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Molecular Mechanisms of Apoptosis in HL-60 Cells Induced by a Nitric Oxide-Releasing Compound

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Nitric oxide (NO) generated from 1-hydroxy-2-oxo-3, 3-bis(2-aminoethyl)-1-triazene (NOC 18), an NO-releasing compound, induced monocytic differentiation of human promyelocytic leukemia HL-60 cells as assessed by expression of nonspecific esterases and morphologic maturation. Simultaneously, DNA fragmentation and morphological alterations typical of apoptosis were also induced. To investigate the mechanisms of apoptosis during differentiation of HL-60 cells induced by NO, the endogenous levels of Bcl-2 and Bax were assessed by immunoblotting. Treatment of cells with NOC 18 slightly reduced the level of Bcl-2 followed by Bax. These changes might be involved in the induction of apoptosis. The involvement of the activation of the interleukin-1β converting enzyme (ICE) family of proteases (caspases), such as ICE and CPP32, in the pathways was also investigated. CPP32, but not ICE, was strongly activated in response to NOC 18 stimulation, thereby implicating CPP32-like activity in the induction of apoptosis. Moreover, the possible involvement of tyrosine phosphorylation in apoptosis was investigated. Pretreatment of cells with herbimycin A, an inhibitor of tyrosine kinases, suppressed DNA fragmentation and CPP32-like activity, whereas pretreatment with vanadate, an inhibitor of tyrosine phosphatases, enhanced both parameters, suggesting that tyrosine phosphorylation might be involved in the pathways of apoptosis in HL-60 cells induced by NO.

Keywords: Nitric oxide, HL-60 cells, cell differentiation, apoptosis, DNA fragmentation, Bcl-2, Bax, caspase, ICE, CPP32, tyrosine phosphorylation

Abbreviations: NO, nitric oxide; NOC 18, 1-hydroxy-2-oxo-3, 3-bis(2-aminoethyl)-1-triazene; PTK, protein tyrosine kinase; PTP, protein tyrosine phosphatase; TPA, 12-O-tetradecanoylphorbol-13-acetate; NSE, nonspecific esterase; PBS, phosphate-buffered saline; TBS, Tris-buffered saline; ICE, interleukin-1β converting enzyme; MCA, 4-methyl-coumaryl-7-amide; AMC, 7-amino-4-methyl-coumarin; PARP, poly(ADP-ribose) polymerase; carboxy-PTIO,2-(4-carboxyphenyl)-4,4,5,5-tetramethyl-imidazoline-1-oxyl 3-oxide.

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INTRODUCTION

Nitric oxide (NO) is a reactive free-radical gas generated from L-arginine by NO synthases in many cell types.[1] It has been shown to play important roles in diverse physiological processes such as vasodilation, inhibition of platelet aggregation, and neurotransmission.[2–4] It is also well known that NO will induce apoptosis in several cell types such as macrophages.[5–7] We reported previously that NO generated from NOC 18, a chemical NO donor, induces rapid apoptosis of human promyelocytic leukemia HL-60 cells under low oxygen tension.[8] Moreover, NO has recently been shown to induce monocytic differentiation of HL-60 cells.[9–11] Thus, NO can both initiate apoptosis and induce monocytic differentiation of HL-60 cells. However, the precise molecular mechanisms responsible for the induction of cell death of HL-60 cells by NO remain largely unknown and a possible correlation of apoptosis with cell differentiation has not been established.

One of the major regulators of apoptosis is the protein Bcl-2 whose gene was first isolated at the t(14;18) chromosomal breakpoint in follicular B cell lymphoma.[12–14] Many studies have shown that Bcl-2 can block cell death induced by a wide variety of stimuli.[15,16] Recently, several homologs of Bcl-2 have been identified, such as Bax, which was found in Bcl-2 immunoprecipitates.[17] It has been proposed that Bax counters Bcl-2 activity by forming heterodimers with Bcl-2 and that higher levels of Bax than Bcl-2 accelerate cell death.[17,18] Moreover, Bcl-2 and Bax have been shown to be down-regulated during TPA-induced monocytic differentiation of HL-60 cells.[19]

