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Journal of Orthopaedic Research 23 (2005) 462–468

Journal of Orthopaedic Research

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Molecular mechanism of nitric oxide-induced osteoblast apoptosis ☆

Ruei-Ming Chen [a,b,*], Ta-Liang Chen [a], Wen-Ta Chiu [c], Chia-Chen Chang [a,b]

[a] *Department of Anesthesiology, Wan-Fang Hospital, College of Medicine, Taipei Medical University, No. 111, Hsing-Lung Rd., Sec. 3, Taipei 116, Taiwan*

[b] *Graduate Institute of Medical Sciences, College of Medicine, Taipei Medical University, No. 250, Wu-Hsing St., Taipei 110, Taiwan*

[c] *Department of Surgery, Division of Neurosurgery, Wan-Fang Hospital, College of Medicine, Taipei Medical University, Taipei 116, Taiwan*

Accepted 5 August 2004

Abstract

Nitric oxide (NO) can regulate osteoblast activities. Our previous study showed that NO induced osteoblast apoptosis [Chen RM, Liu HC, Lin YL, Jean WC, Chen JS, Wang JH. Nitric oxide induces osteoblast apoptosis through the de novo synthesis of Bax protein. J Orthop Res 2002;20:295–302]. This study was further aimed to evaluate the mechanism of NO-induced osteoblast apoptosis from the viewpoints of mitochondrial functions, intracellular oxidative stress, and the anti-apoptotic Bcl-2 protein using neonatal rat calvarial osteoblasts as the experimental model. Exposure of osteoblasts to sodium nitroprusside (SNP), an NO donor, significantly increased amounts of lactate dehydrogenase in the culture medium, and decreased cell viability in concentration- and time-dependent manners. Administration of SNP in osteoblasts time-dependently led to DNA fragmentation. The mitochondrial membrane potential was significantly reduced following SNP administration. SNP decreased complex I NADH dehydrogenase activity in a time-dependent manner. Levels of cellular adenosine triphosphate (ATP) were suppressed by SNP. In parallel with the mitochondrial dysfunction, SNP time-dependently increased levels of intracellular reactive oxygen species. Immunoblotting analysis revealed that SNP reduced Bcl-2 protein levels. Exposure to lipopolysaccharide (LPS) and IFN-γ significant increased endogenous nitrite production. In parallel with the increase in endogenous NO, administration of LPS and IFN-γ suppressed cell viability, mitochondrial membrane potential, and ATP synthesis. Results of this study show that NO released from SNP can induce osteoblast insults and apoptosis, and the mechanism may involve the modulation of mitochondrial functions, intracellular reactive oxygen species, and Bcl-2 protein.

Keywords: Osteoblasts; Nitric oxide; Apoptosis; Mitochondrial functions; Reactive oxygen species; Bcl-2 protein

Introduction

Nitric oxide (NO), synthesized from L-argenine by NO synthases, contributes to the regulation of tissue/cell activities, including vasodilation, neurotransmission, immunoresponses, and death control [4,27]. NO can also modulate bone remodeling [11]. In untreated osteoblasts, NO is constitutively produced [11]. Following pretreatment with inflammatory cytokines

☆ Part of this study was presented at the 47th Annual Meeting of the Orthopedic Research Society, February 2001, San Francisco, CA, USA.

* Corresponding author. Address: Graduate Institute of Medical Sciences, College of Medicine, Taipei Medical University, No. 250, Wu-Hsing St., Taipei 110, Taiwan, ROC. Tel.: +886 2 29307930x2159; fax: +886 2 86621150.

E-mail addresses: rmchen@tmu.edu.tw, rmchen@wanfang.gov.tw (R.-M. Chen).

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doi:10.1016/j.orthres.2004.08.011

or mechanical stress, high levels of NO in osteoblasts are synthesized [8,26]. NO has biphasic effects on osteoblast activities [11,27]. Constitutive NO can be an effective mediator to regulate osteoblast proliferation and differentiation [29]. However, overproduction of NO leads to osteoblast injuries [12,24,26].

