NITRIC OXIDE PROTECTS HUMAN OSTEOBLAST-LIKE MG63 CELLS FROM OXIDATIVE STRESS-INDUCED CELL APOPTOSIS THROUGH SUPPRESSION OF BAX PROTEIN

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Introduction: Osteoblasts play a crucial role in bone metabolism. Various systemic and local effectors modulate osteoblast-mediated bone formation. Nitric oxide (NO), produced by constitutive or inducible NO synthase, is a diatomic free radical and contributes to various biological activities. Osteoblasts can constitutively or inducibly produce NO with or without the treatment of proinflammatory cytokines or endotoxin lipopolysaccharide (Collin-Osdoby et al., 1995). NO can act as an autocrine stimulator of osteoblast growth and cytokine production, but overinduction of the free radical could cause osteoblast apoptosis (Chen and Liu, 2000). Low concentration of NO can protect oligodendrocytes, cardiomyocytes and hepatocytes from oxidative stress-induced cell damage (Li et al., 1999). The present study is designed to investigate the protective effects of NO on oxidative stress-induced osteoblast apoptosis and their possible mechanisms from the aspects of cell morphology, cell viability, alkaline phosphatase (ALP) activity, immunoblotting analysis of Bax protein.

Methods: The human osteoblast-like MG63 osteosarcoma cells were maintained in DMEM medium supplemented with 10% heat-inactivated fetal bovine serum and 100 units/ml penicillin and 100 µg/ml streptomycin at 37°C in a humidified atmosphere of 5% CO2. NO in culture medium of MG63 cells exposed to sodium nitroprusside (SNP), an NO-donor, was determined by the Greiss reaction system. Cell viability was analyzed by MTT assay. ALP activity was assayed using the Sigma Diagnostics Alkaline, Acid and Prostatic Acid Phosphatase kit. Analysis of apoptotic cells from propidium iodide-stained cells was determined by a flow cytometry method. Cytosolic proteins from untreated and drug-treated cells were electrophoretically separated and blotted to a nitrocellulose membrane. Immunodetection of Bax protein was carried out using a rabbit polyclonal antibody against human Bax protein.

Results: NO in human MG63 cells was time- and concentration-dependently augmented following SNP treatment. SNP at 2 mM was cytotoxic to human MG63 cells, but SNP at 100 µM was not cytotoxic. Pretreatment of 100 µM SNP blocked 2 mM SNP-induced decrease of ALP activity and cell viability. SNP at 2 mM alternated MG63 cells into shrinkage morphologies (Fig. 1). Pretreatment of 100 µM SNP completely protected human MG63 cells from 2 mM SNP-caused morphological changes. SNP at low concentrations significantly inhibited 2 mM SNP-induced apoptosis of human MG63 cells (Fig. 2). Immunoblotting analysis using a rabbit polyclonal antibody revealed that 100 µM SNP inhibited Bax protein of human MG63 cells and significantly decreased 2 mM SNP-induced apoptosis of human MG63 cells (Fig. 3).

Discussion: NO has a biphasic role in modulating cell function. Low concentration of NO is beneficial to maintain normal cellular physiology, but high concentration of NO will damage cells (Collin-Osdoby et al., 1995). SNP can be decomposed to NO under the presence of biological tissues, reducing agents, or visible light. The amounts of NO released by SNP are dependent on the dosage of the NO donor. SNP at 2 mM releases massive amounts of NO, increases oxidative stress, and leads to cell apoptosis of human osteoblast-like MG63 cells. SNP at 100 µM releases much less amounts of NO than at 2 mM. The level of NO released by 100 µM SNP is not cytotoxic to human MG63 cells but promotes the tolerance of the osteoblast-like cells against oxidative stress. Therefore, pretreatment with low concentration of SNP can protect human MG63 cells from more oxidative stress-induced cell apoptosis. Bax protein is a proapoptotic protein. Our previous study has shown that high concentration of NO induces osteoblast apoptosis through induction of Bax protein (Chen and Liu, 2000). The present study reveals that SNP at low concentration inhibits the basal level of Bax protein and completely suppresses high concentration of SNP-caused increase of the protein. From the present data, we propose that NO could protect osteoblast-like cells from oxidative stress-induced cell apoptosis through suppression of Bax protein. During the inflammation, appropriate elevation of intracellular NO may protect osteoblasts against more oxidative stress-induced cell death.


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