hypertensive rats and human hypertensives (Liu et al.,2005; 2006). In this study, we investigate whether TRPC3 up-regulation in aorta from SHR is associated with angiotensin II receptor (AT1R) mediate calcium influx. **Methods:** Blood pressure was measured using tail-cuff plethys-mography and a pressure transducer. Vasoconstriction of aortic rings was measured by organ chamber. Aortic smooth muscle cells (VSMCs) was cultured. Cytosolic calcium concentration was measured by the fluorescence technique. TRPC3 and AT1R expressions were detected by western blotting. **Results** TRPC3 expression was significantly increased in aorta form SHR compared to WKY (1.48±0.05 vs. 1.00±0.06, $p<0.01$). AT1R expression was no significantly difference in aorta from SHR compared to WKY ($p>0.05$). Administration of Ang II significantly increased TRPC3 expression in VSMCs from SHR compared to SHR control conditions ($p<0.05$). Administration of telmisartan, an AT1R blocker, significantly down regulated TRPC3 expression in cultured VSMCs ($p<0.05$). Immuno-fluorescence showed that TRPC3 and AT1R coexisted in cultured VSMC. Ang II significantly increased mean arterial blood pressure in SHR compared to WKY (53±3 vs. 22±4 mmHg, $p<0.01$). AngII-induced vasoconstriction was significantly higher in aortic rings from SHR compared to WKY (82±3% vs. 54±6%, $p<0.01$). After administration of telmisartan (5mg/kg/day) in SHR for 4 weeks systolic blood pressure was significantly reduced from 202±20 mmHg to 124±18 mmHg ($n=6$, $p<0.01$). Upregulation of TRPC3 by overexpressing TRPC3 gene in the cultured VSMCs significantly increased TRPC3 expression ($p<0.01$), and AngII-induced calcium influx was significantly increased to 155±12% ($n=6$; $p<0.01$). After siRNA against TRPC3 significantly reduced TRPC3 expression by 30±5% in the cultured VSMCs and Ang II-induced calcium influx was accompanied reduced ($p<0.05$). **Conclusion** This study for the first time highlights the importance of AT1R mediated the calcium influx through TRPC3 channel in genetic hypertension (supported by 973 program 2006CB503804).

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Effects of Recombinant Human Brain Natriuretic Peptide on a Porcine Model of Acute Pulmonary Hypertension

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OBJECTIVES: The effects of brain natriuretic peptide (BNP) on the pulmonary hypertension remains unknown. The aim of the present study was to evaluate the effects of recombinant human BNP (rhBNP) on pulmonary and systemic haemodynamics in a porcine model of thromboxane-induced acute pulmonary hypertension. **METHODS:** Acute pulmonary hypertension was induced with a continuous infusion of the thromboxane analogue, U46619. Fifteen adult swine were randomized into two groups. Group 1 ($n = 8$) received an intravenous bolus rhBNP at 0.3 mg/kg/min, followed by an infusion at a rate of 0.015 mg/kg/min, and group 2 ($n = 7$) received a saline solution instead of rhBNP. **RESULTS:** Acute pulmonary hypertension was achieved in all animals. The administration of rhBNP reversed pulmonary hypertension, with a significant decrease in mean pulmonary artery pressure and pulmonary vascular resistance, a moderate decrease in systemic arterial pressure and a concomitant increase in cardiac output, but no marked changes in heart rate. **CONCLUSIONS:** The administration of rhBNP is associated with predominant pulmonary vasodilatation with moderate systemic vasodilatation in this porcine model of acute pulmonary hypertension. This study was supported by Natural Science Foundation of China (No. 30600242 and 30670880)

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HMG-CoA Reductase Inhibitor Ameliorates Aortic Stiffness in Spontaneously Hypertensive Rats