Apoptosis, an energy-dependent type of programmed cell death, has a critical role in evolutionarily conserving physiologic cell death [14,15]. Hock et al. reported that apoptosis determines osteoblast populations in the postanal and adult skeleton [18]. A variety of intrinsic and extrinsic factors are involved in the regulation of cell apoptosis [10,15,28]. NO can be an effector for death regulation [4,9]. In inflammation-induced osteoporosis, elevated levels of NO have been shown to induce osteoblast apoptosis, and to decrease bone mineral density [1]. Several lines of evidence were provided by our previous study to demonstrate that the NO-induced osteoblast insult occurs via an apoptotic mechanism [6].

Mitochondria are energy-producing organelles. Maintenance of the mitochondrial membrane potential and metabolizing enzyme activities is critical to adenosine triphosphate (ATP) synthesis [32,38]. Depolarization of the mitochondrial membrane potential increases the release of apoptotic factors from the mitochondria to the cytoplasm and leads to cell apoptosis [3,19,34]. Intracellular reactive oxygen species (ROS), one of several apoptotic factors, can augment oxidative stress and damage cells [9,17,22]. Bcl-2 is an anti-apoptotic protein [20,35]. A decrease in the ratio of Bcl-2 over Bax, an apoptotic protein, increases the risk that cells will undergo apoptosis [16]. Our previous study showed that NO increases Bax protein production and induces osteoblast apoptosis [6]. In this study, we further hypothesized that NO-induced osteoblast apoptosis may occur through modulation of the mitochondrial functions, intracellular oxidative stress, and Bcl-2 protein levels.

Materials and methods

Cell isolation and drug treatment

Rat osteoblasts were prepared from 3-day-old Wistar rat calvaria according to the method of Partridge et al. [31]. Osteoblasts were seeded in Dulbecco's modified Eagle's medium (Gibco-BRL, Grand Island, NY, USA) supplemented with 10% heat-inactivated fetal bovine serum, L-glutamine, penicillin (100 IU/ml), and streptomycin (100 μg/ml) in 75-cm^2 flasks at 37 °C in a humidified atmosphere of 5% CO_2. Osteoblasts were grown to confluence prior to drug treatment. Only first passage of rat osteoblasts was used in the present study. Each osteoblast isolation represents a determination. Each experiment was repeated at least three times.

Sodium nitroprusside (SNP) purchased from Sigma Corporation (St. Louis, MO, USA) was freshly dissolved in phosphate-based saline (PBS) buffer (0.14 M NaCl, 2.6 mM KCl, 8 mM Na_2HPO_4, and 1.5 mM KH_2PO_4) and protected from light. Concentration-and time-dependent effects of SNP on osteoblasts were determined.

Quantification of lactate dehydrogenase

For evaluating the toxicity of SNP to osteoblasts, amounts of lactate dehydrogenase released in culture medium were determined. Osteoblasts (1×10^5) were seeded in 24-well tissue culture plates (Corning-Costar, Cambridge, MA, USA). After administration of SNP, the culture medium was collected and centrifuged. Levels of lactate dehydrogenase in supernatants were analyzed using a model 7450 automatic autoanalyzer system of Hitachi (Tokyo, Japan).

Assay of cell viability

A trypan blue exclusion method was carried out to determine the cytotoxicity of SNP to osteoblasts. Briefly, osteoblasts (2×10^5) were cultured in 24-well tissue culture plates. After SNP administration, osteoblasts were trypsinized by 0.1% trypsine-EDTA (Gibco-BRL). Following centrifugation and washing, osteoblasts were suspended in 1× PBS buffer and stained with an equal volume of trypan blue dye (Sigma). Fractions of dead cells with a blue signal were determined using a reverse-phase microscope.