Another mediator of apoptosis is interleukin-1β converting enzyme (ICE) that is a cytoplasmic aspartate-specific cysteine protease.[20] It is initially synthesized as an inactive proenzyme and requires proteolytic processing to generate an active heterodimeric enzyme. Overexpression of ICE in mammalian cells induces apoptosis, suggesting that this proteolytic processing event is an essential component of the cell death pathway.[21] To date, ten ICE homologues of human origin have been identified which comprise an emerging "caspase" family,[22] including CPP32/Yama/apopain (caspase-3).[23–25]

Tyrosine phosphorylation is often implicated in signal transduction for cell growth and differentiation.[26] Recent studies using HL-60 cells have suggested that protein tyrosine kinases (PTKs) regulate apoptosis both positively and negatively.[27,28] Hence, PTKs may also be implicated in NO-induced apoptosis of HL-60 cells.

In this study, we demonstrated that NOC 18 induces apoptosis during monocytic differentiation of HL-60 cells. In addition, we demonstrated that NOC 18 slightly down-regulates Bcl-2 followed by Bax and strongly activates CPP32 but not ICE. We further suggest the possible involvement of tyrosine phosphorylation in the pathway. These data contribute to our knowledge of apoptosis induced by NO.

MATERIALS AND METHODS

Chemicals

NOC 18 (Dojindo Laboratories, Inc., Kumamoto, Japan)[29] was freshly dissolved in 100 mM NaOH, filtered through 0.22-μm membranes (Millipore Co.), and added to cell cultures. Herbimycin A (Kyowa Medex Co., Tokyo, Japan) was dissolved in DMSO to create a 0.1 mM stock solution. Vanadate (Sigma Co.) was dissolved in H_2O to create a 10 mM stock solution. Aliquots of the above stock solutions were used for each assay. All other chemicals used were of the highest grade commercially available.

Cell Line

The human promyelocytic leukemia HL-60 cells[30] were kindly donated by Dr. M. Saito, Hokkaido University, Japan. The cells were maintained at densities of 0.1–1 × 10^6 cells/ml in RPMI-1640 medium (Nissui Co., Ltd., Tokyo, Japan) supplemented with 10% heat-inactivated

fetal bovine serum (GIBCO BRL), 100 U/ml penicillin and 100 μg/ml streptomycin at 37°C under a 5% CO_2/95% air atmosphere. Cells in the logarithmic growth phase were used for assays. Cell density and viability were determined by trypan blue exclusion.

Evaluation of Cell Differentiation

Cultures were initiated at a density of 1.5×10^5 cells/ml in the presence or absence of 150 μM NOC 18. After 1–4 days of incubation, the cells were centrifuged onto slides by the cytospin system (Kubota, Inc., Tokyo, Japan). The monocytic differentiation was evaluated by staining for nonspecific esterase (NSE) activity.[31] On each slide, at least 200 cells were counted under a light microscope.

Analysis of DNA Fragmentation

The extent of DNA fragmentation was determined by the diphenylamine method.[32,33] The cells (6×10^5) were harvested by centrifugation at 250g for 5 min, and washed with PBS. The pellet was lysed in 200 μl of lysis buffer (10 mM Tris-HCl, pH 7.4, 10 mM EDTA, and 0.5% Triton X-100) on ice for 20 min. The lysate was centrifuged at 13,000g and 4°C for 20 min to separate intact and fragmented chromatin. Both the pellet and the supernatant were precipitated at 4°C for over 30 min with 6% perchloric acid. The precipitates were sedimented at 13,000g and 4°C for 20 min. The DNA precipitates were heated at 70°C for 20 min in 50 μl of 6% perchloric acid, and were mixed with 100 μl of diphenylamine solution (1.5% (w/v) diphenylamine, 1.5% sulfuric acid, and 0.01% acetaldehyde in glacial acetic acid). After overnight incubation at 30°C in the dark, both ODs were measured at 600 nm, and the percentage of DNA fragmentation was calculated as the ratio of DNA in the supernatant to the total DNA.

