Nitric Oxide Protects Osteoblasts from Oxidative Stress-Induced Apoptotic Insults via a Mitochondria-Dependent Mechanism

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Abstract

Nitric oxide (NO) contributes to the regulation of osteoblast activities. In this study, we evaluated the protective effects of NO pretreatment on oxidative stress-induced osteoblast apoptosis and its possible mechanism using neonatal rat calvarial osteoblasts as the experimental model. Exposure of osteoblasts to sodium nitroprusside (SNP) at a low concentration of 0.3 mM significantly increased cellular NO levels without affecting cell viability. However, when the concentration reached a high concentration of 2 mM, SNP increased the levels of intracellular reactive oxygen species and induced osteoblast injuries. Thus, administration of 0.3 and 2 mM SNP in osteoblasts were respectively used as sources of NO and oxidative stress. Pretreatment with NO for 24 h significantly ameliorated the oxidative stress-caused morphological alterations and decreases in alkaline phosphatase activity, and reduced cell death. Oxidative stress induced osteoblast death via an apoptotic mechanism, but NO pretreatment protected osteoblasts against the toxic effects. The mitochondrial membrane potential was significantly reduced following exposure to the oxidative stress. However, pretreatment with NO significantly lowered the suppressive effects. Oxidative stress increased cellular Bax protein production and cytochrome c release from mitochondria. Pretreatment with NO significantly decreased oxidative stress-caused augmentation of Bax and cytochrome c protein levels. In parallel with cytochrome c release, oxidative stress induced caspase-3 activation and DNA fragmentation. Pretreatment with NO significantly reduced the oxidative stress-enhanced caspase-3 activation and DNA damage. Results of this study show that NO pretreatment can protect osteoblasts from oxidative stress-induced apoptotic insults. The protective action involves a mitochondria-dependent mechanism.