Quantification of DNA fragmentation

DNA fragmentation in osteoblasts was quantified to evaluate if SNP damaged nuclear DNA. The BrdU-labeled histone-associated DNA fragments in the cytoplasm of cell lysates were detected according to the instructions of the cellular DNA fragmentation enzyme-linked immunosorbent assay (ELISA) kit (Boehringer Mannheim, Indianapolis, IN, USA). Briefly, osteoblasts (2×10^5) were sub-cultured in 24-well tissue culture plates and labeled with BrdU overnight. Cells were harvested and suspended in the culture medium. One hundred microliters of cell suspension was added to each well of 96-well tissue culture plates. Osteoblasts were cocultured with SNP for another 8 h at 37 °C in a humidified atmosphere of 5% CO_2. Amounts of BrdU-labeled DNA in the cytoplasm were quantified using an Anthos 2010 microplate photometer (Anthos Labtec Instruments, Lagerhausstrasse, Wals/Salzburg, Austria) at a wavelength of 450 nm.

Quantification of the mitochondrial membrane potential

The mitochondrial membrane potential was determined following the method of Chen [5]. Briefly, osteoblasts (5×10^5) were seeded in 12-well tissue culture plates overnight, and then treated with SNP for different time intervals. After SNP administration, osteoblasts were harvested and incubated with 3,3′-dihexyloxacarbocyanine ($DiOC_6(3)$), a positively charged dye, at 37 °C for 30 min in a humidified atmosphere of 5% CO_2. After washing and centrifuging, cell pellets were suspended with 1× PBS buffer. Intracellular fluorescent intensities were analyzed using a flow cytometer (FACS Calibur, Becton Dickinson, San Jose, CA, USA).

Assay of mitochondrial NADH dehydrogenase activity

NADH dehydrogenase activity was determined using a colorimetric 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assay following the method of Wu et al. [37]. Briefly, osteoblasts (5×10^5) were seeded in 96-well tissue culture plates overnight. After drug treatment, cells were cultured with new medium containing 0.5 mg/ml 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide for another 3 h. A blue formazan product in cells was dissolved in dimethyl sulfoxide and spectrophotometrically measured at a wavelength of 570 nm.

Quantification of cellular adenosine triphosphate (ATP) levels

Levels of cellular ATP in osteoblasts were determined with a bioluminescence assay described previously [7]. This assay was based on luciferase's requirement for ATP in producing emission light according to the protocol of Molecular Probes' ATP determination kit (Molecular Probes, Eugene, OR, USA). Luminent light (560 nm) emitted by the

luciferase-mediated reaction of ATP and luciferin was detected using a WALLAC VICTOR$^2_{TM}$ 1420 multilabel counter (Welch Allyn, Turku, Finland).

Determination of intracellular ROS

Levels of intracellular ROS were quantified following a method described previously [23]. Briefly, 5×10^5 osteoblasts were cultured in 12-well tissue culture plates overnight, and then cotreated with SNP and 2′,7′-dichlorofluorescin diacetate, an ROS sensitive dye. After drug treatment, osteoblasts were harvested and suspended in 1× PBS buffer. Relative fluorescence intensities of osteoblasts were quantified using a flow cytometer (FACS Calibur).

Gel electrophoresis and immunoblotting analysis

After SNP treatment, osteoblasts were washed with 1× PBS buffer. Cell lysates were prepared in ice-cold radioimmunoprecipitation assay (RIPA) buffer (25 mM Tris–HCl pH 7.2, 0.1% SDS, 1% Triton X-100, 1% sodium deoxycholate, 0.15 M NaCl, and 1 mM EDTA). To avoid protein degradation, a mixture of proteinase inhibitors, including 1 mM phenyl methyl sulfonyl fluoride, 1 mM sodium orthovanadate, and 5 μg/ml leupeptin, was added to the RIPA buffer. Protein concentrations were quantified by a bicinchonic acid (BCA) protein assay kit (Pierce, Rockford, IL, USA). Cytosolic proteins (100 μg per well) were subjected to sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), and transfered to nitrocellulose membranes. Membranes were blocked with 5% non-fat milk at 37 °C for 1 h. Immunodetection of Bcl-2 was carried out using a mouse monoclonal antibody against rat Bcl-2 (Transduction Laboratories, Lexington, KY, USA). Cellular β-actin protein was immunodetected using a mouse monoclonal antibody against mouse β-actin (Sigma) as an internal standard. Intensities of the immunoreactive bands were determined using an UVIDOCMW version 99.03 digital imaging system (Uvtec, Cambridge, England, UK).

