Chromatography Analysis of Endostatin in Normal and Ischemic Kidney

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The mammalian kidney is susceptible to injury by ischemia/reperfusion and nephrotoxins, and regeneration after injury is characterized by hyperplasia and recovery of the epithelial cell lining of the tubules. When the intensity and/or duration of injury is severe, the tubular cells either undergo necrosis or detach from the basement membrane (BM). Collagens type IV, XV and XVIII, together with laminins, nidogens, heparan sulfate proteoglycans (HSPG), 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 analysis of ES in normal and schemic kidney. Male C57 BL/6 were submitted to renal ischemia injury by clamping the renal vein and artery for 45 min. Kidneys harvested from normal and ischemic mice were homogenized in protein extraction buffer. The purification of kidney ES was guide by cation-exchange (Resource-S) and affinity (heparin binding) chromatography using elution with a 0.1 – 1 M gradient of NaCl. ES was immunodetected by Dot blot and western blotting with monoclonal ES antibody. The analysis of chromatogram areas revealed an increase of 9.4% in cationic proteins and 20.5% of heparin binding proteins in ischemic kidney. The purified ES corresponds of 5.3 % and 12.5 % of heparin binding proteins in normal and ischemic kidney respectivaly. Immunoreactive bands for Es were identified, by NH^2-terminal sequencing, as the fragment derived from noncollagenous domain 1, with 28KDa. The results indicate that Es is locally produced in response to acute injury and may be an important factor involved in recovery after acute renal injury.

Key words: ischemia/reperfusion; endostatin; kidney injury; chromatography.