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Systemic hypertension is associated with elevated cardiovascular morbidity and mortality and is at least partly due to large artery stiffening. Cholesterol-lowering therapy has been efficacious in reducing arterial stiffness in patients with hypercholesterolemia, and thus may be beneficial in systemic hypertension. The present study was to examine whether HMG CoA reductase inhibitor plays a role in the regulation of vascular stiffness. Pulse wave velocity (PWV) was determined as the time delay between the foot of pressure waves recorded simultaneously at the aortic arch and abdominal aorta (just above the bifurcation) in anesthetized Spontaneous Hypertensive Rats (SHR). Eight-week-old SHRs were given standard chow or chow containing rosuvastatin at a dose of 10 mg/kg or for 8 weeks: an untreated control group (CON, $n = 12$), and an HMG-CoA reductase inhibitor, rosuvastatin (ROS, $n=12$). PWV was measured at the same blood pressure (BP) level as in the control group, and the level of collagen content and advanced glycated end products (AGE) was measured in aortic wall. PWV was improved after treatment (CON vs. ROS: 10736.53± 2531mm/sec vs. 8742.94±1593mm/sec, $p<0.05$). Aortic systolic blood pressure was not changed after rosuvastatin treatment (180±17 vs. 189.0±22 mm Hg, p=ns), as were mean (151.3±16 vs. 156.1±24 mm Hg, p=ns), diastolic blood pressures (137.0±16 vs. 139.6±17 mm Hg, p =ns) and pulse pressure (42.9±8 vs. 49.4±13 mm Hg, p=ns). Hydroxyproline content was significantly reduced by treatment (CON vs. ROS: 14.8± 5mg/g vs. 10.4±3, $p<0.05$), whereas AGE content was not changed (315.89± 9076U/mg vs. 291.20±8861, p=ns). This study demonstrates that rosuvastatin reduces collagen content in the aortic wall and is also associated with a concomitant reduction in aortic PWV. This suggests that HMG CoA reductase inhibitor has effect modifying arterial stiffness and improves vascular function in hypertension.

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Arterial and Venous Endothelin-1 Content in Humans: Interactions with Other Predictors of Hypertension

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Introduction: Local production of endothelin-1 (ET-1) by vascular endothelial cells may contribute to hypertension development by causing vascular constriction or hypertrophy. Most studies have shown, however, that arterial content of ET-1 is not higher in patients with mild to moderate essential hypertension. Nevertheless, such measurements are possibly confounded by effects of hyperlipidemia, type II diabetes, and other factors on ET-1 content. In addition, ET-1 is a potent venoconstrictor, but relatively few studies report venous ET-1 content. **Hypothesis**: We tested the hypothesis that arterial or venous ET-1 content can predict hypertension when adjusted for these confounding variables. **Methods:** We determined ET-1 content in internal mammary artery and saphenous veins of 57 subjects, who underwent coronary artery bypass graft surgery for atherosclerotic disease. A chemiluminescent ELISA was used to measure ET-1 content. Other patient data was obtained from medical records and patient interviews. Univariate comparisons, correlation analyses and logistic regression modeling were used to analyze the data. **Results**: No statistically significant differences in arterial or venous ET-1 content were found when comparing patients according to blood pressure, age, sex, smoking, lipids, fasting glucose levels, or type II diabetes status. Also no differences were found in patients taking statins, ACE inhibitors, or beta-blockers. Arterial ET-1 content did not predict hypertension when added to any logistic regression model; but the data revealed a strong correlation between arterial ET-1 content and plasma triglyceride levels. The only logistic model that strongly predicted ($p= 0.0083$) hypertension included sex and venous ET-1 content as predictor variables. **Conclusions**: Two important conclusions are drawn from these results. First, in confirmation of earlier work, arterial ET-1 content is not higher in hypertension, but such measures may be confounded by an influence of plasma triglycerides on arterial ET-1. Second, venous ET-1 may have a larger effect on hypertension development than previously appreciated.

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Regulation of Blood Pressure by Prostaglandin F_{2a} Receptor Gene (FP)

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Aspirin reduces renin and blood pressure in renovascular hypertension and there is a rebound rise in renin after reversible inhibition of cyclooxygenases by salicylates in humans. It has been assumed that these effects reflect modulation of prostacyclin (PGI_2) a potent renin secretagoge. However, recent development of novel methodology indicates that prostaglandin (PG) $F_{2\alpha}$ is a far more abundant prostaglandin in urine of mice and humans and little is known of its cardiovascular function. Intravenous $PGF_{2\alpha}$ results in a dose dependent elevation of blood pressure in wild type mice. This response is lost in mice lacking the F prostanoid (FP) receptor, while the hypertensive response to angiotensin II is augmented, consistent with increased expression of AT1a and AT1b receptors in large arteries. Deletion of the FP reduces blood pressure retards atherogenesis in hyperlipidemic mice and also reduces blood pressure in normolipidemic mice on either a chow or a high fat diet. Curiously, expression of the FP, while evident in arterioles and the renal collecting ducts, is not detectable in the heart or large arteries of mice. Deletion of the FP depressed plasma renin activity, angiotensin and aldosterone under basal conditions and following salt depletion. These findings demonstrate that $PGF_{2\alpha}$ plays a critical role in maintenance of blood pressure homeostasis by regulating the renin - angiotensin system in the kidney of mice. Antagonism / deletion of the I prostanoid receptor (IP) which accelerates atherogenesis. Pharmacological blockade of the FP may represent a novel therapeutic strategy in syndromes of renin dependent hypertension with a more cardioprotective profile than suppressing synthesis or disrupting activation of the IP by PGI_2.