Statistical analysis

Statistical differences between the control and SNP-treated groups were considered significant when the P value of Duncan's multiple-range test was less than 0.05. Statistical analysis between groups over time was carried out using two-way ANOVA.

Results

Table 1 presents the concentration-dependent effects of SNP on osteoblast viability. Administration of 0.5 and 1 mM SNP in osteoblasts did not affect lactate dehydrogenase release. SNP at 1.5 and 2 mM significantly increased amounts of lactate dehydrogenase by 85% and 154%, respectively. Analysis by a trypan blue exclusion method revealed that SNP at 0.5 and 1 mM was not cytotoxic to osteoblasts (Table 1). However, after administration of 1.5 and 2 mM SNP, viability of osteoblasts was decreased by 38% and 62%, respectively.

Table 2 shows the time-dependent effects of SNP on osteoblast viability. In 4-h-treated osteoblasts, SNP did not affect lactate dehydrogenase release. After SNP administration for 8 and 16 h, amounts of lactate dehydrogenase were significantly augmented by 65% and 151%, respectively. Exposure to SNP for 4 h was not cytotoxic to osteoblasts (Table 2). Viability of osteoblasts was significantly reduced by 40% and 55%, respectively, following SNP administration for 8 and 16 h.

Table 1
Concentration-dependent effects of sodium nitroprusside on osteoblast viability

SNP, mM	Lactate dehydrogenase (U/L)	Cell viability (cell number × 10^3)
0	41 ± 11	188 ± 44
0.5	38 ± 10	187 ± 38
1	35 ± 8	179 ± 45
1.5	76 ± 8*	116 ± 18*
2	104 ± 13*	72 ± 21*

Rat osteoblasts were exposed to 0.5, 1, 1.5, and 2 mM sodium nitroprusside (SNP) for 16 h. Amounts of lactate dehydrogenase in the culture medium were determined by an autoanalyzer as described in "Materials and Methods". Cell viability was assayed by the trypan blue exclusion method. Each value represents the mean ± SEM for $n = 12$ from four independent cell preparations.
* Values significantly differ from the respective control, $P < 0.05$.

Table 2
Time-dependent effects of sodium nitroprusside on osteoblast viability

Time, h	Lactate dehydrogenase (U/L)	Cell viability (cell number × 10^3)
0	37 ± 10	179 ± 31
4	30 ± 6	167 ± 58
8	61 ± 9*	107 ± 30*
16	93 ± 11*	81 ± 19*

Rat osteoblasts were exposed to 2 mM sodium nitroprusside for 4, 8, and 16 h. Amounts of lactate dehydrogenase in the culture medium were determined by an autoanalyzer as described in "Materials and Methods". Cell viability was assayed by the trypan blue exclusion method. Each value represents the mean ± SEM for $n = 12$ from four independent cell preparations.
* Values significantly differ from the respective control, $P < 0.05$.

Fig. 1 presents the effects of SNP on DNA damage. Exposure of osteoblasts to 0.5 and 1 mM SNP for 16 h did not cause DNA injury (*top panel*). SNP at 1.5 and 2 mM significantly increased levels of DNA fragments by 92% and 267%, respectively. In 4-h-treated osteoblasts, SNP did not damage nuclear DNA (*bottom panel*). After SNP administration for 8 and 16 h, levels of DNA fragments were enhanced by 65% and 205%, respectively.