The laddering pattern of the DNA fragmentation was detected by agarose gel electrophoresis as described previously.[8] Briefly, the supernatant obtained as described above was incubated at 37°C for one hour with 400 μg/ml RNase A and for another hour with 400 μg/ml proteinase K. The fragmented DNA was then extracted with phenol/chloroform/isoamyl alcohol (25:24:1), and was subjected to 2% agarose gel electrophoresis in TBE buffer (89 mM Tris, pH 8.3, 89 mM boric acid, and 2 mM EDTA) containing 0.1 μg/ml ethidium bromide. The DNA bands were visualized by ultraviolet illumination.[34]

Immunological Detection of Proteins

The cells (10^6) were washed with PBS twice, and solubilized in 100 μl of ice-cold RIPA buffer (50 mM Tris-HCl, pH 8.0, 150 mM NaCl, 1 mM EDTA, 1% Nonidet P-40, 0.5% sodium deoxycholate, 0.1% SDS, and 1 mM phenylmethylsulfonyl fluoride) on ice for one hour. The lysates were clarified by centrifugation at 13,000g and 4°C for 20 min. The supernatants were assayed for protein concentrations by the method of Lowry *et al.*[35] to normalize protein concentrations across samples. The samples were diluted in 2 × SDS-sample buffer (125 mM Tris-HCl, pH 6.8, 4% SDS, 10% β-mercaptoethanol, 20% glycerol, and bromophenol blue), boiled for 5 min, and then subjected to SDS-polyacrylamide gel electrophoresis (15% gel in most cases) using Tris-glycine buffer.[36] The proteins resolved were transferred on to Immobilon polyvinylidene difluoride membrane (PVDF, Millipore Co.) using a semidry blotting apparatus (AE-6675, ATTO Co., Tokyo, Japan) with 2 mA/cm^2 applied for 1.5 h. The blotted filter was incubated overnight at 4°C with TBS-T (20 mM Tris-HCl, pH 7.5, 150 mM NaCl, and 0.05% Tween 20) containing 5% non-fat dry milk (blocking buffer) to block nonspecific binding of the antibodies. The filter was washed twice with TBS-T, and then incubated in blocking buffer containing primary rabbit polyclonal antibodies against Bcl-2 or Bax (0.1 μg/ml, Santa Cruz Biotechnology, Inc.) at 37°C for 1.5 h. The filter was washed three times with TBS-T, and next incubated in blocking buffer containing secondary horseradish peroxi-

dase-linked goat anti-rabbit IgG (1:4000 dilution, Amersham Co.) at 37°C for one hour. After washing three times with TBS-T, the blotted proteins were developed by enhanced chemiluminescence (ECL, Amersham Co.).

The relative amounts of Bcl-2 and Bax were determined by measuring the intensity of the bands using the public domain NIH Image program (written by Wayne Rasband at the U.S. National Institutes of Health) without manipulation.

Protease Activity Assay

The cells (10^5) treated with or without NOC 18 were lysed in 50 µl of lysis buffer (50 mM Tris, pH 7.5, 0.5% Nonidet P-40, 0.5 mM EDTA, and 150 mM NaCl) on ice for 30 min. The lysates were then centrifuged at 15,000g for 10 min. The supernatants were incubated with 10 µM fluorogenic peptide substrates, acetyl-Tyr-Val-Ala-Asp-MCA (Ac-YVAD-MCA) specific for ICE[37] or acetyl-Asp-Glu-Val-Asp-MCA (Ac-DEVD-MCA) specific for CPP32[25] (Peptide Institute, Inc., Osaka, Japan), in 200 µl of reaction buffer (20 mM HEPES, pH 7.5, 0.1 M NaCl, and 5 mM DTT) at 37°C for 1 h. The reactions were halted by the addition of stopper (0.2 M Gly-HCl, pH 2.8). The fluorescence of released AMC was measured by a fluorospectrophotometer (Hitachi 650-10 LC, Hitachi, Inc., Tokyo, Japan). The wavelengths for excitation and emission were 355 and 460 nm, respectively. One unit was defined as the enzyme activity that liberates 1 µmol of AMC during one hour.

