Development of a Two-Step Chromatography Procedure for Circulating Endostatin Purification

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Collagens type IV, XV and XVIII, together with laminins, nidogens, heparan sulfate proteoglycans, fibulins, dystroglycan and other glycoproteins, are major constituents of BM. Endostatin (ES), C-terminal fragment, is generated by cleavage of the ECM involving matrix metalloproteases, cathepsins 2 and elastases. Free, soluble Es was demonstrated to inhibit angiogenesis, whereas immobilized form of ES supports the survival and migration of endothelial cells. We recently reported the presence and upregulation of ES mRNA and protein in a mouse model of ischemia-reperfusion injury and obstructive nephropathy. The aim of this study was to developed a two-step chromatography procedure for circulating ES analysis. Male Wister rats with 200-250 g of weight were used as donors of blood plasma. A total of 1mL of plasma was treated with dextran sulfate (10%) for the removal of lipids, before purification. The purification of circulating ES was guide by cation-exchange (Resource-S) and affinity (heparin binding) chromatography using elution with a 0.1 – 1M gradient of NaCl. ES was immunodetected by Dot blot and western blotting with monoclonal ES antibody. The analysis of chromatogram areas showed that 47.0%±0.1 of total protein present in plasma have cationic character. Of this total, the ES corresponds to 49.1%±0.20. The analysis of affinity chromatogram areas showed that of total cationic proteins 34.2%±0.28 have affinity for heparin. The purified ES corresponded to 80.7%±0.27 of the heparin binding proteins in the plasma. Immunoreactive band for ES were identified as a 28KDa isoform. In conclusion, we have developed a simple and reliable procedure for high recovery of circulating ES.

Key words: Endostatin, Protein Purification, Two-Step Chromatography, Circulating Endostatin.