The mitochondrial membrane potential of osteoblasts was determined and shown in Fig. 2. In 1-h-treated osteoblasts, SNP did not change the membrane potential of mitochondria. After administration of SNP for 2 and 4 h, the mitochondrial membrane potential was significantly decreased by 16% and 36%, respectively.

Table 3 shows the effects of SNP on NADH dehydrogenase activity and ATP synthesis. In 4-h-treated osteoblasts, SNP did not affect NADH dehydrogenase activity. Administration of SNP for 8 and 16 h significantly reduced activities of NADH dehydrogenase by

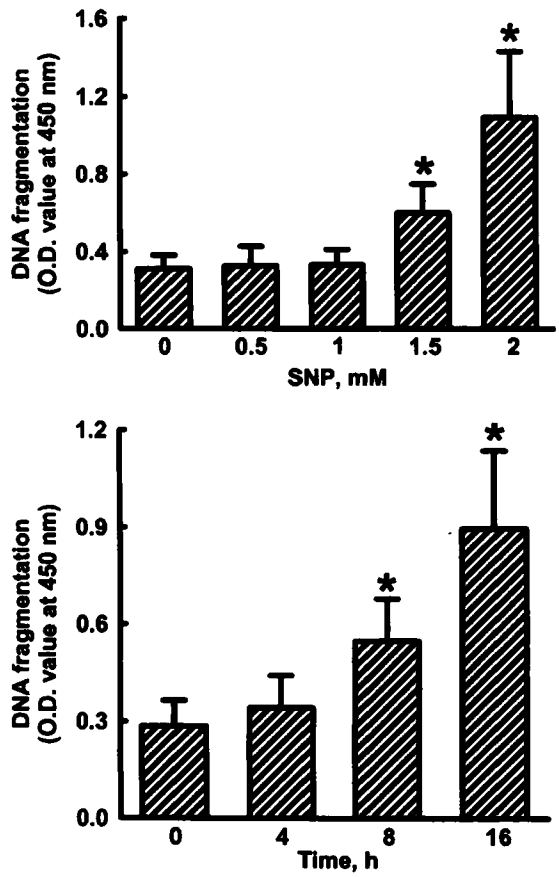

Fig. 1. Effects of SNP on DNA fragmentation. Osteoblasts prepared from neonatal rat calvaria were exposed to 0.5, 1, 1.5, and 2mM SNP for 16 h (*top panel*) or 2mM SNP for 4, 8, and 16h (*bottom panel*). DNA fragments in osteoblasts were quantified using a BrdU-labeled histone-associated DNA fragmentation ELISA kit as described in "Material and Methods". Each value represents the mean ± SEM for $n = 9$ from three independent cell preparations. * Values significantly differ from the respective control, $P < 0.05$.

Table 3
Effects of sodium nitroprusside on NADH dehydrogenase activity and cellular adenosine triphosphate levels

Time, h	NADH dehydrogenase (OD value at 550nm)	ATP (pmol)
0	1.03 ± 0.21	38 ± 8
4	0.98 ± 0.17	31 ± 9
8	0.74 ± 0.18*	24 ± 4*
16	0.62 ± 0.14*	17 ± 5*

Rat osteoblasts were exposed to 2mM sodium nitroprusside for 0, 4, 8, and 16h. NADH dehydrogenase activity was assayed by a colorimetric 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide method as described in "Materials and Methods". Cellular adenosine triphosphate (ATP) levels were determined by a bioluminescence assay. Each value represents the mean ± SEM for $n = 12$ from four independent cell preparations.
* Values significantly differ from the respective control, $P < 0.05$.

