Dimerization of Mitogen-Activated Protein Kinase and Auto-Phosphorylation of Akt Occur in the Newly Differentiated Endothelial-Like Cells From Adult Bone Marrow Stem Cells in Vitro

Zhenguo Liu, Rong Lou, Yuehua Jing, Kai Gu, Catherine Verfaillie, Robert J. Bache, University of Minnesota Medical School, Minneapolis, MN

The present experiments were conducted to investigate the differences of biological behavior and signal transduction pathways between the newly differentiated endothelial cells and normal mature endothelial cells. Cultured mouse adult bone marrow multipotent progenitor cells (MAPCs) were used as the source of stem cells in this study. The MAPCs were induced to differentiate into endothelial cells in serum-free medium in the presence of vascular endothelial growth factor. The course of stem cell differentiation into endothelial cells was monitored with endothelial cell specific markers including von Willebrand factor (vWF). The differentiating cells started to express vWF 10 days after initiation of differentiation. At day 14 of cell differentiation, it was found that the newly-differentiated endothelial-like cells derived from MAPCs formed much denser networks of branching and anastomosing processes on growth-factor reduced Matrigel than did normal mature endothelial cells. It was also observed that the newly-differentiated endothelial cells had much greater growth potential than did normal mature endothelial cells. Phosphorylated mitogen-activated protein kinase (MAPK) dimers of 84 kD were identified in the newly differentiated endothelial cells without stimulation. Phosphorylation of the serine/threonine protein kinase Akt was also observed in these unstimulated newly differentiated cells. Neither the MAPK dimers nor auto-phosphorylation of Akt was found to be present in normal cultured mature endothelial cells. These results suggest that both MAPK and Akt are constitutively activated in the newly differentiated endothelial cells. The presence of phosphorylated MAPK dimers and constitutive phosphorylation of Akt in the newly-differentiated endothelial-like cells may contribute to their unique biological behaviors.

Marked Upregulation of Lipopiggyenase-1, a Receptor for Ox-Low-Density Lipoprotein in Atherosclerosis, and Its Total Ablation by Candesartan and Rosuvastatin Given Concurrently

Jiawei Chen, Dayuan Li, Robert Schaefer, Jawahar L. Mehta, University of Arkansas for Medical Sciences, Little Rock, AR

Background: LOX-1 is a receptor for ox-LDL, which is upregulated in atherosclerosis. Recent studies show its upregulation by ox-LDL and angiotensin II type 1 (AT1) receptor activation. We postulated that control of dyslipidemia with rosuvastatin, an HMG CoA reductase inhibitor, blocks of AT1 activation with candesartan, would have a synergistic inhibitory effect on LOX-1 expression and evolution of atherosclerosis.

Methods and Results: Apo-E knockout mice were fed high-cholesterol diet (1% cholest- erol) alone, or with candesartan (10 mg/kg) or rosuvastatin (10 mg/kg/d) or both. Twelve weeks later, the extent of atherosclerosis was determined by Sudan IV staining. Apo-E knockout mice with high-cholesterol diet had extensive atherosclerosis. Candesartan and rosuvastatin each decreased the extent of atherosclerosis (P < 0.002, n = 7 and 5 for each group independent of reduction in total- and LDL-cholesterol. However, the combined feeding of candesartan and rosuvastatin reduced atherosclerosis in a synergistic fashion (P < 0.01 vs., control and P < 0.05 vs. candesartan and rosuvastatin alone). The expression of LOX-1 was upregulated (10-fold) by high-cholesterol diet in apo-E knockout mice (col. 2 vs. 1, figure below). While candesartan and rosuvastatin (col. 3 and 4) each had a small inhibitory effect on the expression of LOX-1, the combination therapy (col. 5) ablated the expression of LOX-1 below the levels seen in normocholesterolemic C57Bl/6J mice (background for apo-E knockout mice). Combination of candesartan and rosuvastatin also ablated the expression of NF-κB and p38 MAPK, whereas candesartan and rosuvastatin alone had only a modest effect.

Conclusion: This study, for the first time, demonstrates that the combination of candes- artan and rosuvastatin markedly affects the expression of p38 MAPK, redox-sensitive NF-κB and LOX-1. These alterations then lead to a marked anti-atherosclerotic effect.

Angiotensin II Induces TRAF-2 in Human Vascular Smooth Muscle Cells and Sensitizes CD40 Without Upregulation of CD40 Expression

Michael Bradowitzki, Caroline AH Pfeiffer, Joachim Schmidt, Roger Kranzhoefer, University of Heidelberg, Heidelberg, Germany

Background: Chronic inflammation of the vessel wall is a hallmark of atherosclerosis. Besides secreted cytokines and direct cell-cell contact e.g. via the CD40/CD154 receptor-ligand-dyad, it contributes to this inflammatory reaction. CD40 is expressed on human vascular smooth muscle cells (SMC) and CD154 on lymphocytes. Intracellular CD40 signaling depends on TNF-receptor associated factors (TRAFs). Aim of the present study was to investigate if the proatherogenic vasococeptive peptide angiotensin II (ANG II) stimulates the inflammatory response in human vascular smooth muscle cells via a CD40/CD154-dependent pathway.

Methods and Results: Human SMC were preincubated with ANG II (100 nM) and afterwards stimulated by recombinant CD154 (5 ng/ml). These cells secreted higher amounts of IL-6 into the culture medium than control cells not primed with ANG II (271 +/- 13 vs. 158 +/-14 pg/ml, p < 0.05). This stimulation of cytokine secretion was not due to increased expression of CD40 protein on the cell surface as shown in Western blots but was associated with higher expression of TRAF-2 protein. The effect could be blocked by the specific AT1-receptor antagonists losartan (10 µM) and candesartan (100 nM). Moreover, ANG II time-dependently activated the proinflammatory transcription factors NF-κB and AP-1, as shown in electrophoretic mobility shift assay.

Conclusion: ANG II induces a functionally relevant inflammatory response in human SMC by induction of TRAF-2. This mechanism may contribute to the proatherogenic effect of ANG II.