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Angiotensin II-induced vascular stiffness is reversed by antioxidants through effects on lysil oxidase and matrix metalloproteinases.

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Introduction: Changes in elastin structure in resistance vessels are associated to the impaired mechanical properties associated to hypertension. Oxidative stress is implicated among others processes in hypertrophy, apoptosis, migration and fibrosis, all involved in vascular remodeling in hypertension. **Aim**: To analyze the contribution of reactive oxygen species (ROS) and the elastin cross-linking enzyme lysil oxidase (LOX) to the increased vascular stiffness and elastin alterations of resistance arteries from hypertensive animals. **Methods**: Mesenteric resistance arteries from mice and Wistar Kyoto normotensive (WKY) and spontaneously hypertensive rats (SHR) were used. Mice were divided into four groups: 1) Control; 2) infused with Angiotensin II (1.44 mg/Kg/day, 2 weeks, subcutaneously by osmotic minipumps); 3) infused with Angiotensin II and the NADPH Oxidase inhibitor apocynin (1.5 mmol/l, drinking water), and 4) infused with Angiotensin II and the mitochondria-targeted antioxidant mito-TEMPO (0.7 mg/Kg/day i.p). All treatments started 24 h before Angiotensin II-infusion. WKY and SHR were treated with the Angiotensin II receptor 1 antagonist, losartan (15 mg/kg/day, drinking water, 12 weeks), apocynin (17 days), mito-TEMPO (17 days) or the LOX inhibitor β-aminopropionitrile BAPN (100 mg/kg/day, drinking water, 5 weeks). Blood pressure was measured by tail-cuff pethysmography. Structure and mechanics of mesenteric arteries were studied by pressure myography. ROS production was assessed by dihidroetidium induced fluorescence and by enhanced lucigenin chemiluminiscence. Gene expression was studied by RT-PCR and elastin structure by confocal microscopy. All data are expressed as mean values ± SEM. Results were analyzed by using unpaired Student's t-test or one-way or two-way ANOVA followed by Bonferroni's post hoc test. A $p<0.05$ was considered significant. **Results**: Losartan ($P<0.01$), but not apocynin, mito-TEMPO or BAPN, reduced systolic blood pressure in SHR (WKY: 151.9±3; SHR: 219.9±7.6, $P<0.05$ vs WKY; SHR LOSARTAN: 160.1±4.2; SHR APOCYNIN: 215.5±8.1; SHR mito-TEMPO: 212.9±5; SHR BAPN: 211.9±2.5 mm Hg, n=8 for each group). In addition, apocynin and mito-TEMPO treatments prevented, in part, the increased blood pressure induced by Angiotensin II infusion in mice ($P<0.01$). Losartan, apocynin and mitoTEMPO treatments decreased the increased vascular ROS production (WKY: 100.9±2; SHR: 151.7±3, $P<0.05$ vs WKY; SHR LOSARTAN: 85.1±8; SHR APOCYNIN: 59.1±2.5; SHR mito-TEMPO: 80.2±5%, all treatments $P<0.05$ vs SHR, n=5 for each group) and NADPH Oxidase activity observed in SHR. Similarly, apocynin and mito-TEMPO prevented Angiotensin II induced ROS production and NADPH Oxidase activation. Vascular LOX gene expression was not affected by Angiotensin II; however, apocynin treatment reduced vascular LOX expression in Angiotensin II infused mice. Losartan and BAPN improved the increased wall:lumen ratio in SHR. However, none of the antioxidants had effect in wall:lumen ratio in the SHR or in the Angiotensin II infused model. In the SHR model, losartan, apocynin, mito-TEMPO and BAPN treatments improved the increased vascular stiffness (slope of the incremental elastic modulus: WKY: 4.73±0.08; SHR: 6.26±0.26, $P<0.05$ vs WKY; SHR LOSARTAN: 4.71±0.21; SHR APOCYNIN: 4.69±0.3; SHR mito-TEMPO: 5.28±0.14; SHR BAPN: 5.28±0.14, all treatments $P<0.05$ vs SHR, n=8 each group) and the alterations in elastin structure (fenestrae number and size: WKY: 17.6±2; SHR: 9.4±0.5, $P<0.05$ vs WKY; SHR LOSARTAN: 13.3±1.2; SHR APOCYNIN: 12.8±0.7; SHR mito-TEMPO: 23.4±1.6; SHR BAPN: 28.1±2.6 µm, all treatments $P<0.05$ vs SHR, n=8 each group). In the Angiotensin II infused model, apocynin and mito-TEMPO also improved altered vascular stiffness and elastin structure. The gene expression of the elastin-degrading enzymes, matrix metalloproteinases (MMP) 2 and 9, was similar in WKY and SHR (MMP2, WKY: 0.98±0.1; SHR: 0.78±0.1, n=9). However, apocynin and mito-TEMPO increased MMP2 gene expression (SHR APOCYNIN: 3.41±0.9; SHR mito-TEMPO: 1.3±0.2 relative expression, $P<0.05$ vs SHR, n=5-7) without any effect on MMP9. **Conclusions**: Angiotensin II-induced oxidative stress from NADPH Oxidase and/or mitochondria is responsible, at least in part, of the mechanical alterations of resistance arteries in hypertension by affecting elastin structure. An altered equilibrium in elastin synthesis and degradation by changes in LOX and/or MMPs expression and/or activity might participate in these alterations induced by oxidative stress.

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