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Induction of B-Type Natriuretic Peptide Gene Expression by Oxidized Low-Density Lipoprotein

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B-type natriuretic peptide (BNP) has been shown to be powerful diagnostic and prognostic markers in heart failure (HF). The factors that influence the synthesis of BNP are poorly understood. It is generally believed that its increase reflects the changes in pressure-volume characteristics of the left ventricle. In this study, we hypothesize and test that the left ventricle of subjects with HF would have increased levels of oxidized LDL (Ox-LDL) and that it would induce the expression of BNP. This hypothesis is based on the assumption that longer left ventricular residence time of LDL as a result of poor ejection fraction of subjects with HF would increase the propensity of LDL to undergo local oxidation. LDL was isolated from human plasma and was oxidized. Oxidation was monitored spectroscopically and was arrested at various stages of oxidation. HL-1 myocytes were cultured and exposed to varying concentrations of native LDL, mildly ox-LDL, extensively ox-LDL, and oxidatively modified LDL. RNA was extracted and the levels of BNP gene expression were quantitized by Real Time PCR. Venous and left ventricular blood was obtained from subjects undergoing various surgical procedures. The presence of Ox-LDL in the left ventricular blood was established using a commercially available kit. **Results:** Blood from left ventricle of human subjects with HF contained increased amounts of Ox-LDL as compared to venous blood. Mildly and extensively Ox-LDL, and not native LDL, induced BNP gene expression several fold over BNP gene in control cells that were not exposed to any forms of LDL. Oxidatively modified LDL that represented decomposition of the peroxidized lipids, was less effective as compared to mildly or extensively ox-LDL. **Conclusions:** The current study might suggest that left ventricular flow dynamics during HF might result in the generation of Ox-LDL and such LDL might be responsible for the increase in BNP levels in HF subjects. This is the first report of the influence of an atherogenic lipoprotein (Ox-LDL) on an important HF marker. As atherosclerosis itself is an important risk factor for HF, the formation of Ox-LDL in the LV might contribute to HF by additional mechanisms.

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Dehydroepiandrosterone Reverses Systemic Vascular Remodeling Through the Inhibition of an Akt/GSK-3β/NFAT Axis

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Vascular remodeling diseases (VRD) are characterized by proliferative and apoptosis-resistant vascular smooth muscle cells (VSMC). As in cancer, proliferative VSMC have hyperpolarized mitochondria membrane potential ($\Delta\Psi$m), downregulated K+ channels (especially Kv1.5) and increased [Ca2+]i. We found that this proliferative, anti-apoptotic phenotype was associated with Akt activation, decreasing glycogen synthase kinase-3β (GSK-3β) activity, resulting in both the activation of the transcription factor NFATc1 and the translocation of hexokinase-II on mitochondrial membranes, causing increased $\Delta\Psi$m. Dehydroepiandrosterone (DHEA) is a naturally occurring and clinically used steroid known to inhibit the Akt axis in cancer. We hypothesized that DHEA treatment reverses VRD. We used cultured human carotid artery VSMC and saphenous vein grafts in tissue culture, stimulated by PDGF to induce VSMC proliferation; and the rat carotid injury model in vivo. DHEA decreased proliferation and increased apoptosis in VSMC both in vitro and in vivo, reducing vascular remodeling while sparing healthy tissues. Through pharmacological (Akt and GSK-3β agonists and antagonists) and molecular (forced-expression of constitutively active Akt1) approaches we showed that DHEA effects were mediated by inhibition of Akt and a subsequent activation of GSK-3β leading to $\Delta\Psi$m depolarization and inhibition of NFAT reversing Kv1.5 donwregulation, repolarizing VSMC, and normalizing [Ca2+]i. In vivo, DHEA reversed established carotid stenosis, increasing flow and decreasing vascular wall thickness. The effects on Akt were independent of any steroid-related effects since they were not altered by androgen and estrogen inhibitors. Our data suggest that DHEA might be an attractive candidate for VRD therapy in humans.

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Smooth Muscle-Specific PPARγ Deficiency Augments Angiotensin II-Induced Atherosclerosis but Does Not Affect Abdominal Aortic Aneurysms in Male LDL Receptor-Deficient Mice

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Objective: Chronic infusion of angiotensin II (AngII) augments the development of atherosclerosis and induces abdominal aortic aneurysms (AAAs) in hypercholesterolemic mice. Smooth muscle cells (SMCs) play a pivotal role in the development of these two pathologies. AngII-induced AAA is associated with the downregulation of peroxisome proliferator-activated receptor gamma (PPARgamma). In addition, activation of PPARgamma by ligands attenuates atherosclerosis in male mice. The purpose of the study was to define whether deficiency of SMC-specific PPARgamma influences AngII-induced vascular pathologies. **Methods and Results:** Male and female LDL receptor −/− mice were bred with homozygous floxed PPARgamma alleles (PPARgammaf/f). Parental males were also developed that were hemizygous for Cre under the control of the SM22 promoter. Littermates were generated that did not express Cre (Cre0/0, n=20) or were hemizygous for Cre(Cre1/0, n =16) and fed a saturated fat-enriched diet (21% wt/wt milk fat; 0.15% wt/wt cholesterol). Mice were infused with AngII (1,000 ng/kg/min) by osmotic minipump for 4 weeks. AngII increased systolic blood pressure equivalently in both groups. SMC-specific deficiency of PPARgamma had no effect on serum cholesterol concentrations (Cre0/0, 1544±112; Cre1/0, 1548±87 mg/dL) or lipoprotein-cholesterol distributions. AngII infusion led to a similar incidence of AAA formation (84% of male Cre0/0 versus 80% of male Cre1/0 mice). Ex vivo measurement of maximal diameter of abdominal aorta showed a comparable dilation in Cre0/0 and Cre1/0 mice (2.16±0.16 versus 2.17±0.31 mm, *P*=NS). Male Cre1/0 mice had significantly larger atherosclerotic lesion areas in both arch and thoracic aortic regions by en face measurement compared to male f/fCre0/0 mice (Arch area intimal lesions; Cre0/0 = 4.74±1.13%; Cre1/0 = 10.24±1.67%, Thoracic area intimal lesions; Cre0/0 = 4.12±0.69%; Cre1/0 = 8.99±2.28%, $P<0.05$, n=12–18 per group). SMC specific PPARgamma deficiency in female LDL receptor −/− mice did not influence either AngII-induced atherosclerosis or AAAs. **Conclusion:** SMC specific PPARgamma deficiency augments atherosclerosis but does not contribute to the formation of AAAs induced by AngII in male hyperlipidemic mice.

