

## **Extraction and purification of glycosaminoglycans of the primitive cordate *Styela plicata* and their antithrombotic activity using arterio-venous shunt model**

*Santos, J.C.<sup>1</sup>; Mesquita, J.M.F.<sup>1</sup>; Gandra, M.<sup>1</sup> and Pavão, M.S.G.<sup>1</sup>*

*1- Laboratório de Tecido Conjuntivo, Hospital Universitario Clementino Fraga Filho and Departamento de Bioquímica Médica, Instituto de Ciências Biomédicas (ICB), Universidade Federal do Rio de Janeiro (UFRJ), Rio de Janeiro, RJ, Brazil*

The presence of sulfated polysaccharides in different species of ascidians has been reported by our group in previous works. In the ascidian *Styela plicata*, a dermatan sulfate is located in the extracellular matrix of the connective tissue in the intestine, heart, pharinx, and cloak. Heparinoids are present in the basal laminae and inside cytoplasmatic granules of epidermic-like cells of the intestine, heart and pharinx (Gandra et al, Glycobiology 2000 Dec;10(12):1333-40). In this work, we report an optimal purification method to obtain molecules. Repeated protease extraction (in a total of 5 reincubations) of the total polysaccharides from the dry tissues were analysed by agarose gel electrophoresis. Glycosaminoglycan-containing extractions were fractionated in a Q-Sepharose column (anion exchange) under a NaCl linear gradient (0-2M) and elution was monitored by metachromasy. The peaks were dialysed and analyzed by agarose gel electrophoresis. Two heparin and one dermatan sulfate enriched fractions were individually re-applied to the same column for fine purification. When subjected to a size exclusion chromatography, the dermatan sulfate molecule was clearly larger than the heparin-like compounds. The antithrombotic effect of these sulfated polysaccharides were investigated in Wistar rats, using an extracorporeal arterio-venous shunt after intravascular administration of each glycan. Overall, a more efficient purification methodology was developed and these polymers were able to prevent thrombus formation on this experimental model.