18% and 58%, respectively. In 4-h-treated osteoblasts, SNP did not change cellular ATP levels (Table 3). After

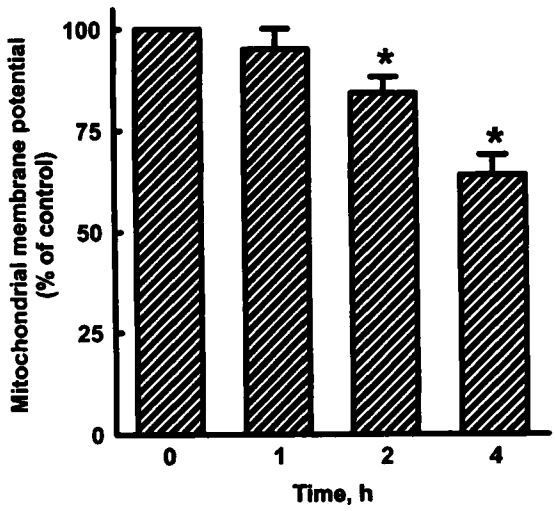

Fig. 2. Effects of SNP on the mitochondrial membrane potential. Osteoblasts prepared from neonatal rat calvaria were exposed to 2mM SNP for 1, 2, and 4h. The mitochondrial membrane potential of osteoblasts was analyzed using the fluorescent dye, $DiOC_6(3)$, and quantified by a flow cytometer. Each value represents the mean ± SEM for $n = 9$ from three independent cell preparations. * Values significantly differ from the respective control, $P < 0.05$.

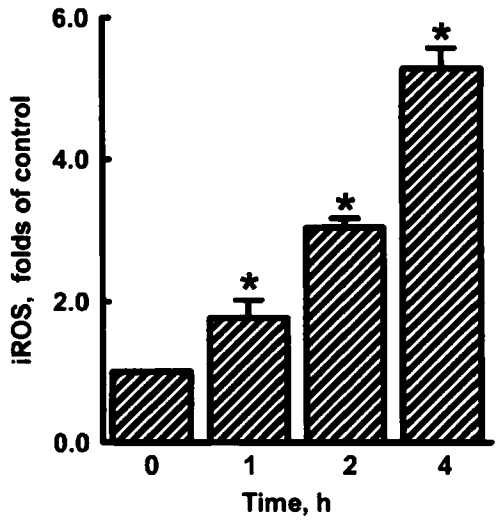

Fig. 3. Effects of SNP on intracellular reactive oxygen species (ROS). Osteoblasts prepared from neonatal rat calvaria were exposed to 2mM SNP for 1, 2, and 4h. Levels of intracellular ROS were caught using the ROS-sensitive dye, 2′,7′-dichlorofluorescin diacetate, and quantified by a flow cytometer. Each value represents the mean ± SEM for $n = 9$ from three independent cell preparations. * Values significantly differ from the respective control, $P < 0.05$.

administration of SNP for 8 and 16h, cellular ATP levels were significantly decreased by 37% and 55%, respectively.

Intracellular levels of ROS were determined and are shown in Fig. 3. In 1-h-treated osteoblasts, SNP caused a significant increase in ROS levels by 76%. Levels of intracellular ROS in osteoblasts were respectively

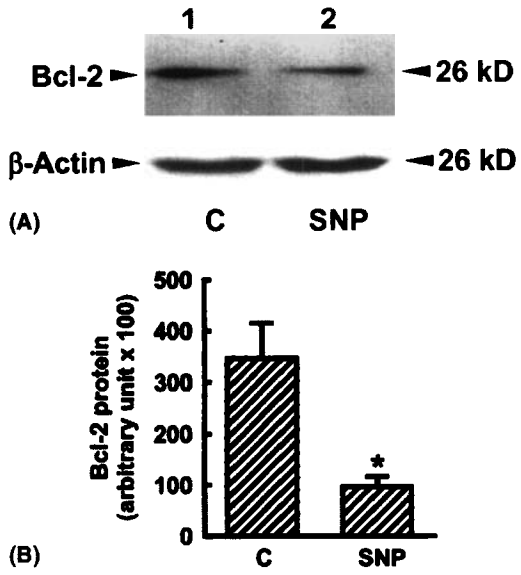

Fig. 4. Immunoblotting analysis of Bcl-2 protein. Osteoblasts prepared from neonatal rat calvaria were exposed to 2mM SNP. Cytosolic protein was prepared, separated by SDS-PAGE, and transferred to nitrocellulose membranes. Immunodetection of Bcl-2 protein was carried out using a monoclonal antibody against rat Bcl-2 protein (A, *top panel*). β-Actin was immunodetected as an internal control (*bottom panel*). Intensities of protein bands were quantified by a digital imaging system (B). Each value represents the mean ± SEM for $n = 6$ from three independent cell preparations. * Values significantly differ from the respective control, $P < 0.05$.

augmented by 3- and 5-fold following SNP administration for 2 and 4h.