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Air Pollution and Cardiac Remodeling: A Role for RhoA/Rho-Kinase

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Objectives: Exposure to ambient air pollution is a risk of cardiovascular diseases. We had previously demonstrated vascular activation of the RhoA/ROCK pathway by exposure to concentrated particulate matter $<2.5\mu$ (CAPs). In this investigation we examined if the RhoA/ROCK pathway mediated the cardiac effects of CAPs. **Methods and Results:** C57BL/6 mice were exposed to CAPs or filtered air (FA) for 12 weeks. In the last two weeks of CAPs exposure, mice were infused with Vehicle, angiotensin II (AII), or AII in conjunction with fasudil, a Rho-kinase antagonist. Results were expressed as mean±SD (FA vs. CAPs). Blood pressure during AII infusion was monitored through tail cuff method. CAPs exposure potentiated AII induced hypertension, and this effect was abolished by fasudil treatment. Western blot analysis revealed that CAPs increased cardiac RhoA translocation (Membrane/Cytosol: 0.58±0.25 vs. 1.12±0.33 in vehicle groups; 1.85±0.82 vs. 4±1.98 in AII groups). The enhanced RhoA translocation by CAPs was accompanied by increased mRNA expression of the guanine exchange factors, PDZ-RhoGEF (0.42±1.14 vs. 0.81±1.18 in vehicle groups; 1.5±0.54 vs. 2.77±0.2 in AII groups) and p115 RhoGEF (9±5.02 vs. 10.46±4.66 in Vehicle groups; 13.85±1.27 vs. 17.96±2.22 in AII groups). CAPs exposure also increased AII induced cardiac hypertrophy and collagen deposition, with these increases being normalized by fasudil (Heart/Body: 0.0046±0.0001 vs. 0.0049±0.0003 in vehicle groups; 0.0059±0.0002 vs. 0.0064±0.0001 in AII groups; and 0.0055±0.0001 vs. 0.0055±0.0002 in AII-fasudil groups. Nuclear/Cytosol ratio of cardiocytes: 0.12±0.04 vs. 0.1±0.04 in vehicle groups; 0.08±0.01 vs. 0.06±0.01 in AII groups; 0.1±0.02 vs. 0.1±0.03 in AII-fasudil groups. Collagen by Picrosirius red staining: 23.41±5.18 vs. 31.19±8.19 in vehicle groups; 39.68±9.11 vs. 59.54±11.34 in AII groups; 33.10±6.74 vs. 37.61±6.14 in AII-fasudil groups). Real-time RT-PCR analyses revealed that MMP2, but not MMP9, mRNA was increased by CAPs, consistent with gelatin zymography results that MM2, but not MMP9, activity was increased by CAPs. **Conclusion:** CAPs potentiates cardiac remodeling in response to AII through RhoA/Rho-kinase dependent mechanisms.

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Significant Pharmacodynamic Variability in P2Y12 Receptor-Mediated Platelet Response Persists in Healthy Volunteers as Assessed by Comprehensive Platelet Function Analysis

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Objective: Variability in clopidogrel response is associated with adverse cardiovascular events. Differences in baseline platelet function may contribute to this variability. We hypothesize that variability in P2Y12 receptor-mediated platelet function persists in non-medicated, healthy volunteers. **Methods:** Whole blood from 29 volunteers was utilized for platelet analysis by light transmittance aggregometry (LTA), VASP phosphorylation, P-selectin expression (CD62P), GP IIb/IIIa receptor activation (PAC1), and the VerifyNow P2Y12 assay. **Results:** Extensive variation in ADP-induced platelet response was observed in non-medicated, healthy subjects. ADP EC50s differed by ~8-fold for LTA, PAC1, and CD62P (range = 0.46–3.6, 1.1–8.6, and 0.54–3.9 uM, respectively). Notable variability persisted in the presence of an *ex vivo* P2Y12 antagonist as cangrelor IC50s varied from 22.1–185 ng/mL for PAC1 and CD62P. In the VASP assay, platelet reactivity ranged from 55.4–100%. P2Y12 reaction units of the VerifyNow assay varied from 174–410. Cangrelor, SQ (TxA2 receptor blocker), and MRS (P2Y1 receptor blocker) significantly inhibited ADP response in most assays ($P<0.05$); however, MRS had the reverse effect on VASP phosphorylation (Figure 1A). Significant assay correlations are shown in Figure 1B. **Conclusions:** These findings suggest that even in the absence of clopidogrel, sizeable pharmacodynamic variability is evident in healthy volunteers and may be important for explaining differences in antiplatelet response. The range of inter-individual differences was consistent with and without cangrelor and at all tested components of the ADP pathway from signaling to aggregation.