On the Cusp: The effects of immobilized pyridoxamine and methylglyoxal on platelet-material interactions in bioprosthetic heart valves

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Introduction

Though bioprosthetic heart valves (BHV) do not require lifelong therapeutic anticoagulation, it was recently reported that 12% of BHVs have significant leaflet thrombosis.

Recently our group identified pyridoxamine (Vitamin B6, abbreviated PYR) as a potential pretreatment agent to reduce advanced glycation end-product (AGE) accumulation within bovine pericardial (BP) BHVs. AGEs contribute to valvular degeneration through collagen crosslinking and immune cell signaling via the receptor for AGEs (RAGE). PYR is also known to inhibit platelet aggregation. We evaluated the anti-platelet effect of PYR on collagen surfaces simulating BHV tissue as well as BP in an ex-vivo whole blood perfusion system.

Methods

Glutaraldehyde-fixed collagen-coated polyvinyl chloride (PVC) bypass tubing was used as a leaflet surface model. Untreated collagen surfaces were compared to PYR pretreated (PYR tx) collagen surfaces and PYR supplementation of perfusate (+PYR). Additionally, collagen surfaces were aggressively glycated with methylglyoxal (MGO); untreated glycated controls were compared to PYR treated (PYR tx) glycated collagen surfaces, PYR supplementation (+PYR), and RAGE antagonism. Fresh citrated human whole blood was perfused over the surfaces for 4 hours at 37 °C and arterial shear stress. Platelet activation was measured via flow cytometry (CD62P expression). Tubing sections were viewed using scanning electron microscopy (SEM).

Results

PYR treatment of glutaraldehyde fixed collagen surfaces significantly reduced CD62P expression (13.12% v 23.7%, $p<0.001$) in circulating platelets after perfusion when compared to the control. Glycation of the collagen surface resulted in increased circulating platelet CD62P expression (30.6% v 23.7%, $p<0.001$), but PYR treatment of the glycated surface (11.99%, $p=0.001$) as well as RAGE antagonism (9.04%, $p<0.001$) mitigated the effect of glycation on platelet CD62P expression.

Platelet-collagen surface adhesion was increased by glycation (105 platelets/field v 58, $p=0.006$) compared to the non-glycated collagen surface (Figure 1a-b). PYR treatment of glutaraldehyde fixed BP tissue reduced platelet adhesion compared to untreated control fixed BP ($p=0.002$) (Figure 1d-f).

Conclusions

Pyridoxamine pretreatment and supplementation demonstrate significant anti-platelet effects by reducing platelet activation and adhesion to blood-contacting material. This effect appears to be preserved in glycation prone environments. RAGE antagonism resulting in decreased platelet activation suggests that glycation of blood contacting surfaces significantly contributes to platelet activation via RAGE; AGE neutralization may be one mechanism by which PYR exerts its antiplatelet effect.

Takeaway

Pyridoxamine improves hemocompatibility of collagen surfaces by reducing platelet activation and adhesion to blood-contacting materials, even in highly glycated environments, making it an attractive potential pretreatment and supplementation agent to reduce BHV thrombosis and degeneration.

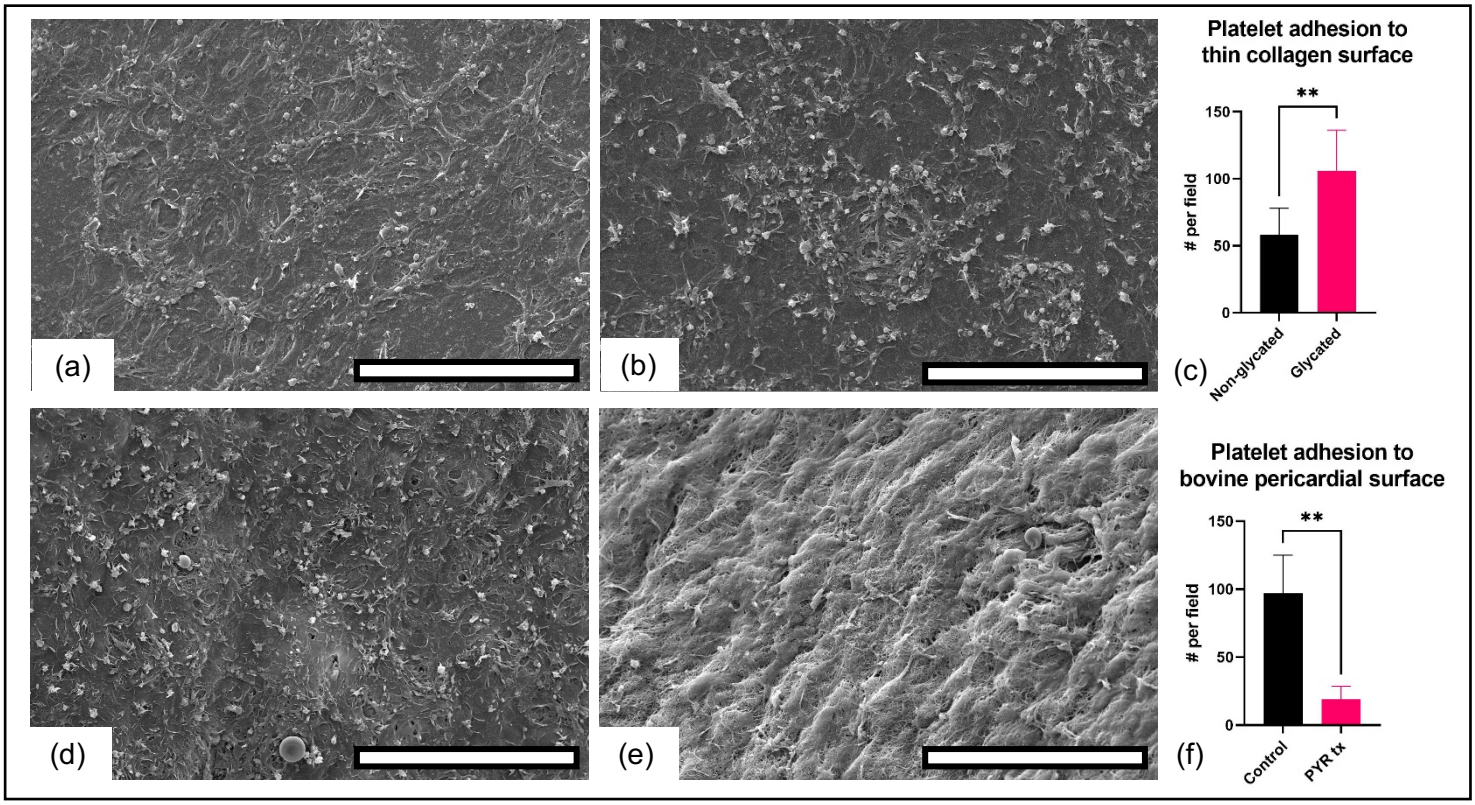

Figure 1 – Scanning electron microscopy (1000x magnification, scale bar = 50μm) of (a) non-glycated and (b) glycated collagen surfaces after whole blood perfusion, demonstrating a significant increase in platelet adhesion due to surface glycation (c); (d) untreated control and (e) PYR treated glutaraldehyde fixed bovine pericardium after blood exposure, demonstrating decreased platelet adhesion with PYR treatment (f)