Flow Induced Platelet Activation in Mechanical Heart Valves -- *in vitro* Studies in a LVAD

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ABSTRACT

Thromboembolism is a common complication of mechanical heart valves (MHV). The objective of this study was to elucidate mechanisms of thromboembolism in flow past MHV in vitro. Gel-filtered platelets were recirculated in a buffer through MHV mounted in a model of a left ventricular assist device (LVAD), and their activation measured by an innovative platelet activity state (PAS) assay. In a first series of experiments, the motion of one of the MHV in LVAD was restricted, simulating thrombus formation on the valve inducing pathologic flow conditions, while a LVAD with free MHV served as a control. The platelet activation increased as a function of the recirculation time past the valve, and the restricted MHV had a higher activation rate as compared to control. A comparative study of a monoleaflet MHV (Bjork-Shiley) and a bileaflet MHV (St. Jude Medical) indicated that the latter induces a higher platelet activation rate. Comparative studies with flow cytometry indicated that PAS assay is more sensitive in quantifying flow induced platelet activation.

Keywords – thromboembolism, mechanical heart valve, LVAD, platelet activity state, flow cytometry

INTRODUCTION

Approximately 120,000 prosthetic heart valves are currently implanted each year in United States. In all implanted mechanical heart valves (MHV), thromboembolism is the main complication, requiring lifelong anticogulant treatment, with its attendant risk of bleeding yet not eliminating thromboembolic complications [1]. The thrombogenicity of MHV is primarily due to platelet activation induced by the non-physiological flow patterns generated by the valve. After MHV implantation, the collar stenosis created and the leaflets non-physiologic geometry may induce jet flow, elevated shear stresses, shed vortices in the wake of the valve, and turbulence. Platelets may get activated under these conditions, potentially leading to the formation of free emboli that may move away from the valve, increasing the risk of cardioembolic stroke.

METHODS

To study flow induced platelet activation *in vitro*, gel filtration was used to separate unactivated platelets from the plasma, and to remove the normal prothrombin. Gel filtration did not involve centrifugation nor resuspension of pellets, thus less damaging to cells and inducing minimal platelet activation – a crucial point in the experimental design. Once separated, the platelets were added into Hepes-modified Ca^{2+}-free Tyrode's buffer and adjusted to physiological platelet count and viscosity. Platelets were then circulated through MHV mounted in a model of a left ventricular assist device (LVAD) for 30 minutes inside an incubator at normal physiologic temperature. Platelet samples were removed every 3 minutes their activity state quantified by measuring thrombin generation rates in the assay. Based on a modification of the standard prothrombinase assay, the PAS assay [2] uses acetylated prothrombin, which produces thrombin species that does not feed back to reactivate platelets. Thus, the thrombin generation rates measured in the assay are a direct result of the flow induced platelets procoagulant activity.

Flow cytometry was conducted to compare the results obtained from PAS assay. Fluorescein-FITC labeled Annexin V suitable for the counting of activated platelets by flow cytometry was used. The protein annexin V binds to anionic, but not neural phospholipids, and this binding can be used to identify activated platelets. As with other methods that rely on this technique, the result is gLVAD abstractenerally reported as the percentage of platelets in the sample that binds annexin V, rather than the average extent of anionic phospholipid exposure. The methodology developed by Holme et al [3] for measuring shear induced platelet activation in stenostic flows was applied to the MHV recirculation studies.

RESULTS

Two Bjork-Shiley MHV were mounted within the LVAD model (Fig. 1). Gel-filtered platelets (plasma-free) were circulated through a free MHV and a suture-restricted MHV. Different sutures were added to the MHV strut to generate a mild restriction and a severe restriction. The mildly restricted MHV could open fully but could not close tightly, while the severely restricted MHV, whose leaflet trajectory was restricted to approx. half of its free motion, could not open nor close entirely. The amount of acetylated thrombin generated in the PAS assay was measured using a chromogenic assay. The results (Fig. 2) indicated a

