New ex vivo calcification model for intact murine aortic valves

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Introduction: Calcific aortic valve disease (CAVD) is a common, progressive disease of the aortic valves for which no medical treatment exists and surgery is the only therapeutic solution. The discovery of novel pharmacological treatment for CAVD has been hampered due to the lack of suitable test-systems.

Purpose: We aimed to establish an ex vivo calcification model for intact mouse aortic valves.

Methods: We used the ex vivo flow model for mouse hearts (Miniature Tissue Culture System) to induce calcification with culture media supplemented with 1) β-glycerophosphate, ascorbic acid, and dexamethasone (OSM) or 2) inorganic phosphates (PI) and compared this with in vitro calcification of mouse valvular interstitial cells (mVICs).

Results: Ex vivo cultured aortic valve leaflets calcified in the presence of PI, but not OSM. RUNX was upregulated in both OSM and PI conditions, whereas alkaline phosphatase (ALP) and cyclooxygenase-2 (COX2) were differentially expressed. Apoptosis was not observed, together indicating that osteogenic calcification took place. Interestingly, both OSM and PI were able to induce calcification in vitro, revealing in vitro-ex vivo differences. In addition to the calcification in the aortic valves, endochondral calcification was present in the sinus near the hinge of the aortic valve in both PI and OSM conditions as evidenced by the expression of collagen II, aggrecan and ALP.

Conclusions: Together these data show that we have established an ex vivo calcification model for mouse aortic valves. Osteogenic calcification is induced in the aortic leaflets, whereas endochondral calcification is induced in the aortic sinus, reflecting the calcification found in human CAVD. Using this model, we can now study the initiation and progression of aortic valve calcification.