In vitro techniques to study valvular interstitial cells of the mitral valve in mammals /domestic animals. A cellular approch for the investigation of valvular insufficiency.

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The study of cellular and molecular mechanisms of valvular interstitial cells (VICs) is an emerging research area for veterinary medicine and comparative pathology. Despite many clinical investigations have been done to study canine mitral insufficiency, several doubts remain concerning the underlying etiopathological mechanisms. In case of degenerative disease of the mitral valve, the leaflets accumulate myxomatous tissue in spongiosa and fibrosa layers, characterized by proliferation of VICs with neovascularization, increased matrix, fibrosis and calcification. In order to study these hypotheses, the authors decided to perform assays from bovine mitral valves, collected at the slaughter house, immediately after the animal's death. Valves were conserved in cold and sterile phosphate-buffered saline (PBS). In the lab, the atrial aspect of the mitral leaflets was removed by scraping with a scalpel: when the endocardial surface was opaque, subendocardial material was collected and seeded with complete medium, and routinely incubated. Cell suspension (5×105 cells/mL) was seeded on glasses: at 80% confluence, they were washed three times with PBS, rinsed with 4% formalin (10 min) and conserved at 4°C with 0.03% sodium azide solution. Glasses were treated for immunocitochemistry (ICC) for vimentin, factor VIII, and actin smooth muscle antibodies. Other cell aliquots were seeded in 96 well plates to perform a proliferation assay using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT). Wells were divided in: blank (only medium), control (cells and medium), and treated (cells with 0.05%, 0.1% and 0.5% of hydrogen peroxide - H2O2). Plates were routinely developed at the prefixed time point (2, 24, 48, and 72 hours). Other cells were seeded in Petri dishes and treated using the same H2O2 concentrations, fixed and dyed with Diff Quick staining. The ICC showed a strong and diffuse cytoplasmic positivity for vimentin and occasional for actin smooth muscle. All cells were negative for factor VIII. These results demonstrated that this isolation method is able to primary isolate VICs from bovine mitral valve. The results obtained by proliferation assays demonstrated that high concentrations of H2O2 were lethal, but the lowest concentration induced morphological modifications. The data obtained by this preliminary assay demonstrated that:1) it is possible to isolate and start a primary culture from bovine mitral valve leaflet, 2) ICC was able to confirm point 1), 3) a noxious stimulus mimicking oxidative stress (H2O2) is able to induce morphological modification. Next steps should include the ICC on H2O2-treated cells in order to monitor the antibody expression, receptor identification, study concerning inhibiting/stimulating drugs acting on VICs differentiation to evaluate new pharmacologic treatment approaches. In the authors' opinion, VICs investigation will give a new understanding of comparative pathology of the mitral valve disease. 1) Cushing et al. Matrix Biol. 2005; 6:428-37. 2)Heaney et al. J Vet Cardiol. 2009, 11:1-7. 3). Liu et al. Am J Pathol. 2007, 171:1407-18. 4) Pedersen et al. Cardiovasc Res. 2000, 47:234-43. 5) Pomerance and Witney. Cardiovasc Res. 1970, 4: 61-6.