N-ACETYLCYSTEINE DOSE-DEPENDENTLY REDUCES TNF- α CELLULAR TOXICITY

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BACKGROUND

Circulatory inflammatory cytokine tumor necrosis factor-alpha (TNF) is increased in pathological conditions, such as diabetes, which initiate or exacerbate existing cardiovascular complications. Both nitric oxide (NO) and reactive oxygen species (ROS) may play a dual role (i.e., inhibiting or promoting) in TNF-induced endothelial cell apoptosis. We investigated the effects of antioxidant N-acetylcysteine (NAC) on TNF-induced human umbilical vein endothelial cells (HUVECs) apoptosis and the production of NO and antioxidant enzymes.

METHODS

Cultured HUVECs (cell line ECV304) were either not treated (control), or treated with TNF-alpha (40 ng/mL) alone (T) or TNF-alpha in the presence of the NAC at 30 mmol/L (NAC30+T) or 1 mmol/L (NAC1+T), respectively, for 24 hr. Cell viability was measured by MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide) assay, a widely used method in assessment of cytotoxicity and cell viability. The MTT assay result was compared to another cytotoxicity test, the LDH (lactate dehydrogenase) release assay. HUVECs apoptosis was assessed by flow cytometry, and compared to DNA ladder assay. Data were expressed as mean $\pm$ SEM and analyzed with one-way ANOVA with multiple comparisons.

RESULTS

TNF induced HUVEC apoptosis is associated with increased NO production (63.4 $\pm$ 3.4 μ mol/L in T vs 18.4 $\pm$ 4.4 μ mol/L in control, $P < 0.01$) and decreased antioxidant enzymes (superoxide dismutase, SOD; glutathione peroxidase, GSH-Px) and increased lipid peroxidation product malondialdehyde (MDA, 1.03 $\pm$ 0.03 nmol/mg protein in T vs 0.69 $\pm$ 0.04 in control, $P < 0.05$). Treatment with NAC at 30 mmol/L further increased NO production (78.3 $\pm$ 4.39 μ mol/L in NAC30+T) as compared to TNF alone ($P < 0.01$), but ended up with improved cell viability and reduced apoptotic cell death. This is accompanied by increased SOD and GSH-Px production and reduced MDA content. NAC at 1 mmol/L, however, did not have significant effects on TNF-induced endothelial cell apoptosis and cell viability, despite it slightly enhanced GSH-Px production.

CONCLUSIONS

N-acetylcysteine attenuation of TNF induced human umbilical vein endothelial cell apoptosis is dose-dependent that may involve the stimulation of endothelial cell NO production.