Penta-Galloyl Glucose Stabilizes Elastin and Reduces Calcification in Bioprosthetic Heart Valves

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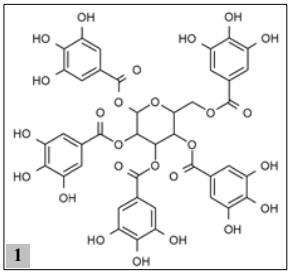
Introduction: Glutaraldehyde (Glut) fixed tissues are used in cardiovascular surgery to replace diseased heart valves with excellent short-term results. This achievement is largely the result of effective Glut-mediated stabilization of the collagen component within heart valve cusps and adjacent aortic wall segments. However, long-term clinical performance of these devices is limited by structural and non-structural dysfunction, including matrix degeneration and calcification. This may be attributable in part to the inability of Glut to adequately stabilize and protect the elastin component from enzymatic attack. Elastin is an important functional component of the aortic wall segment and the cusp constituent in bioprosthetic heart valves (BHVs)[1, 2], aiding in natural tissue recoil during the cardiac cycle. In previous research we have shown that polyphenols such as tannic acid (a deca-galloyl glucose) bind tightly to pure elastin and in doing so, protect it from in vitro digestion by elastase. More recently, we showed that penta-galloyl glucose (PGG, Figure 1) is an equally effective, yet more stable and less cytotoxic derivative when compared to tannic acid.[3]

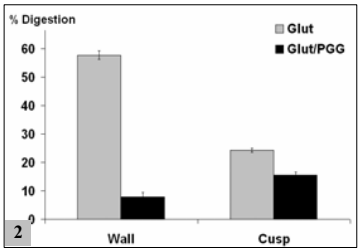
By virtue of its elastin-stabilizing properties, we hypothesized that PGG treatment of Glut-fixed BHVs would extend their durability by targeting elastin stabilization in both the aorta and cusp components.



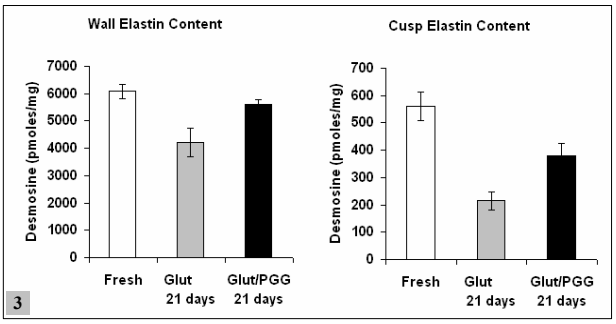
To test this hypothesis, we treated Glut-fixed porcine aortic wall and cusps with PGG and tested their resistance to elastase in vitro and their degradation and calcification potential in a clinically relevant animal model. Glut-treated tissues served as controls in both the in vitro and in vivo experiments.

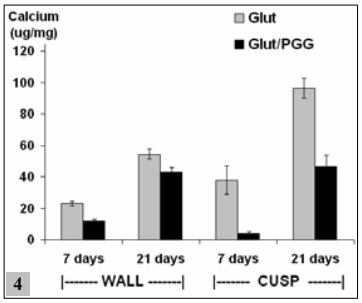
Materials and Methods: PGG was synthesized from tannic acid by methanolysis as described before.[3] Samples of fresh porcine aorta and separately fresh porcine aortic cusps were treated with 0.6% Glut for 1 day followed by 0.2 % Glut for another 6 days. Randomly selected tissue samples were further treated with 0.3% PGG containing 0.6% Glut for 4 days (Glut/PGG group). Control Glut samples were incubated in same solution without PGG for 4 days (Glut group). Following the PGG treatment, samples were rinsed and subjected to elastase digestion. Gravimetric analysis and histology using elastin-specific stains were used to assess elastin fiber stability after elastase. To test for degeneration and calcification, samples (n=12) were implanted subdermally in juvenile Sprague-Dawley rats for 7 and 21 days and analyzed for elastin integrity by histology and desmosine (elastin-specific amino acid) radioimmunoassay, and calcification by histology and atomic adsorption spectrophotometry.

Results and Discussion: Glut-fixed controls exhibited significant mass loss (% digested) after elastase in vitro (Figure 2), indicating that majority of elastin in Glut-fixed wall and cusps is not protected from degeneration. Conversely, Glut/PGG treatment significantly ($p<0.05$) increased resistance to elastase of Glut-fixed aortic wall and cusp (Figure 2).



In vivo results showed that Glut-fixed cusp and wall lost significant ($p<0.05$) amounts of elastin (Figure 3, grey bars), confirming biological instability of elastin in Glut-fixed tissues, and accrued large amounts of calcium (Figure 4, grey bars). In contrast, Glut/PGG-treated wall and cusps exhibited excellent elastin preservation (Figure 3, black bars, $p<0.05$) and lower ($p<0.05$) calcium levels at both 7 and 21 days (Figure 4, black bars) as compared to Glut controls. Notably, Glut/PGG-mediated elastin stabilization was accompanied by reduced tissue calcification in this animal model.





Conclusions: PGG treatment stabilizes elastin in Glut-fixed BHVs and may delay the onset and/or diminish the rate of progression of BHV degeneration and calcification after implantation. PGG could exhibit potential use as an elastin-targeted stabilizing agent for cardiovascular bioprostheses.

References

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3. Isenburg JC, et al.: Biomaterials 2006, 27:3645-3651

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