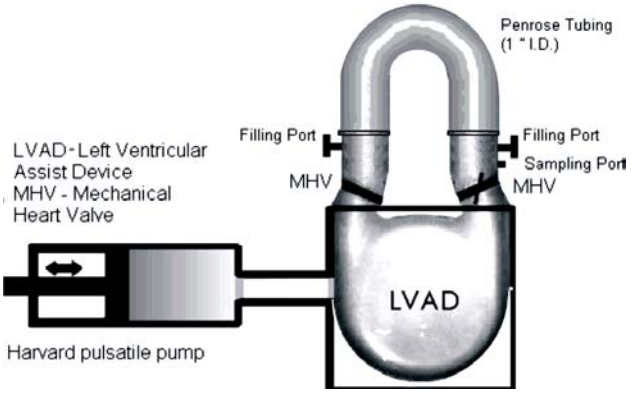

Fig. 1 LVAD system for platelet activation measurements

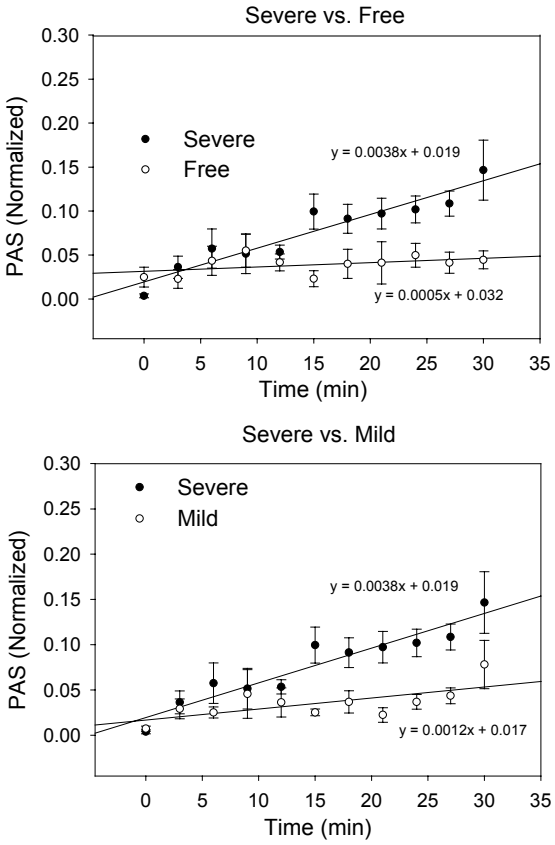

Fig. 2 Platelet activity measurements in severely and mildly restricted MHV, and a control (P<0.001)

significant increase in platelet activation compared to the free valve, with the severely restricted valve activating platelets at a rate approx. 6 times higher than the free valve, and 3 times higher than that of the mildly restricted valve. In all cases platelet activation increased monotonically as a function of the recirculation time past the valve, demonstrating the efficacy of the method for measuring flow induced platelet activation in devices.

A comparative study of a monoleaflet MHV (Bjork-Shiley) and a bileaflet MHV (St. Jude Medical) indicated that the latter induced a higher platelet activation rate. This may be explained in part by the fact that the monoleaflet valve's leaflet is better oriented with the major axis of the flow field, generating a smaller wake, and the fact that the bileaflet MHV produces twice the number of shear layers, in which elevated stresses contribute to platelet activation.

Comparative studies of the PAS assay and flow cytometry were conducted, using platelets stained with fluorescein (FITC)-labeled annexin V (a specific marker for platelet activation). Annexin V binds to anionic phospholipid, but does not involve FV. It is thus expected to be in part correlated with PAS activity. The agonist used was Ca^{2+} ionophore A23187 of varying concentrations. The results indicated a reasonably good correlation between the two methods, with the PAS assay clearly indicating a higher sensitivity and consistency across the ionophore concentrations range.

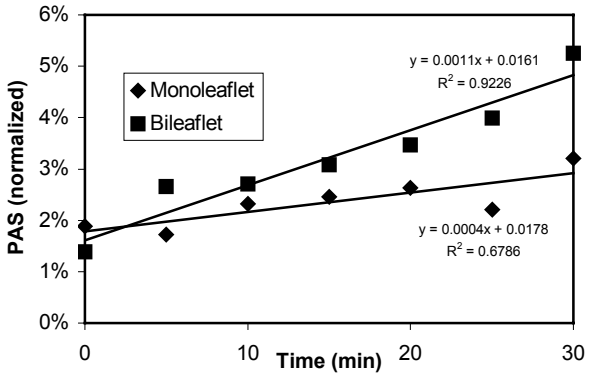

Fig. 3 A comparison of platelet activation between a bileaflet and monoleaflet MHVs

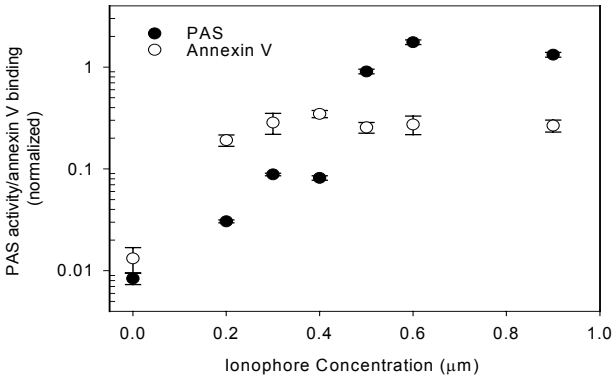

Fig. 4 Comparison between flow cytometry and PAS assay

CONCLUSIONS

We have demonstrated that the PAS assay is a robust technique for in vitro measurement of flow induced platelet activation in prosthetic cardiovascular devices. The ability to perform such sensitive measurements of platelet activity in flow through cardiovascular devices *in vitro* may have a major impact on the design process of these and other vascular devices.

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