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Enhanced Nuclear Translocation of Nuclear Factor-κB in Micro-G Stimulated Cardiomyocyte Cells

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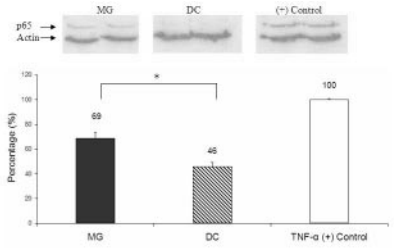
Introduction: Rotating Wall Vessel (RWV) bioreactors developed by NASA have been used in the laboratory to create suspension cell culture environments for simulating microgravity (MG) conditions on earth, and to study the effect of shear stress and gravity on the evolution, growth, and physiological changes of mammalian cells. Nuclear factor-kappa B (NF-κB) is one of the most prevailing oxidation-sensitive transcription factors. The objective of this study is to investigate the activation of NF-κB in the rat cardiac cells (H9c2) under MG. **Method**: H9c2 were cultured in the RWV under MG and unit-gravity dynamic control (DC) conditions. Western blots and enzyme-linked immunosorbent assay (ELISA) were done using the nuclear extracts for the evaluation of differential NF-κB p65 protein detection. **Results**: NF-κB p65 protein revealed differential expression under MG and DC conditions. Mean activations of p65 protein were 69% for MG and 46% for DC as compared to positive control which were stimulated with TNF-α for 30 min (* $p<0.05$, $n=3$, Figure). The results from western blots were confirmed by ELISA which showed 66% for MG and 45% for DC. **Conclusions**: The present result showed significantly differential expression of NF-κB p65, a pro-inflammatory transcription factor that is sensitive to oxidative stress, under MG as compared to DC. The study may be linked to explain the physiological changes such as muscle atrophy and further to identify the regulatory pathways and effector molecules under exposure to MG.



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Nuclear Shp-2 Is One Important Negative Regulator of the Nuclear Export of Telomerase Reverse Transcriptase Induced by Oxidative Stress: Implication for Aging Processes and Vascular Diseases

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In the western society one major risk factor associated with aging are vascular diseases. The risk of heart attack and coronary diseases increases with aging. Therefore, it is important to understand aging on a cellular level. One factor of cell aging is the shortening of telomeres, the ends of the chromosomes. The enzyme telomerase reverse transcriptase (TERT) counteracts this shortening. Therefore, TERT can exert the life span of different cell types. One important regulatory mechanism of TERT is its localisation. In this context, we recently showed that in endothelial cells oxidative stress induced Src kinase family-dependent tyrosine phosphorylation of TERT, which resulted in nuclear export of TERT and reduction of nuclear TERT activity. This led to accelerated senescence and enhanced apoptosis sensitivity of endothelial cells. Therefore, the aim of this study was to investigate the mechanism inhibiting nuclear export of TERT. One potential "inhibitor" is the tyrosine phosphatase Shp-2, which can reduce the activity of the Src kinase family. For inhibiting the nuclear export of TERT the nuclear localisation of Shp-2 is necessary. Thus, we first demonstrated that endogenous Shp-2 was located in the nucleus and in the cytosol. Next, we showed that oxidative stress reduced Shp-2 protein levels and activity. Nuclear Shp-2 associated with TERT and overexpression of Shp-2 wildtype inhibited H2O2-induced nuclear export of TERT. This inhibition was dependent on Shp-2 phosphatase activity, since a dominant negative Shp-2 mutant (Shp-2C459S) reduced nuclear TERT protein and activity already under basal conditions. For identification of the member of the Src kinase family, which is responsible for the export of TERT, we used mouse embryonic fibroblasts deficient in the Src kinase family members Src, Fyn and Yes. In these cells the oxidative stress-induced nuclear export of TERT was completely abolished. Taken together, these data demonstrate for the first time that a function for nuclear Shp-2 exists in inhibiting nuclear export of TERT. Thus, increasing the amount of nuclear Shp-2 may a useful therapeutic to delay/inhibit vascular aging processes.

