A Non-anticoagulant Heparin-like Glycan from Ascidian Phallusia nigra is a Potent Antimetastatic Agent by Inhibition of P-selectin

Eliene O. Kozlowski¹; Wallace S. Abreu¹; Christiane F. S. Silva¹; Lubor Borsig² and Mauro M. S. Pavão¹

¹Laboratório de Bioquímica e Biologia Celular de Glicoconjugados, Instituto de Bioquímica Médica, UFRJ, Brazil.
²Institute of Physiology, University of Zurich, Switzerland

Heparin has been used clinically as an anticoagulant and antithrombotic agent for over 50 years, during surgeries, rehabilitation of patients and other treatments. However, recently it has been shown that heparin also inhibits the binding of P- and L-selectin to its ligands, resulting in attenuation of metastasis and inflammation. In this work, a heparin-like polymer was extracted from ascidian *Phallusia nigra* by proteolitic digestion and purified by ion-exchange chromatography. The glycan was identified by agarose gel electrophoresis and enzymatic degradation. Polyacrylamide gel electrophoresis showed that its molecular weight is around 30 kDa. The anticoagulant activity was measured by activated partial thromboplastin time (apTT), showing an effect around 73-fold lower than bovine heparin. Such activity was shown to be mediated by inhibition of thrombin via heparin cofactor II (HCII). In vivo assays demonstrated that ascidian heparin prolongs the occlusion time of the carotid artery after FeCl₃-induced lesion at the dose of 4 mg/kg, without activating factor XII. In vitro experiments also showed that ascidian heparin can bind to P-selectin, inhibiting the binding of other ligands. Short-term animal models confirmed that this effect can inhibit the formation of platelet-tumor cell aggregates, a strategy used by tumor cells to avoid being recognized by leukocytes while migrating inside the blood vessels. These analyses were done by macroscopic and microscopic comparison of the lungs from animals treated with saline solution or doses of the studied glycan. The present work shows a heparin-like polymer which does not have a great effect on coagulation or arterial thrombosis, but it is a potent inhibitor of metastasis by binding to P-selectin from platelets in lower concentrations than bovine heparin.

Keywords: anticoagulant, heparin-like, metastasis, P-selectin.