The effect of apocynin on endothelial cells under conditions of sepsis

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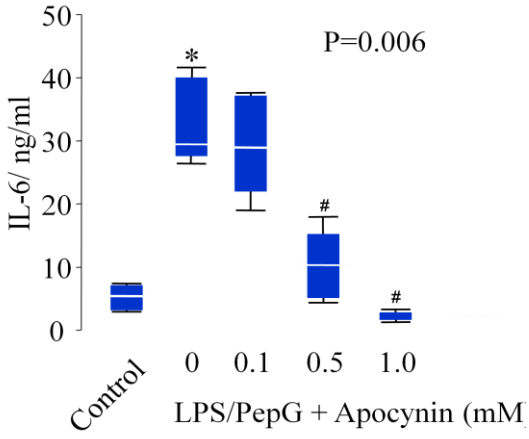
Sepsis is the most common cause of mortality in the intensive care unit. Overproduction of inflammatory mediators and reactive oxygen (ROS) and nitrogen (RNS) species have been linked to mitochondrial dysfunction and reduced cellular ATP production during sepsis. NADPH oxidase enzymes (NOX 1-5 and Duox 1-2) are membrane-associated enzymes that catalyze molecular oxygen to superoxide. Although NADPH oxidase is better known as the source of the phagocyte respiratory burst, NOX proteins modulate cell signalling pathways for transcription factor activation and cytokine production via intracellular superoxide and hydrogen peroxide production. We assessed the effect of apocynin, a naturally occurring vanillin-like NOX inhibitor, on mitochondrial function, oxidative stress and inflammation in human endothelial cells under conditions mimicking sepsis.

Human umbilical vein endothelial cells (HUVEC) were cultured for up to 7 days with 2µg/ml lipopolysaccharide (LPS) and 20µg/ml peptidoglycan G (PepG), plus 0-1mM apocynin. Constitutive NOX 1-3 mRNA expression was confirmed in HUVEC using RT-PCR. Mitochondrial membrane potential was analysed using the fluorescent probe JC-1 and metabolic activity was determined using Alamar Blue. Total glutathione was measured as a marker of oxidative stress and total radical production was measured using carboxy-dichloro-dihydro-fluorescein diacetate. Interleukin-6 (IL-6) concentrations were measured in medium as an index of inflammation using enzyme immunoassay. Cell viability was assessed using acid phosphatase.

Apocynin had no effect on cell viability at any concentration. Under conditions of sepsis, apocynin inhibited ROS production in a dose dependent manner ($p<0.05$) associated mitochondrial membrane potential and glutathione were higher at all concentrations ($p<0.05$). However, apocynin had no effect on metabolic activity at any concentration.



Figure

Interleukin-6 (IL-6) concentrations in culture media from HUVEC exposed to 2µg/ml LPS and 20µg/ml PepG for 24h. Data are shown as median, interquartile and full range (n=6). P value shown is Kruskal Wallis.

* = higher than control cells ($p<0.05$)

= lower than LPS/PepG alone, both $p<0.05$, Mann Whitney U test.

Apocynin protects against ROS production and oxidative stress and inhibits IL-6 secretion, but had mixed effects on mitochondrial function.

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