F-D9

Uric Acid Induced Endothelial Senescence Associated with an Increased Production of ADMA and ROS in HUVEC

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Introduction : Endothelial dysfunction is an early and reversible event for the development of cardiovascular disease. Recent experimental and clinical data revealed a firm link between uric acid (UA) and endothelial dysfunction with a demonstration of UA-induced decrease in nitric oxide (NO) release from cultured endothelial cells and an impaired flow-mediated vasodilatation in hyperuricemic patients. Cell senescence, the limited ability of primary human cells to divide when cultured in-vitro, is considered an essential contributor to endothelial dysfunction, possibly associated with an alteration in NO bioavailability and oxidative stress. In order to investigate the mechanism of UA-induced endothelial dysfunction, we studied whether UA per se induced endothelial cell senescence and whether it would be related to oxidative stress and the change of an endogenous eNOS inhibitor, asymmetric dimethylarginine (ADMA) production.

Methods : Intracellular ROS generation in human umbilical venous endothelial cells (HUVECs) incubated with UA (6 to 12 mg/dL) was measured by DCF-DA staining and FACscan analysis. ADMA production was measured by high- performance liquid chromatography (HPLC) after derivatization with naphthalene-2,3-dicarboxaldehyde. For detection of cell senescence, cells were fixed and stained for senescence-associated β-galactosidase (SA β-gal) activity. The percentage of SA β-gal positive cells was determined by counting the amount of blue cells. In order to investigate the effect of NO or ROS on UA-induced endothelial cell senescence, HUVECs were treated with NO donor, DETA-NONOate (100 mM) or antioxidant, tempol (1 mM) combined with UA stimulation.

Results : UA induced a 2.3-fold increase in the activity of SA β-gal of HUVEC compared to control from 48 hours of stimulation. Intracellar ROS generation was also increased from 30 minutes. At 24 hours of UA (9 mg/dL) stimulation, the concentration of ADMA in cell culture media was 5.9 ± 1.4 pM/μg of cell protein which was significantly higher than control media (2.4 ± 1.7 pM/μg, $p<0.05$). Co-incubation of DETA-NONOate or tempol of HUVEC with UA abolished the UA-induced increase in SA β-gal activity, suggesting that UA-induced senescence of HUVEC was mediated by NO and/or ROS system.

Conclusion : UA-induced cell senescence may be one of the mechanisms of endothelial dysfunction. Increased oxidative stress and/or decreased NO bioavailability with an increased ADMA production, which are already known as key factors of endothelial dysfunction, seems to be responsible for endothelial senescence by UA.