RESULTS

Effect of NOC 18 on the Growth of HL-60 Cells

The effects of NO on the growth of HL-60 cells using NOC 18 as NO donor were investigated. In the absence of NOC 18, HL-60 cells showed a normal logarithmic pattern of growth with a doubling time of about 20 h. However, treatment of cells with NOC 18 resulted in a concentration-dependent inhibition of cell growth (Fig. 1). The cells treated with 50 and 100 µM NOC 18 barely doubled within 22 and 38 h, respectively, whereas those treated with 200 and 250 µM NOC 18 gradually declined in cell numbers. Interestingly, an intermediate effect was obtained with only 150 µM NOC 18 which caused growth-arrest of HL-60 cells after approximately 48 h.

NOC 18 Induces Monocytic Differentiation of HL-60 Cells

Since NO has been shown to induce monocytic differentiation of HL-60 cells,[9–11] NOC 18 was investigated for similar properties. Monocyte-like cells which displayed spreading and polarization (but not adherence) appeared in substantial numbers 72 h after stimulation with 150 µM NOC 18. As shown in Figure 2A, monocytic cells were evaluated by staining for NSE activity, a specific marker of monocytic cells.[31] The percentage of NSE positive cells steadily increased up to about 14% at 96 h, whereas control cells remained unstained (Fig. 2B), indicating that NOC 18 can induce monocytic differentiation of HL-60 cells.

NOC 18 Induces Apoptosis of HL-60 Cells

Cell differentiation preceding maturation has been shown to correlate with apoptotic cell death.[38] To examine whether or not growth inhibition by NOC 18 was caused by apoptosis, cellular DNA was extracted from HL-60 cells treated with 150 µM NOC 18. Figure 3A shows a laddering pattern of DNA, typical of apoptosis, with the appearance of multiples of about 180 base pair units on agarose gel electrophoresis. Figure 3B shows a time course of change in the percentage of fragmented DNA. At 24 h after the addition of NOC 18, the DNA fragmentation was drastically increased, and at 36 h, reached about 30%. Characteristic morphological changes such as

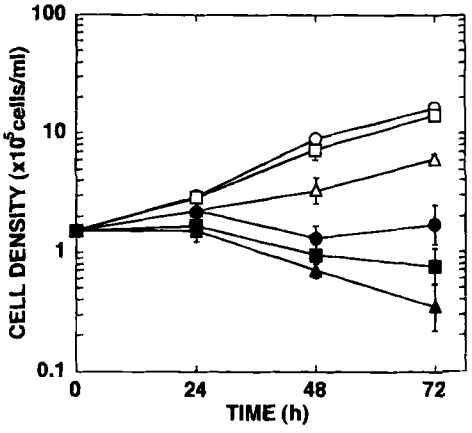


FIGURE 1 Effect of various concentrations of NOC 18 on growth of HL-60 cells. The cells were exposed to 0 μM (○), 50 μM (□), 100 μM (△), 150 μM (●), 200 μM (■), and 250 μM (▲) NOC 18 under the experimental conditions described in "MATERIALS AND METHODS." At the indicated time, the viable cell density was determined by trypan blue dye exclusion. Each value represents the mean ± S.D. of three independent experiments.

cell shrinkage, chromatin condensation and nuclear fragmentation were also recognized by Wright-Giemsa staining (data not shown). These results indicate that NOC 18 rapidly induces apoptosis during cell differentiation of HL-60 cells and that growth inhibition by NOC 18 was due to apoptosis. Moreover, NOC 18-induced DNA fragmentation was suppressed by adding NO-trapping agents, such as carboxy-PTIO and oxyhemoglobin (data not shown) as reported before,[8] suggesting that NO released from NOC 18 might be responsible for the induction of apoptosis. Whether or not the differentiated cells undergo apoptosis or two different populations

