Concomitant Delivery of Copper Nanoparticles and Hyaluronan Benefits Vascular Elastin Matrix Synthesis, Crosslinking and Fiber Formation

C. R. Kothapalli[1], A. Ramamurthi[1, 2]

[1]Clemson University-Medical University of South Carolina Bioengineering Program, [2]Department of Cell Biology and Anatomy, Charleston, SC 29425, USA.

Introduction: Elastin and elastic fibers are crucial to maintain the elastic structure of vascular tissues and to regulate cell-signaling pathways important to injury response and morphogenesis. Vascular tissue defects are typically associated with nutritional copper deficiency, which biochemically results in a reduction in lysyl oxidase (LOX) functional activity [1], which in turn compromises oxidative deamination of lysyl and/or hydroxylysyl residues in collagen and elastin and their ability to form covalent stabilizing crosslinks [2]. The failure to reinstate a healthy elastin matrix in such cases, and when damaged by injury or disease can severely compromise vessel homeostasis, though efforts to regenerate degraded or lost elastin structures are limited by unavailability of scaffolds that can provide cellular cues essential to upregulate elastin synthesis. To address this, we have been investigating novel scaffolds based on hyaluronan (HA), a GAG in the vascular ECM. HA has been implicated to play key roles in elastin synthesis by VSMCs [3]. Our recent studies have shown that HA influences the elastin matrix output by VSMCs in a fragment-size specific manner [4, 5]. This study tests the hypothesis that concomitant delivery of copper nanoparticles (CuNP) and HA benefits upregulation of elastin synthesis, cross-linking and fiber formation.

Methods: Adult rat aortic smooth muscle cells (RASMC) (P4) were seeded at $4x10^4$ cells/2.4 cm^2. Except for controls, CuNP (80-100 nm) were added at a dosage of 1 ng/mL and 10 ng/mL (n=3/case). HA fragments of sizes designated as HMW-HA (1500 kDa), VLMW-HA (20 kDa) and Oligomers (0.6 kDa) were exogenously supplemented to RASMC cultures twice weekly at concentrations of 0 (control/ CuNP alone) and 0.2 μg/mL (n=3/case). After 21 days of culture, the cell layers were harvested and analyzed through biochemical assays for DNA, elastin and collagen. Extracellular elastin was quantified as soluble tropoelastin collected in the medium and as soluble and insoluble elastin deposited in the cellular matrix. Trends in soluble tropoelastin and cross-linked elastin production were selectively confirmed through western blot analysis and desmosine assay respectively. The ultrastructural organization of elastin and collagen matrix and their abundance were assessed using transmission electron microscopy (TEM) and confocal microscopy.

Results/Discussion: After 3 weeks of culture, CuNP doubled cell proliferation compared to control ($p<0.05$); tropoelastin production decreased by 28 ± 3% at 1 ng/mL dose of CuNP and by 46±4% at 10 ng/mL compared to controls ($p<0.05$). The output of soluble elastin and collagen also decreased by 25±5% with addition of CuNP alone, though no significant dose effects were observed.

However, no significant difference in the synthesis of insoluble cross-linked elastin was observed on addition of CuNP alone, compared to control. Concomitant delivery of HMW-HA and CuNP decreased tropoelastin output drastically by 75±8 % compared to controls ($p<0.05$). Though addition of HMW HA and CuNP did not impact cell proliferation and collagen production compared to CuNP alone, CuNP dose-specific and HA fragment-size specific effects were observed on cross-linked elastin synthesis. Insoluble cross-linked elastin amounts increased 2.5-3 fold in the presence of HA oligomers and HMW-HA compared to HA-free CuNP controls ($p<0.05$). Moreover, in the presence of CuNP, soluble elastin production decreased (30-50 %) in all the cases ($p<0.05$), except when HA oligomers were present. Based on these results, we hypothesize that larger HA chains provide more anionic sites for Cu^{2+} binding owing to electrostatic attraction. This, in turn, locally upregulates LOX enzyme functional activity, which enhances covalent cross-linking of elastin peptide chains that are coacervated on the surface of the highly anionic HA. CuNP binding by HA fragments on the other hand, appears inadequate. Western blot analysis semi-quantitatively confirmed the observed biochemical trends for tropoelastin in all the cases. Immunocytochemistry of cell layers exhibited the abundance of elastin/collagen matrix engulfing the cells while TEM images of the matrix ultra-structure revealed fibrillin-mediated elastin fiber deposition.

Conclusions: CuNP and HA promote cellular synthesis of cross-linked elastin matrices relative to control cultures, in a CuNP-dosage specific and HA-fragment size-specific manner. CuNP significantly enhanced the cross-linking of soluble tropoelastin into matrix elastin, which is one of the primary objectives of this study. The results clearly demonstrate that the novel approach of exogenous supplementation of CuNP in the presence of HMW HA or HA fragments influences elastin synthesis by RASMC. The current results suggest the advantages offered by the combined supplementation of HA fragments and CUNP in stimulating vascular elastin network regeneration.

References:

1. Rucker R, et al. J Nutr, 1996; 126:51–60.
2. Reiser K, McCormick R, Rucker R. FASEB J, 1992; 6:2439–2449.
3. Merrilees MJ, et al. Circ Res, 2002; 90:481-487.
4. Joddar B, Ramamurthi A. Biomaterials, 2006; 27:2994-3004.
5. Joddar B, Ramamurthi A. Biomaterials, 2006; 27:5698-5707.