Decreased platelet function in aortic valve stenosis: high shear platelet activation then inactivation

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Abstract

***Objective*—To elucidate the mechanism of the bleeding tendency observed in patients with aortic valve stenosis.**

***Design*—A prospective study of high and low shear platelet function tests in vitro in normal controls compared with that in patients with severe aortic valve stenosis with a mean (SD) systolic gradient by Doppler of 75 (18) mm Hg before and at least 4 months after aortic valve replacement.**

***Setting*—District general hospital.**

***Results*—The patients showed reduced retention in the high shear platelet function tests. (*a*) Platelet retention in the filter test was 53·6 (12·6)% in patients with aortic valve stenosis and 84·8 (9·6)% in the controls ($P < 0·001$). (*b*) Retention in the glass bead column test was 49·8 (19·2) in the patients and 87·4 (8·7) in the controls ($P < 0·001$). (*c*) The standard bleeding time was longer in the patients ($P < 0·06$). Results of the high shear tests (*a, b,* and *c*) after aortic valve replacement were within the normal range. The platelet count was low but within the normal range before surgery and increased postoperatively ($P < 0·01$). There were no differences in the results of standard clotting tests, plasma and intraplatelet von Willebrand's factor, or in 15 platelet aggregation tests using five agonists between patients with aortic valve stenosis and controls.**

***Conclusions*—The high shear haemodynamics of aortic valve stenosis modify platelet function in vivo predisposing to a bleeding tendency. This abnormality of platelet function is detectable only in vitro using high shear tests. The abnormal function is reversed by aortic valve replacement. High shear forces in vitro activate and then inactivate platelets. By the same mechanisms aortic valve stenosis seems to lead to high shear damage in vivo, resulting in a clinically important bleeding tendency in some patients.**

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Keywords: aortic valve stenosis; platelet function tests; high shear haemodynamics; haemorrhagic diathesis

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Some patients with aortic valve stenosis are prone to bleeding, especially if they have angiodysplasia of the gut. Surgery on the gut in such patients is often unsuccessful, while replacement of the aortic valve alleviates the bleeding in 90% of the patients.[1] This strongly suggests that high shearing forces across the stenotic aortic valve cause the, usually minor, haemorrhagic diathesis. We have shown that one in vitro test involving high shear is decreased in aortic valve stenosis before surgery.[2 3] We and others[4–7] have also shown that in vitro acute high shearing forces activate normal platelets and that when calcium, von Willebrand's factor, and glycoproteins Ib and IIb/IIIa are present, the platelets aggregate. Thus, there is an apparent paradox between increased platelet activation by an in vitro single exposure to high shear and the in vivo decreased activity occurring in the high shear tests caused by chronic stenosis.

This paper further studies and considerably extends our original observations. The nature of the chronic shear induced abnormality has still not been precisely identified; nevertheless, these findings are potentially important because this defect might contribute to a new approach to the treatment of thrombosis and atherogenesis.[8]

Patients and methods

PATIENTS

Fifteen patients (11 men) aged 66·1 (12·0) years were asked to participate in the study which was approved by the local ethics committee and complied with the Declaration of Helsinki. All had haemodynamically important aortic valve stenosis and were being considered for aortic valve replacement. Two patients were assessed only clinically; the mean (SD) peak systolic gradient in the echo/Doppler study was 85·1 (1·3) mm Hg in 10 patients and the peak to peak catheter withdrawal gradient was 66 (5·5) mm Hg in five. Three patients had mild or moderate aortic reflux and four had angiographically severe coronary artery disease. Body weight ranged from 66 to 108 kg. Two patients gave a history of bleeding from presumed colonic angiodysplasia and one from a peptic ulcer. Six patients were regularly taking aspirin. The 10 controls (5 men) aged 69·1 (7·1) years were healthy volunteers with no history of bleeding and no clinical evidence of valvular disease. There were slightly more males but otherwise patients and controls were well matched for age and smoking; two controls took regular non-steroidal anti-inflammatory drugs.

At operation 11 patients were given a xenograft and four had a prosthetic aortic

valve replacement. Two patients also had coronary bypass surgery. It was possible to restudy seven of these patients 2–6 (mean 4) months postoperatively; this group comprised five patients with a xenograft and two with a prosthetic aortic valve. Six of the 10 controls were re-examined during the same period.