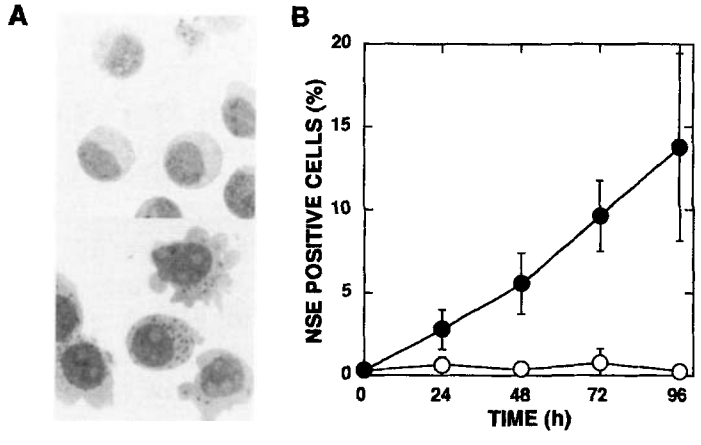
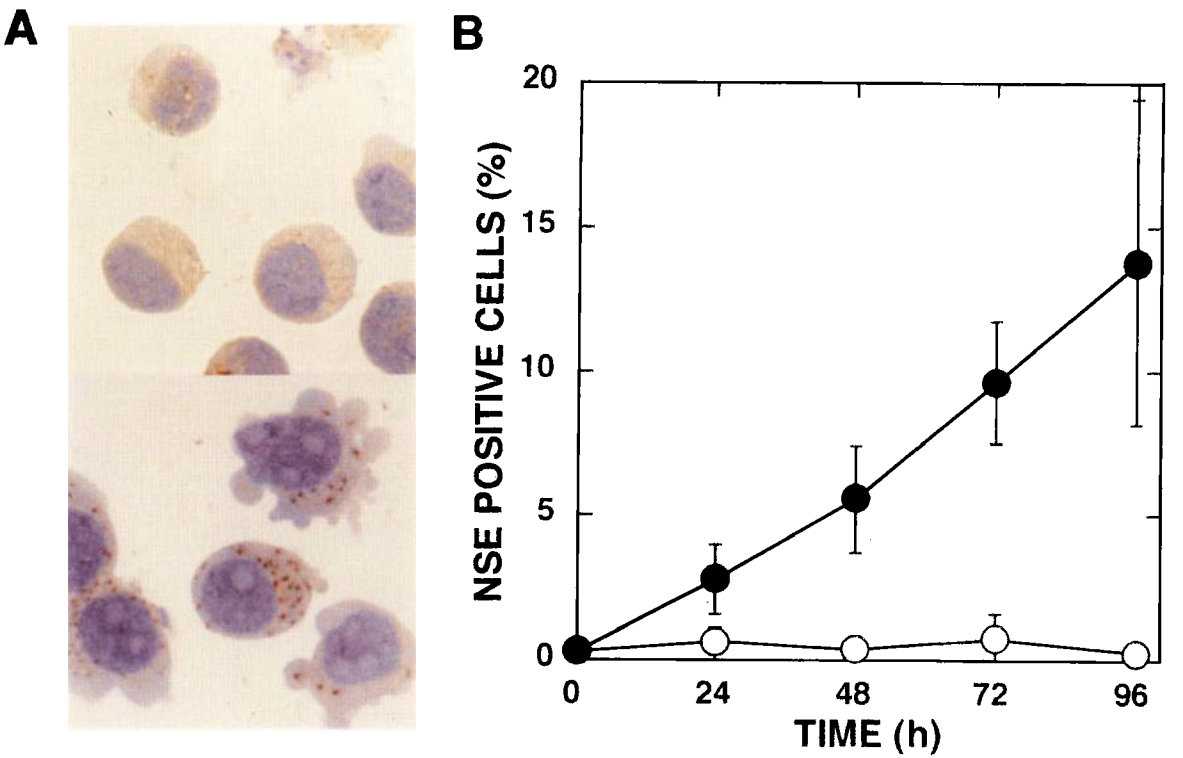


FIGURE 2 NOC 18-induced NSE activity in HL-60 cells. (**A**) Morphological changes in NOC 18-treated HL-60 cells. The cells, at an initial density of 1.5×10^5 cells/ml, were either treated with 150 μM NOC 18 (below) or left untreated (above). After 72 h, the cells were fixed and stained for NSE activity. Magnification × 400. (**B**) A time course of changes in the percentage of NSE positive cells. Cells treated with 150 μM NOC 18 are represented by dark circles; untreated cells by light circles. Each value represents the mean ± S.D. of at least three independent experiments. (See Color Plate V at the back of this issue.)



