

Temporal and Spatial Investigations of the Cellular Mechanisms of H₂O₂-induced Increases in Microvessel Permeability in Individually Perfused Rat Mesenteric Venules

Presenter Zhou, Xueping

Advisor He, Pingnian

Hydrogen peroxide (H₂O₂) and other reactive oxygen species generated at inflammatory sites play a critical role in the pathogenesis of vascular diseases. Previously we demonstrated that H₂O₂ induced Ca²⁺ influx-dependent progressive increases in microvessel permeability, a pattern different from agonist-induced immediate and transient permeability increases. This study aims to investigate the cellular mechanisms of H₂O₂-induced permeability increases. We hypothesized that apoptosis in endothelial cells (ECs) and pericytes as the result of H₂O₂-induced Ca²⁺ overload contributes to the progressive increases in microvessel permeability. Fluorescence labeled annexin-V was used to detect apoptotic cells in H₂O₂-perfused rat mesenteric venules. The activation of apoptotic signaling pathway was examined with caspase-3,7 binding peptide. Cell viability and membrane permeability were examined with calcein and propidium iodide (PI) staining. The temporal and spatial occurrence of apoptosis and the activation of caspase signaling pathways were illustrated in vessels exposed to H₂O₂ for 15-60 min with confocal images. Neither annexin-V nor caspase-3,7 binding was detected in vessels perfused with Ringer-albumin perfusate for 60 min. Apoptosis did not occur in vessels perfused with 100 μM H₂O₂ until after 60 min of perfusion, in which apoptosis occurred only in pericytes, not in ECs. Apoptosis occurred in both ECs and pericytes after perfusion of 500 μM H₂O₂ for 45 min. All cells with annexin-V binding or caspase-3,7 staining colocalized with PI staining. The application of LaCl₃ that blocked H₂O₂-induced Ca²⁺ influx and Lp increases also prevented pericyte apoptosis in 100 μM H₂O₂ perfused vessels and significantly reduced apoptosis in both ECs and pericytes in vessels perfused with 500 μM H₂O₂ for 60 min. The occurrence of apoptosis closely correlated with H₂O₂-induced time and dose dependent Lp increases. These results indicated that Ca²⁺ influx-dependent apoptosis of ECs and/or pericytes might be responsible for H₂O₂-induced progressive increases in microvessel permeability, which was associated with the activation of caspase signaling pathways. Pericytes showed higher sensitivity to lower concentration of H₂O₂ than ECs in apoptosis. Supported by HL56237 and HL084338.