BLOOD COLLECTION

Venous blood was collected from the arm with minimal tourniquet pressure via a 19 gauge butterfly needle (Venisystems, Abbott Ireland, Sligo, Ireland). Some 9 ml blood was immediately transferred into each of five stoppered tubes containing trisodium citrate dihydrate (final concentration 0·32%). The blood in three of the tubes was used for the high shear filter tests and for preparing platelet rich and platelet poor plasma. The other two tubes were used for aggregation, clotting, and multimeric analysis of plasma von Willebrand's factor. Six ml blood was dispensed into a tube containing edetic acid (final concentration 0·17 mol/l) and was also used in the filter. Ten ml blood was collected into a second syringe and immediately tested in the glass bead column.

HIGH SHEAR FILTER TEST

The technique is described in detail elsewhere.[6] Briefly, fresh citrated or edetic whole blood was forced under constant pressure through a 10 μm resin bonded glass fibre filter with tortuous channels (Pall U100; Pall Process Filtration, Portsmouth, UK). The transit time was 8 ms. One aliquot of the citrated blood was forced through the filter at a constant pressure of 40 mm Hg and another aliquot at 100 mm Hg. The edetic blood was tested only at 40 mm Hg. For each of these three tests, the effluent blood was collected between 0 and 5 s and between 20 and 40 s. A full blood count was performed before the filter and on the two timed post filter samples. The percentage of platelets, white cells, and red cells retained in the filter was then calculated. The total number of drops emerging from the filter between 0 and 40 s was also recorded.

GLASS BEAD COLUMN TEST

Native blood was forced through a column of glass beads (Adeplat "S" Immuno, Sevenoaks, UK) at a constant speed; the effluent blood was collected between 15 and 20 s into tubes containing edetic acid for counting. The difference between the initial platelet count and the post-column count expressed as a percentage of the initial count is referred to as glass bead column platelet retention percentage.[9]

PLATELET AGGREGATION

This was performed on a platelet aggregation profiler model PAP-4 (Biodata, Alpha Laboratories, Eastleigh, UK). The following aggregating agents were used: adenosine diphosphate (Sigma, Poole, UK) 0·5, 1·0, 2·5, and 5·0 μmol/l; adrenaline as the sulphate salt 1·5, 5·0, 10·0, and 50·0 μmol/l; collagen (Collagen Reagent, HORM; Hormon-CHEMIE, Munich, GMBH Germany) 1·0, 2·0, 4·0, and 10·0 μg/ml; arachidonic acid (Biodata) 0·5 mg/ml; and ristocetin (Sigma) 1·25 and 0·5 mg/ml. From the aggregation tracings the optical density before aggregation was taken as 100%. The maximum decrease in optical density was then expressed as a percentage of the initial 100%.

CLOTTING STUDIES

The international normal ratio and plasma fibrinogen of platelet poor plasma were tested routinely. (ACL 300 analyser; Instrumentation Laboratories, Warrington, UK).

INTRAPLATELET AND PLASMA VON WILLEBRAND'S FACTOR ANTIGEN

Citrated platelet rich and platelet poor plasma was prepared by centrifugation for 10 min at room temperature at 112 g and 1900 g respectively. A platelet rich plasma count was performed and 2 ml platelet rich and platelet poor plasma were pipetted into Nunc tubes and frozen and thawed six times. The supernatants were then separated and stored at −70°C for up to 3 months before assaying.

von Willebrand's factor antigen was assayed by a general procedure for the quantification of trace amounts of antigens by double antibody sandwich enzyme linked immunosorbent assay (ELISA) on microtitre plates. The coating antibody and peroxidase conjugate were obtained from Dako (High Wycombe, UK).

The intraplatelet von Willebrand's factor antigen was calculated by subtracting the frozen and thawed platelet poor plasma value from the frozen and thawed platelet rich plasma value and the value divided by the platelet count.

VON WILLEBRAND'S MULTIMERS

The von Willebrand's multimers were studied by gel electrophoresis.

SKIN BLEEDING TIME

In a room at 24°C and with a sphygmomanometer cuff inflated to 40 mm Hg two horizontal routine cuts were made with a Simplate II device (Organon Teknika, Durham, NC, USA) blotted every 20 s until the bleeding stopped. The mean of the two results was recorded.

FULL BLOOD COUNT

These investigations were performed on a Coulter "S" plus IV (Coulter Electronics, Luton, UK).

STATISTICAL ANALYSIS

The results were compared using unpaired and paired Student's t tests. A p value of 0·05 was accepted as significant. One standard deviation is given in brackets.