Color Plate V (See page 329 Figure 2) NOC 18-induced NSE activity in HL-60 cells. (**A**) Morphological changes in NOC 18-treated HL-60 cells. The cells, at an initial density of 1.5 x 10^5 cells/ml, were either treated with 150 μM NOC 18 (below) or left untreated (above). After 72 h, the cells were fixed and stained for NSE activity. Magnification x 400. (**B**) A time course of changes in the percentage of NSE positive cells. Cells treated with 150 μM NOC 18 are represented by dark circles; untreated cells by light circles. Each value represents the mean ±S.D. of at least three independent experiments.

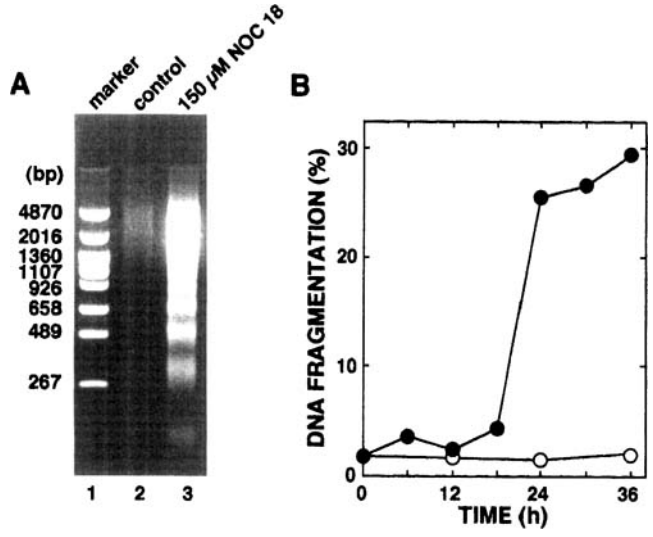


FIGURE 3 NOC 18-induced DNA fragmentation in HL-60 cells. (**A**) Gel electrophoresis of fragmented DNA. The cells (3×10^5) were treated with 150 μM NOC 18 (lane 3) or were untreated (lane 2). After 30 h, fragmented DNA was extracted and subjected to 2% agarose gel electrophoresis. The pHY marker (Takara Shuzo Co., LTD., Otsu, Japan) was used as a DNA molecular size marker (lane 1). (**B**) A time course of changes in the percentage of fragmented DNA. The cells were treated with 150 μM NOC 18 (dark circles) or were untreated (light circles). After the indicated time of incubation, the percentages of fragmented DNA were determined by the diphenylamine method.

of cells undergo either apoptosis or differentiation remains unknown.

Effect of NOC 18 on the Levels of Bcl-2 and Bax in HL-60 Cells

The molecular mechanisms by which NO induces apoptosis during differentiation of HL-60 cells remain to be elucidated. We first examined whether NOC 18 influences Bcl-2 and Bax expression at the protein level. As shown in Figure 4A, the two proteins were constitutively expressed under normal conditions (lane 1). 24 h after the addition of 150 μM NOC 18, Bcl-2 was slightly down-regulated by about 27%, whereas Bax was unaffected or slightly up-regulated (lane 2). Densitometric estimates showed a reduced relative ratio of Bcl-2 to Bax (Fig. 4B), suggesting that NOC 18-induced apoptosis of HL-60 cells might be due, in part, to the down-regulation of Bcl-2. In contrast, both Bcl-2 and Bax were down-regulated at 96 h (lane 3), suggesting that the down-regulation of the two proteins may be part of the monocytic differentiation of HL-60 cells.

