Acute Administration of Asymmetric Dimethylarginine Reduces Insulin Sensitivity in Mice

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Background: Asymmetric dimethylarginine (ADMA) is an endogenous inhibitor of NO synthase. ADMA is metabolized by dimethylarginine dimethylaminohydrolase (DDAH). We have shown that plasma ADMA level inversely correlates with a measure of insulin sensitivity in humans. We now examine if ADMA reduces insulin sensitivity.

Methods: After 4hrs of fasting, wildtype female C57BL/6J mice were injected i.p. with either ADMA (75mg/kg) or PBS; transgenic mice overexpressing DDAH were given the same dose of ADMA (n=3 in each group). After 2hrs, insulin was injected and blood glucose was assayed every 5min for 1hr. The half-time of glucose disappearance from plasma was calculated using a first-order decay model. Plasma ADMA was measured by HPLC.

Results: Wildtype mice given ADMA showed a delayed and attenuated drop in glucose in response to insulin compared to those receiving PBS (see figure). Wildtype mice given ADMA had a significantly higher glucose half-life than those given PBS (47.9±8 vs. 20.9±4 min). Transgenic mice were resistant to the effects of ADMA, and their glucose half-life was not significantly different from wildtype mice receiving PBS. When given ADMA, wildtype mice had significantly higher plasma ADMA compared to transgenic mice (9.2±0.7 vs. 2.9±0.7 µM).

Conclusion: Elevated plasma ADMA acutely reduces insulin sensitivity in mice. We speculate that there is a form of physiological insulin resistance, secondary to ADMA-induced reduction in skeletal muscle blood flow and glucose disposition.

Versican Promoter Is a Target for the β-Catenin/Tcf-4 Signaling Pathway in Vascular Smooth Muscle Cells

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Background: Versican (VCN) is one of the proteoglycans constitutively expressed in atherosclerotic lesions. We have investigated the role of the signaling pathways and cis- and trans-factors in VCN regulation in vascular smooth muscle cells (VSMCs).

Methods and Results: A 752-bp of the human VCN promoter was cloned in luciferase reporter plasmid (VCN-Luc). PI3K inhibitor LY29004 significantly decreased promoter activity. Co-transfection of constitutive active- and dominant negative protein kinase B (PKB) constructs with VCN-Luc increased and decreased VCN promoter activity. Inhibition of the glycogen synthase kinase-3β (GSK-3β) activity by LiCl augmented accumulation of β-catenin and induction of promoter activity. β-catenin has no DNA binding domain therefore it cannot induce transcription of VCN alone. Software analysis revealed the Tcf-4 transcription factor binding site in the VCN promoter. Electrophoretic mobility shift assay and supershift assay revealed specific binding of recombinant hTcf-4 to this site in the VCN promoter. In addition, GSK-3β inhibitor LiCl or β-catenin expression vector did not induce promoter activity when the Tcf-4 binding site was mutated.

Conclusions: Our findings suggest that VCN transcription is predominantly mediated by activation of PKB and subsequent inhibition of GSK-3β pathway via β-catenin/Tcf-4 transcription factor complex in the VCN promoter in VSMCs. Supported by the Canadian Institutes of Health Research and the St. Paul's Hospital Foundation.

ROSIGLITAZONE MODULATES ANGIOGENIC PROGENITOR CELL DIFFERENTIATION AND ATTENUATES INTIMAL HYPERPLASIA AFTER VASCULAR INTERVENTION

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PPAR-γ agonists inhibit vascular smooth muscle proliferation and migration, and improve endothelial function. It is unknown whether PPAR-γ agonists favorably modulate bone marrow-derived angiogenic progenitor cells (APCs) to promote endothelial lineage differentiation and early reendothelialization following vascular intervention.

Methods & Results: C57BL/6 mice, treated with or without rosiglitazone (8 mg/kg/day), a PPAR-γ agonist, underwent femoral angioplasty. Rosiglitazone treatment attenuated neointimal formation (Intima/Media ratio: 0.98 ± 0.12 rosiglitazone vs. 3.1 ± 0.5 control, p<0.001). The contribution of bone marrow-derived cells to neointimal formation was investigated by bone marrow transplantation from eYFP mice to background mice. 58±12% of the cells within the neointima at 4 weeks were derived from the rosiglitazone group, p<0.05) and in human peripheral blood (31±5 vs. 8±2 CD31-positive cells per mm of neointimal surface on day 14, p<0.001).

Conclusions: Rosiglitazone attenuates restenosis after angioplasty.