Results

The reproducibility of these tests was satisfactory. Six of 10 controls and three preoperative patients were studied twice 1 year apart. A minor increase in the platelet

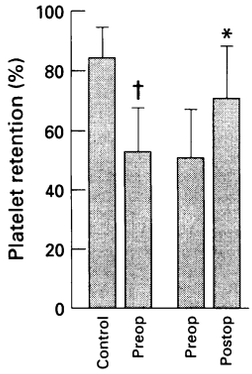

*Figure 1 Platelet retention percentage in citrated blood at 40 mm Hg collected between 20 and 40 s after filtration from 15 preoperative patients with aortic valve stenosis and 10 contemporary controls and seven patients with aortic valve stenosis pre- and post-operatively. Values are means (SD). * P < 0·05; †P < 0·001 (unpaired t test).*

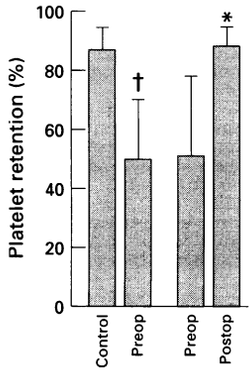

*Figure 2 Platelet retention percentage in native blood at 30°C forced through a column of glass beads and the effluent collected between 15 and 20 s from 15 preoperative patients with aortic valve stenosis and 10 controls and seven patients with aortic valve stenosis pre- and post-operatively. Values are means (SD). *P < 0·01; †P < 0·001 (unpaired t test).*

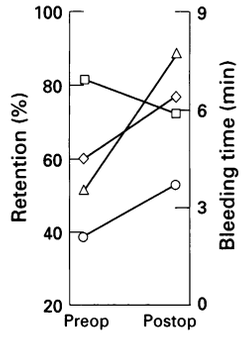

Figure 3 Mean (SD) values for seven patients with aortic valve stenosis pre-operatively and post-operatively. ○ Platelet retention percentage in citrated blood at 40 mm Hg and collected between 0 and 5 s; ◇ platelet retention percentage in citrated blood at 100 mm Hg and collected between 20 and 40 s. △ Glass bead column platelet retention percentage; □ bleeding time.

retention percentage occurred in the filter tests but the size of the increase over the year was negligible compared with results from the pre-operative patients and the controls.

Mean results of the high shear tests are shown for the filter (fig 1) and the glass bead column (fig 2). All the high shear tests before surgery showed that the platelets of the patients were significantly less reactive then the controls (P < 0·001). These included the filter tests carried out in citrated blood at 40 mm Hg and 100 mm Hg and white cell retention at 40 mm Hg. Blood in edetic acid behaved differently from citrated blood in the filter test, but even in this anticoagulant a highly significant decrease was shown at 0–5 s.

The eight controls not receiving aspirin had a mean (SD) bleeding time of 5·3 (1·7) min. The seven of nine preoperative patients not taking aspirin had a bleeding time of 6·9 (1·7) min (P < 0·06). Postoperatively this decreased to 5·9 (1·2) min (fig 3). The five of six preoperative patients given aspirin had a mean (SD) bleeding time of 12·3 (3·5) min.

Fourteen of the 15 agonist induced aggregation tests on citrated platelet rich plasma showed no differences between preoperative patients and the controls; notably ristocetin induced aggregation was normal. The lowest concentration of adenosine diphosphate (0·5 μmol/l) was decreased (P < 0·05) in the patients. The clotting tests, fibrinogen concentration, plasma and intraplatelet von Willebrand's factor antigen, and the multimeric patterns were all similar in controls and pre- and post-operative patients.

The mean (SD) platelet count before surgery was 209·1 (23·0) × 10^9/l. It rose significantly (P < 0·01) to 270·9 (34·1) × 10^9/l when restudied more than 4 months after the operation.

When the seven postoperative patient results were compared with those of six controls studied at the same time all the filter results and the glass bead column were within the normal range and did not differ significantly.

Seven preoperative patient results compared with the seven available postoperative results again confirmed significant differences in the filter tests and the glass bead column (fig 3), with higher retention postoperatively (P varied from < 0·05 to < 0·001).

Discussion

Acute high shearing forces in vivo[10 11] and in vitro[4 7] activate platelets. This however seems to be the first detailed study of the high shear defect in aortic valve stenosis, which was first reported in 1973.[12] Two recent studies using heparin[2 3] and now two independent tests, one using citrate, confirm these results. High shear tests show decreased activity. Low shear tests are normal. The platelet abnormality is presumably causally related to the bleeding diathesis because valve replacement corrects the bleeding tendency and the tests.