NOC 18 Activates CPP32 but not ICE in HL-60 Cells

We next investigated the possible involvement of the ICE family proteases (caspases) such as ICE and CPP32 in NOC 18-induced apoptosis of HL-60 cells. Protease activities in HL-60 cells were measured using fluorogenic peptide substrates, Ac-YVAD-MCA for ICE and Ac-DEVD-MCA for CPP32. As shown in Figure 5, there is hardly any detectable ICE-like activity even after NOC 18 treatment. However, treatment of cells with 150 μM NOC 18 caused a marked increase in CPP32-like activity within 24 h, and at 32 h, the activity reached about 8 mU/10^5 cells. The time course of CPP32-like activity is comparable with that of DNA fragmentation (Fig. 3B). This result suggested that CPP32 and/or CPP32-like proteases were implicated in NOC 18-induced apoptosis of HL-60 cells.

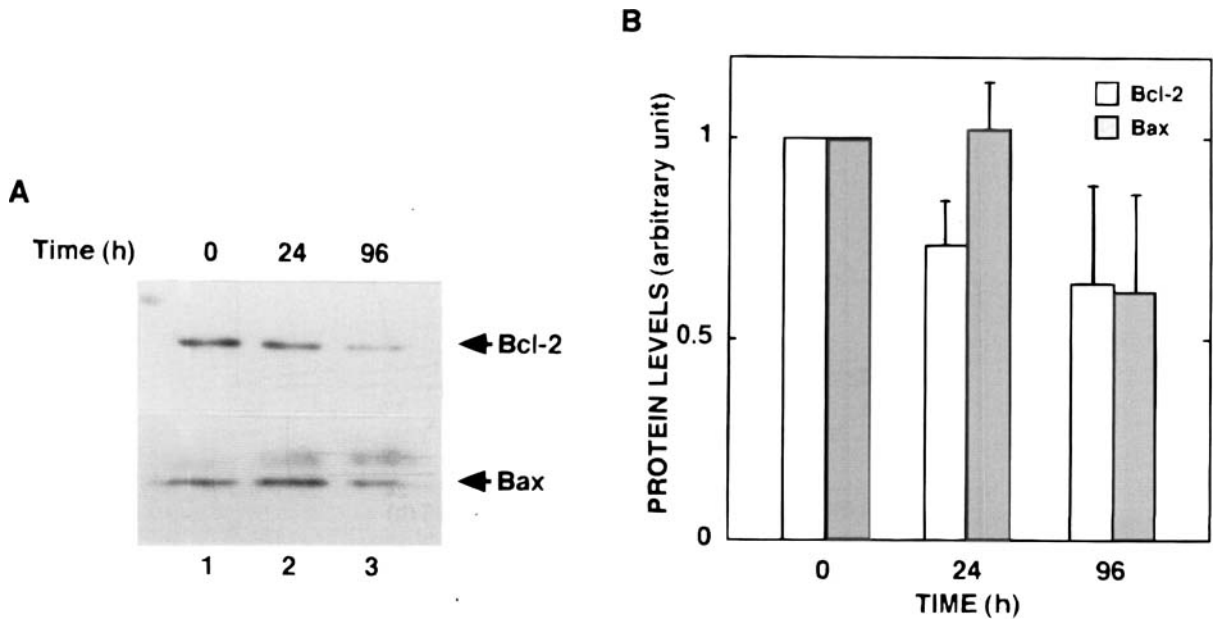


FIGURE 4 Western blot analyses of Bcl-2 and Bax in NOC 18-treated HL-60 cells. The cells were treated with 150 μM NOC 18 for the indicated periods. Total cell lysates (10 μg for Bcl-2, 18 μg for Bax) were subjected to SDS-PAGE, followed by western blotting (**A**). Relative expression levels of Bcl-2 and Bax were expressed as the relative intensity of the bands (**B**). The values represent the mean ± S.D. of five independent experiments.