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The Role of Nrf2 and Unfolded Protein Response in the Induction of Antioxidant Enzymes by Oxidized Phospholipids

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Besides their well-characterized proinflammatory and proatherogenic effects, oxidized phospholipids (oxPLs), such as oxPAPC (oxidized 1-palmitoyl-2-arachidonoyl-sn-glycero-phosphocholine) have been shown to have beneficial responses in vascular cells via induction of antioxidant enzymes such as heme oxygenase-1 (HO-1). We therefore hypothesized that oxPAPC could evoke a general cytoprotective response via activation of antioxidative transcription factor Nrf2. Here we show that oxPAPC increases nuclear accumulation of Nrf2. Using the siRNA approach, we demonstrate that Nrf2 is critical in mediating the induction of glutamate-cysteine ligase modifier subunit (GCLM) and NAD(P)H quinone oxidoreductase-1 (NQO1) by oxPAPC in human endothelial cells, whereas the contribution to the induction of HO-1 was less significant. The induction of GCLM and NQO1 was attenuated by reduction of electrophilic groups with sodium borohydrate as well as treatment with thiol antioxidants, suggesting that the thiol reactivity of oxPAPCs is largely mediating its effect on Nrf2-responsive genes. The PERK-mediated unfolded protein response (UPR) induced by endoplasmic reticulum stress was shown to be important for the induction of these genes by Nrf2. Finally, the oxPAPC-inducible expression of HO-1, GCLM, and NQO1 is lower in Nrf2-null than wild type mice carotid arteries in vivo. We suggest that the activation of Nrf2 by oxPLs provides a mechanism by which their deleterious effects are limited in the vasculature.

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Inhibition of PPAR-γ Agonists on TNF-α-Induced Oxidative Stress in Human Aortic Smooth Muscle Cells by PPAR-γ-Dependent Mechanism

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BACKGROUND AND PURPOSE: Peroxisome proliferator-activated receptors are members of the nuclear hormone receptor superfamily of ligand-activated transcription factors. Recent studies have shown that PPAR-γ agonists reduce the progression of atherosclerotic lesions by direct anti-atherogenic and anti-inflammatory actions with PPAR-γ dependent or independent mechanisms. Oxidative stress and reactive oxygen species (ROS) underpin the pathogenesis of cardiovascular diseases including atherosclerosis. NADPH oxidase is a predominant source of ROS and activation of this enzyme leads to intracellular signaling events causing endothelial dysfunction. We aimed to investigate the inhibition of PPAR-γ agonists rosiglitazone (RGZ) and 15-deoxy$\Delta^{12,14}$-prostaglandin J2 (15d-PGJ2) on TNF-α induced- NADPH oxidase mediated-oxidative stress in human aortic smooth muscle cells (AoSMC) by PPAR-γ dependent mechanism. METHODS: AoSMC were treated with TNF-α (10ng/ml) for 12h and superoxide production was examined by lucigenin-enhanced chemiluminescence assay. AoSMC was pre-treated with RGZ (5μM) or 15d-PGJ2 (10μM) for 12h in the presence and absence of selective PPARγ antagonist GW9662 (10μM) and treated with TNF-α (10ng/ml) for 12h. DMSO (0.1%) was used as vehicle control. Real time RT-PCR analysis was performed to determine the NADPH oxidase subunit Nox4 gene expression level. RESULTS: Superoxide production was detected in AoSMC after treatment with TNF-α through up-regulation of Nox4 (n=4, $P<0.05$). Both RGZ and 15d-PGJ2 significantly inhibited TNFα-induced superoxide production and this effect was reversed by selective PPARγ antagonist GW9962 (n=4, $P<0.05$). RGZ significantly inhibited TNF-α-induced Nox4 gene up-regulation by nearly 50% whilst 15d-PGJ2 showed significant inhibition of more than 80%. The inhibitory effect of RGZ and 15d-PGJ2 was reversed by GW9662. (n=4, $P<0.05$). CONCLUSIONS: We demonstrated that TNF-α stress elicits elevated AoSMC superoxide and that TNF-α induced superoxide production is mediated by Nox4. Our data reveals that, for the first time, both synthetic PPAR-γ agonist RGZ and endogenous PPAR-γ agonist 15d-PGJ2 inhibit TNF-α induced- Nox4 mediated-oxidative stress in AoSMC by PPAR-γ dependent mechanism.