The low normal platelet count before the operation, which has been previously reported,[3] is confirmed and the increase in count persisting for 4 months after the operation suggests preoperative platelet consumption. This supports the concept that the shearing forces in vivo have damaged the plaelets.

von Willebrand's disease is the only other known bleeding disease with subnormal high shear tests and abnormal ristocetin aggregation but with otherwise normal agonist induced low shear aggegation tests. The bleeding in von Willebrand's disease is due to low levels or abnormal functioning of von Willebrand's factor.

von Willebrand's factor in aortic valve stenosis is antigenically normal with normal multimers and the ristocetin aggregation is normal. Thus a von Willebrand-like abnormality is excluded.

It is remarkable that reduced platelet retentions between 0–5 s and 20–40 s are similar in von Willebrand's disease.[5] Retention between 0 and 5 s in aortic valve stenosis is similar to that in von Willebrand's disease but retention between 20 and 40 s is significantly higher (P < 0·001).

The normal low pressure aggregation results in aortic valve stenosis virtually exclude an abnormality of glycoprotein IIb/IIIa. The results from the many variations in the filter test do not support the diagnosis of an abnormality of GP Ib or GP IIb/IIIa.[5] Nitric oxide inhibits low shear agonist induced aggregation and there is minimal shear induced haemolysis,[13] so nitric oxide is probably inactivated. Aspirin does not affect platelet retention in the filter between 0 and 60 s[6 14] so presumably the prostaglandin cyclo-oxygenase pathway is not involved. The results are also unlike those in storage pool deficiency. All the high shear tests even in the absence of calcium[15] (the edetic acid blood) were approximately equally depressed from that of normal. This suggests generalised inhibition of some signalling mechanism that triggers the high shear activation, while leaving low shear agonist induced activation intact. The nature of the shear induced in vivo abnormality in aortic valve stenosis remains obscure and awaits further study.

Alternatively or additionally, or both, to the fore mentioned suggestion it is proposed that low platelet retention in aortic valve stenosis results from platelet activation due to high shear which is followed by decreased activation[16] as occurs in in vivo systems.[17]

Both decreased activity in aortic valve stenosis and in experimental models decreased activation may be related to the "rebleeding" phenomenon, which has recently been reported in an in vitro study using native or anticoagulated blood.[18] In the filter system blood is sheared through a fine filter; this results in platelet activation; they cohere (aggregate), pile up on the filter, and block it. The adherent platelets are so attractive that at 40 s about 90% of the platelets are retained. Between 100 and 200 s "rebleeding" occurs; only about 20% of platelets are retained. The retained adherent platelets have become

unattractive. The nature of this radical change is not fully understood. The unblocking of the filter clearly indicates a dramatic change in the adherent aggregated "felt" of platelets.

Platelets are involved in thrombosis and atherogenesis. Support for this activation-inactivation hypothesis comes from studies of surgically induced coarctation of the aorta in monkeys fed an atherogenic diet. At the site of a constricting band, which is arranged to produce 60–80% coarctation, increased atheroma may be found,[19 20] perhaps reflecting shear induced platelet activation. Decreased atheroma is regularly found downsteam. The motion of the artery wall,[21] its metabolism,[22] flow velocity,[20] and aortic pressure, which was decreased either slightly or not at all in the abdominal aorta,[23] were all considered. In all five studies there was strikingly less atheroma distal to the stenosis.[19–23] For example 30 (27)% (mean (SD)) of the area of the abdominal aortic wall in the controls developed atheroma compared with only 5 (4)% in the monkeys with coarctation ($P < 0.05$).[22] These findings are compatible with a platelet defect induced by high shear.

There are similarities between surgically induced aortic coarctation in monkeys and aortic valve stenosis in humans and it may be asked whether shear induced inactivation of platelets in aortic valve stenosis results in decreased atheroma. Gross atheroma certainly does occur in this syndrome.[24] Two of the 15 patients reported here had coronary bypass surgery. However, no comparison of the degree of atheroma in carefully matched aortic valve stenosis patients and controls has been reported.

Aspirin, heparin, warfarin, and fibrinolytic treatment can all cause haemorrhage but at an appropriate dose they inhibit thrombosis. The animal experiments described earlier strongly suggest that the high shear induced platelet abnormality may result in decreased atherogenesis. When this shear induced abnormality is properly understood it might be manipulated to decrease thrombosis and atherogenesis without causing the bleeding found in aortic valve stenosis.

The clinical importance of these findings is not established. The risk of haemorrhage clearly involves the abnormal platelets. Thus an excessively prolonged bleeding time and very low retention in a high shear system should alert clinicians to increased haemorrhagic risk.

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