

**Pretreatment with low nitric oxide protects osteoblasts
from high nitric oxide-induced apoptotic insults
through regulation of c-Jun N-terminal
kinase/c-Jun-mediated Bcl-2 gene expression and
protein translocation.**

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Abstract

Nitric oxide (NO) can regulate osteoblast activity. In this study, we evaluated the effects of pretreatment with a low concentration of NO on osteoblast injuries induced by a high level of NO and its possible molecular mechanisms. Exposure of osteoblasts to 0.3 mM sodium nitroprusside (SNP), an NO donor, slightly increased cellular NO levels without affecting cell viability. SNP at 2 mM greatly increased the levels of cellular NO and reactive oxygen species, and induced osteoblast death. Thus, osteoblasts were treated with 0.3 and 2 mM SNP as the sources of low and high NO, respectively. Exposure of osteoblasts to high NO decreased alkaline phosphatase (ALP) activity and cell viability, and induced cell apoptosis. With low-NO pretreatment, the high NO-induced cell insults were significantly ameliorated. When the culture medium was totally replaced after pretreatment with low NO, the protective effects obviously decreased. Administration of high NO significantly decreased c-Jun N-terminal kinase (JNK) phosphorylation and nuclear c-Jun levels. Meanwhile, pretreatment with low NO significantly alleviated the high NO-induced reduction in activation of JNK and c-Jun. Sequentially, high NO inhibited Bcl-2 mRNA and protein synthesis. After pretreatment with low NO, the high NO-induced inhibition of the production of Bcl-2 mRNA and protein significantly decreased. Imaging analysis from confocal microscopy further revealed that high NO decreased translocation of the Bcl-2 protein from the cytoplasm to mitochondria. However, pretreatment with low NO significantly ameliorated the high NO-induced suppression of Bcl-2's translocation. Exposure of human osteoblasts to high NO significantly decreased ALP activity and cell viability, and induced cell apoptosis. Pretreatment with low NO significantly lowered the high NO-induced alterations in ALP activity, cell viability, and cell apoptosis. This study shows that pretreatment

with low NO can protect osteoblasts from high NO-induced cell insults via JNK/c-Jun-mediated regulation of Bcl-2 gene expression and protein translocation.