Fig. 4 shows the effects of SNP on Bcl-2 protein. In untreated osteoblasts, Bcl-2 protein was detectable (Fig. 4A, *top panel*, lane 1). Administration of SNP in osteoblasts decreased Bcl-2 protein production (lane 2). β-actin was immunodetected as an internal control (*bottom panel*). Quantificantion of these immunodetected protein bands revealed that levels of Bcl-2 protein were significantly decreased by 82% following SNP administration (Fig. 4B).

Table 4
Effects of lipopolysaccharide and IFN-γ on nitrite production, cell viability, mitochondrial membrane potential, and ATP synthesis

Analysis	Control	LPS + IFN-γ
Nitrite, μM	4 ± 1	28 ± 8*
Cell viability, cell number × 10^3	137 ± 39	58 ± 15*
Mitochondrial membrane potential (% of control)	100	61 ± 18*
ATP, pmol	46 ± 13	24 ± 6*

Rat osteoblasts were exposed to a mixture of 1 μg/ml lipopolysaccharide (LPS) and 100IU/ml IFN-γ for 16h. Amounts of nitrite in the culture medium were determined by the Griess reaction. Cell viability was assayed by the trypan blue exclusion method. The mitochondrial membrane potential was quantified by a flow cytometer. Levels of adenosine triphosphate (ATP) were assayed by a bioluminescence assay. Each value represents the mean ± SEM for $n = 6$ from three independent cell preparations.
* Values significantly differ from the respective control, $P < 0.05$.

Table 4 presents the effects of endogenous NO production on cell viability, the mitochondrial membrane potential, and ATP synthesis. Administration of lipopolysaccharide (LPS) and IFN-γ significantly increased nitrite production by 6.5-fold. Viability of osteoblasts was decreased by 58% following administration of LPS and IFN-γ. After administration of LPS and IFN-γ, the mitochondrial membrane potential and cellular ATP levels were reduced by 39% and 48%, respectively.

Discussion

SNP can be decomposed to NO under light exposure or in the presence of a biological reducing system [2,21]. As presented in our previous study, administration of SNP significantly enhances the amounts of nitrite, which corresponds to an increase in NO [6]. In parallel with the increase in NO, the amounts of lactate dehydrogenase released from osteoblasts into the culture medium were significantly augmented. Analysis by the trypan blue exclusion method showed that the membrane permeability of osteoblasts was disturbed following SNP administration. The breakage of cell membranes increased the release of lactate dehydrogenase. Thus, NO decomposed from SNP broke down plasma membranes, increasing levels of lactate dehydrogenase in the culture medium, and leading to osteoblast insults or even death.

Our previous study provided several lines of evidence which identified that NO-induced osteoblast death mainly occurs via an apoptotic pathway [6]. This study used a cellular DNA fragmentation ELISA to further demonstrate that the nuclear DNA of osteoblasts was fragmented following SNP administration. Fragmented breakage of chromosome DNA is a critical characteristic which indicates that cells are undergoing apoptosis [15,34]. Our data reveal that NO can damage osteoblast DNA and induce cell apoptosis. Dypbukt et al. reported that high concentrations of NO donors increased the release of lactate dehydrogenase and caused cell necrosis [13]. This does not rule out the possibility that SNP partially induces osteoblast necrosis.