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Novel Nox1-Mediated Mechanism of SSH1L Activation in VSMC: Role in Cell Migration

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Platelet-derived growth factor (PDGF) activates a Nox1-based NADPH oxidase and contributes to atherosclerosis and restenosis by stimulating VSMC migration in a hydrogen peroxide (H_2O_2)-dependent manner. Migration requires rapid turnover of actin filaments, which is partially controlled by cofilin. Cofilin is activated by dephosphorylation by the Slingshot phosphatase1L (SSH1L). We recently demonstrated that SSH1L activity is required for PDGF-induced cofilin activation and migration in VSMC. However, the mechanism leading to SSH1L activation is mostly unknown. We hypothesize that Nox1-derived H_2O_2 participates in SSH1L activation. We found that in VSMC exposed to H_2O_2, cofilin is dephosphorylated and SSH1L activity is increased (2124$\pm$132 vs 3489$\pm$880 pmoles PO_4/300 μg) after 30 min. Moreover, VSMC derived from Nox1 KO animals have impaired PDGF-induced migration (150$\pm$28 vs 49$\pm$13 cells/field p=0.03) and SSH1L activation (205 $\pm$26 vs 151 $\pm$14), and this is accompanied by a decrease amount of active cofilin. Of relevance, PDGF-induced migration in Nox1 KO cells is recovered following transfection with a constitutively active form of cofilin (49 $\pm$13 vs 135 $\pm$27 cells/field p=0.04). In addition, we observed that Nox1 KO cells completely failed to activate Rac1 after 5 min of PDGF stimulation while wild type do robustly (182 $\pm$32 % percent control). One proposed mechanism of SSH1L regulation involves the release from an inhibitory complex with 14–3–3 proteins in a Rac1-dependent manner. Indeed, SSH1L and 14–3–3 coimmunoprecipitate in VSMC. Since 14–3–3 protein has potential redox-sensitive cysteines, we hypothesized that in addition to its effects on Rac, Nox1 may also regulate SSH1L by oxidizing its interacting partners. We found that both H_2O_2 and PDGF completely oxidize 14–3–3 at 30 min, suggesting that 14–3–3 contains redox-sensitive SH groups. Taken together, these data show that SSH1L activation is mediated by Nox1-produced H_2O_2 and suggest that the activation mechanism involves Rac1 activation and oxidation of 14–3–3. These findings suggest new therapeutic targets for vascular pathologies such as restenosis and atherosclerosis in which migration is a significant component.

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Gene Transfer of Angiotensin-Converting Enzyme-2 Reverses Angiotensin II- and IV-Induced Expression of Macrophage Migration Inhibitory Factor in Insulin Resistance of Endothelial Cells

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Insulin resistance is a proinflammatory state associated with enhanced oxidative stress, which has been closely linked to abnormalities in the renin-angiotensin system (RAS) . As predominant effectors of RAS, angiotensin (Ang) II and its fragment Ang IV exert various deleterious effects by promoting the productions of proinflammatory cytokines. Macrophage migration inhibitory factor (MIF) is now known as a proinflammatory cytokine that was recently associated with insulin resistance and exhibited previously to be induced by angiotensin (Ang) II. Our aim was to investigate whether Ang II, its fragment Ang IV and related enzyme angiotensin-coverting enzyme 2 (ACE2) could modulate the expression of MIF and insulin signaling in cultured human endothelial cells. A recombinant plasmid encompassing human ACE2 cDNA was constructed and transfected into these cells. The mRNA, phosphorylation and protein levels of p22phox, MIF and Akt in cells were determined by real-time polymerase chain reaction and Western blotting, respectively. The results showed that exposure of endothelial cells to Ang II and Ang IV resulted in a time-dependent increase in MIF mRNA and protein expressions, respectively. ACE2 gene transfer strikingly suppresses the expressions of p22phox and MIF induced by Ang II and IV in endothelial cells. In addition, Ang II diminished insulin-stimulated phosphorylation of Akt at serine 473 and eNOS at serine 1177 and NO generation, which were reversed by ACE2 gene transfer and anti-MIF treatment in endothelial cells. Our findings reveal that gene transfer of ACE2 regulates Ang II-mediated impairment of insulin signaling involving Akt-eNOS phosphorylation pathway. These beneficial effects of ACE2 overexpression are thought to result mainly from blocking MIF expression in endothelial cells,