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Hypoxanthine Induces Human Primary Umbilical Vein Endothelial Cell Apoptosis Through Oxidative Stress

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Introduction: Hypoxanthine is the end product of purine metabolism. The high level of excretion is associated with increased oxidative stress and DNA damage. Our previous report indicated that hypoxanthine is elevated in hemodialysis (HD) patients compare to peritoneal dialysis (PD) patients. In this study we investigated the effects of hypoxanthine on human primary umbilical vein endothelial cells (HUVECs) in relation to reactive oxygen species (ROS).
Methods: HUVECs were treated with 1 mM hypoxanthine in the presence or absence of an antioxidant, N-acetyl cysteine (NAC). Intracellular ROS generation was assessed by using 2',7'-dichlorofluorescin diacetate (DCFH-DA). To investigate the effect of hypoxanthine on the apoptosis of HUVECs, we used propidium iodide (PI) staining and annexin V-fluorescein isothiocyanate (FITC) staining assays. The protein levels of Bcl-2, Bad and Bax were analyzed by Western Blot. The activity of caspase 3 was detected by kits.
Results: Hypoxanthine treatment (1 mM) induced apoptosis assessed by PI/Annexin V-FITC staining assays and increased ROS generation in HUVECs. Hypoxanthine decreased the intracellular expression of anti-apoptotic proteins of the Bcl-2 while the intracellular expression of pro-apoptotic proteins of the Bax was increased in the hypoxanthine-treated HUVECs. The activity of caspase-3 was increased hypoxanthine-treated HUVECs. NAC decreased general ROS levels and inhibited apoptosis in hypoxanthine-treated HUVECs. The ability of hypoxanthine to induce apoptosis is dependent on the generation of intracellular ROS in HUVECs.
Conclusions: Hypoxanthine can induce apoptosis of HUVECs mediated by oxidative stress. It suggests that elevated hypoxanthine level in HD patients may play a role in cardiovascular diseases.

Key Words: Hypoxanthine, Reactive oxygen species, Apoptosis