SNP significantly reduced cellular ATP levels. Mitochondria are critical ATP-synthesizing organelles. This study shows that SNP can decrease the mitochondrial membrane potential of osteoblasts. Previous studies reported that disruption of the mitochondrial membrane potential results in mitochondrial depolarization and blocks the respiratory chain reaction [30,38]. Thus, one of the possible mechanisms involved in the NO-induced depletion of ATP levels in osteoblasts is through the suppression of the mitochondrial membrane potential. Administration of SNP significantly decreased mitochondrial complex I NADH dehydrogenase activity. NADH dehydrogenase contributes to the respiratory

chain reaction and ATP synthesis [34]. A decrease in NADH dehydrogenase activity is another possible mechanism involved in the NO-induced ATP depletion in osteoblasts. Reduction of ATP synthesis has been shown to induce cell apoptosis [3]. Therefore, NO can decrease cellular ATP levels through suppression of the mitochondrial membrane potential and complex I enzyme activity in osteoblasts and thus induce cell apoptosis.

This study shows that levels of intracellular ROS were significantly augmented following SNP administration. ROS is one of the mitochondrial apoptotic factors [24]. Previous studies reported that depolarization of the mitochondrial membrane potential enhances the release of apoptotic factors, including ROS and cytochrome c, from mitochondria to the cytoplasm and drives cells undergoing apoptosis [21,32,37]. Li et al. showed that rotenone, an inhibitor of NADH dehydrogenase, enhanced mitochondrial ROS and induced cell apoptosis [22]. Therefore, SNP can increase intracellular ROS through suppression of the mitochondrial membrane potential and NADH dehydrogenase activity. However, the NO radical is one ROS. DCFH-DA was used in this study to catch ROS. Previous studies demonstrated that NO and peroxynitrite ($NOOO^-$) can also directly react with DCFH-DA [17,33]. Therefore, the elevation of intracellular ROS in SNP-treated osteoblasts is partially due to the enhancement of intracellular NO.

SNP decreased Bcl-2 protein production. Bcl-2, an anti-apoptotic protein, can determine if cells undergo apoptosis [35,36]. A decrease in Bcl-2 protein levels will increase the risk that cells will undergo apoptosis. Bax is a pro-apoptotic protein [25]. Immunocytochemical and immunoblotting analyses were carried out in our previous study to validate that NO increased Bax protein levels in osteoblasts [6]. Mitochondrial apoptotic factors, including ROS and cytochrome c, can regulate the cellular Bcl-2/Bax ratio [9,10]. Therefore, SNP can modulate mitochondrial functions and increase the release of mitochondrial apoptotic factors. The SNP-caused suppression of Bcl-2/Bax proteins can induce osteoblasts apoptosis.

Osteoblasts were exposed to a mixture of LPS and IFN-γ to determine if endogenous NO has similar effects as those of SNP. After administration of LPS and IFN-γ, levels of nitrite in osteoblsts were significantly augmented. In parallel with the increase in NO, administration of LPS and IFN-γ disrupted the mitochondrial membrane potential, reduced cellular ATP levels, and ultimately induced cell death. This study has shown that the exogenous and endogenous forms of NO have the same effects on the induction of mitochondrial dysfunction and cell death.

In summary, NO decomposed from SNP can cause DNA fragmentation, thus the NO-induced death mechanism mainly occurs via an apoptotic pathway. SNP can modulate mitochondrial functions through inhibition of the mitochondrial membrane potential, NADH dehydrogenase activity and cellular ATP levels. Levels of intracellular ROS and Bcl-2 protein are also regulated by SNP. The modulating effects of NO decomposed from SNP are also observed with endogenous NO. Therefore, this study presents further data to validate our hypothesis that NO can modulate mitochondrial functions, intracellular oxidative stress, and Bcl-2 protein production to induce osteoblast apoptosis. The roles of caspases in NO-induced osteoblast apoptosis will be a further study in our laboratory.

Acknowledgements

This study was supported by grants NSC91-2314-B-038-031 and NSC92-2314-B-038-010 from the National Science Council, Taipei, Taiwan, ROC.

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