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Effects of Oxidative Stress versus Cytokine-dependent Inflammation on the release of Endothelial Microparticles

James M. Heilman[1], William S. Evans[1], Steven J. Prior[1,2]. [1]University of Maryland, College Park, MD, [2]Baltimore Veterans Affairs Geriatric Research, Education and Clinical Center and Research and Development Service, Baltimore, MD.

Endothelial microparticles (EMPs) are released by endothelial cells in response to apoptosis and/or endothelial cell activation. As such, EMPs are considered markers of endothelial dysfunction and atherosclerosis that may be detected before clinical manifestation of vascular diseases. Even though certain mechanisms of EMP release are fairly well-known, EMP release following common stressors such as inflammation and oxidative stress remains less clear. **PURPOSE:** Determine the effects of lipopolysaccharide-induced cytokine-dependent inflammation (LPS) and hydrogen peroxide-induced oxidative stress (H_2O_2) on activated and apoptotic EMP release in cultured endothelial cells. **METHODS:** Three lots of passage 4-5 human umbilical vein endothelial cells (HUVECs) were incubated with either 1 µg/mL LPS for 24h or 400 µM H_2O_2 for 1 hour in endothelial growth medium 2 (EGM2). Each condition was performed in each cell lot and processed in duplicate. After 1 hour of H_2O_2 incubation, old medium was removed and new EGM2 was added to the cells, then collected after 24 hours. This was done because H_2O_2 incubation for long periods will kill the cells and degrade EMPs. Endothelial microparticles were isolated from the medium of each condition and stained with fluorescent conjugated antibodies for flow cytometry to quantify $CD31^+42b^-$ (apoptosis) and $CD62E^+$ (endothelial cell activation) EMPs. Analysis of variance was used to test for differences among H_2O_2, LPS, and control conditions. **RESULTS:** There was a significant increase in the amount of $CD31^+42b^-$ EMPs per µL of media following the H_2O_2 condition compared with the LPS condition (5.00 ± 1.84 vs. 1.31 ± 0.64, $P < 0.01$); however, there were no statistically significant differences between conditions and control. **CONCLUSION:** Microparticle release occurs in quiescent and stimulated endothelial cells. H_2O_2-induced oxidative stress enhances generation of apoptotic EMPs compared with cytokine-dependent LPS inflammation. As such, oxidative stress may be a more potent, specialized stimulus for endothelial cell dysfunction at the onset of chronic vascular disease compared to a cytokine-dependent stimulus, which instead works primarily through endothelial cell activation.

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