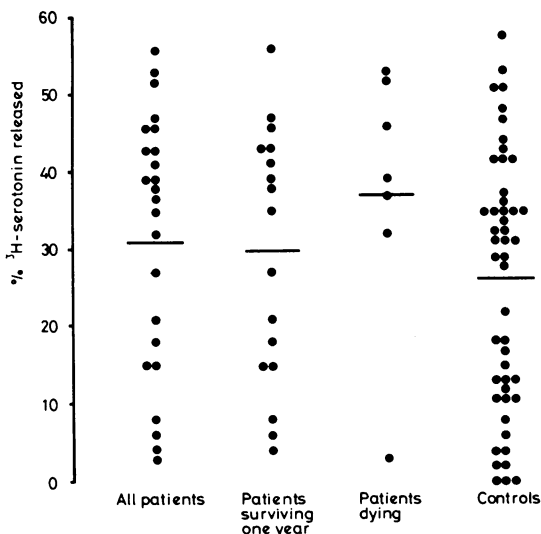


FIG 2—Extent to which adenosine diphosphate induced release of 3 H-serotonin from platelets in heparinised platelet-rich plasma to which citrate had been added. Horizontal line represents mean percentage of 3 H-serotonin released.

Discussion

In these studies we were unable to show any significant difference in the extent of the release reaction between patients who had experienced a recent myocardial infarction and age-matched controls. Differences in platelet aggregation observed previously²⁻¹¹ would therefore appear to relate to other aspects of platelet behaviour.

A surprising finding was the significant difference in the intensity of release observed between patients who subsequently died within a year after infarction and those who survived

($P=0.02$). Of the eight patients who died, seven had platelets that released 20% or more of their 3 H-serotonin; of the 18 who survived, only four had platelets that released this amount. Interestingly, these differences were observed only when citrate was excluded from the experimental procedures (figs 1 and 2). The stimulatory effect of citrate on the release reaction thus masks the differences observed in the absence of citrate. This effect of citrate might explain why the relation between platelet reactivity and prognosis has not been observed before.

In these particular patients high platelet activity after myocardial infarction appeared to identify a high-risk group. Why should this be the case? Do highly reactive platelets contribute to death after infarction or are they simply a marker for some other factor? Enhanced release cannot simply relate to the severity of the admitting infarct, for in our studies the maximal cardiac enzyme concentrations were no higher in the group of patients who subsequently died or the patients who had platelets that released 20% or more of their 3 H-serotonin than in the survivors or the patients with lower platelet reactivity. The group of patients who died did, however, differ from the survivors in respect of their previous history in that more were already receiving digoxin and diuretics when admitted to hospital and a greater proportion had experienced a previous myocardial infarction.

Further studies are therefore needed to clarify whether myocardial infarction causes platelets to become highly reactive in patients whose subsequent prognosis is unfavourable or whether the patients with an unfavourable prognosis and highly reactive platelets after infarction would also have had enhanced platelet reactivity before their infarction. Only by answering this question can we determine whether individuals at high risk may be identified during the period before thrombosis.

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