

P30

Extracellular nucleotide metabolism in porcine heart valve leaflets and its modification during processing by decellularisation and irradiation

Lara Mainetti¹, Ada Yuen¹, Patricia M Taylor¹, Steve Goldstein², Marialuisa Lavitrano³, Al E Heacox², Magdi H Yacoub¹, Ryszard T Smolenski¹

¹Heart Science Centre, Harefield, United Kingdom, ²Cryolife Ltd, Kennesaw, United States, ³University of Milano-Bicocca, Milan, Italy

Objectives:

Extracellular nucleotide metabolism is important for the regulation of thrombosis and inflammation, and we recently reported that it may play a significant role in valve calcification. This study determines the activities of enzymes in valve leaflets that could affect concentrations of extracellular nucleotides and adenosine. Furthermore, we studied how valve processing by decellularisation and gamma irradiation affects these activities.

Method:

Decellularisation was carried out by hypotonic lysis, DNaseI/RNaseA digestion and isotonic washout. Gamma irradiated valves were subjected to 25-40 kGy. Pieces of native (n=3) and decellularised/irradiated/cryopreserved (n=3) porcine pulmonary valve leaflets (10mm² surface area), were incubated in Hanks balanced salt solution containing ATP, AMP or adenosine, and aliquots of medium were sequentially analysed by reversed-phase HPLC for nucleotides and their catabolites. Rate of conversion of substrates into products was used to calculate enzyme activities in intact valves.

Results:

Results demonstrated an exceptionally high activity of ecto-5'-nucleotidase (converts AMP to adenosine) in native leaflets. The rate of leaflet adenosine production was 0.22±0.03 nmol/min, which greatly exceeded previously established cultured endothelial cell and cardiac activity. Catabolism of ATP to ADP and AMP (ecto-ATPD-ase) and conversion of adenosine to inosine (adenosine deaminase) were also detected. Adenosine deaminase was almost completely inactivated in decellularised/irradiated/cryopreserved valves, whereas ecto-5'-nucleotidase activity was decreased by 30%.

Conclusions:

We conclude valve leaflets actively control nucleotide metabolism and decellularisation/irradiation/cryopreservation disrupts this. Further studies may help elucidate the role of nucleotide metabolism in the susceptibility of valves to inflammation, thrombosis and calcification and have implications for valves processed for clinical implantation.