Possible Involvement of Tyrosine Phosphorylation in NOC 18-Induced Apoptosis of HL-60 Cells

We also investigated the possible involvement of tyrosine phosphorylation in apoptosis as well as monocytic differentiation of HL-60 cells induced by NOC 18. The cells were treated for 30 min with 0.1 μM herbimycin A, an inhibitor of PTKs, or 10 μM vanadate, an inhibitor of protein tyrosine phosphatases (PTPs), followed by exposure to 150 μM NOC 18. Treatment with only NOC 18 significantly induced NSE positive cells (~10% at 72 h) and DNA fragmentation (~30% at 24 h). However, herbimycin A pretreatment reduced both, whereas vanadate pretreatment enhanced both (Fig. 6A and B), suggesting that tyrosine phosphorylation might be involved in the pathway of apoptosis as well as monocytic differentiation of HL-60 cells induced by NOC 18.

Herbimycin A and Vanadate Modulate CPP32-Like Activity in NOC 18-Treated HL-60 Cells

Since protein tyrosine phosphorylation might be involved in the pathways of NO-induced apoptosis in HL-60 cells, we next examined the effects of herbimycin A and vanadate on the activities of ICE and CPP32 in NOC 18-treated cells. As described above, NOC 18 strongly induced CPP32-like activity in HL-60 cells (Fig. 5). However, pretreatment with 0.1 μM herbimycin A significantly suppressed the NOC 18-induced CPP32-like activity, whereas pretreatment with 10 μM vanadate significantly enhanced the activity (Fig. 7). ICE-like activity was not detected. These data suggested that the modulation of NOC 18-induced apoptosis in HL-60 cells by herbimycin A or vanadate might be due to the modulation of CPP32-like activity, and that tyrosine phosphorylation might regulate the activation of CPP32 and/or CPP32-like proteases in NOC 18-treated HL-60 cells.

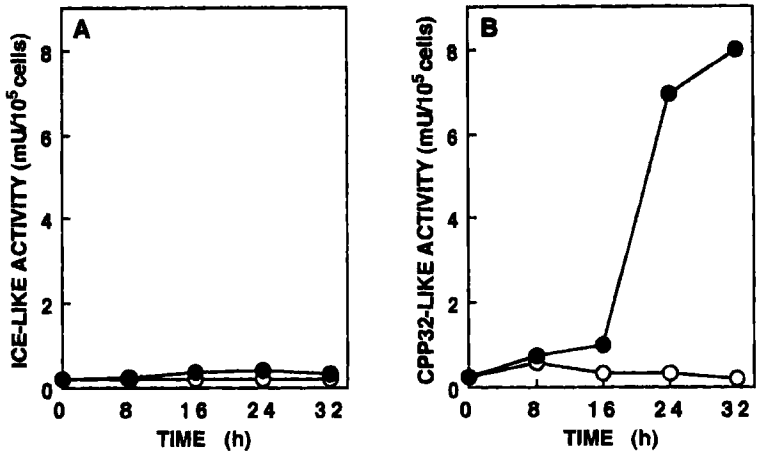


FIGURE 5 NOC 18-induced CPP32-like activity in HL-60 cells. A time course of changes in the activities of ICE (**A**) and CPP32 (**B**). The cells were treated with 150 μM NOC 18 (dark circles) or were untreated (light circles) for the indicated periods. Total cell extract was incubated with 10 μM fluoregenic peptide substrates, Ac-YVAD-MCA for ICE or Ac-DEVD-MCA for CPP32, at 37°C for 1 h. The fluorescence of released AMC was measured (excitation, 355 nm; emission, 460 nm). One unit was defined as the enzyme activity that liberates 1 μmol of AMC during 1 h.

DISCUSSION

NOC 18-induced monocytic differentiation and apoptosis of HL-60 cells occurred concomitantly. Kuo *et al.* previously demonstrated that sodium nitroprusside, another NO donor, had similar effects.[11] However, how cells decide between the two fates is still unknown. Although NO must be a potent differentiation-inducer, since chemical NO donors including NOC 18 are unstable in solution, a relatively high initial concentration of the compounds would be necessary to induce sufficient differentiation. Consequently, cells might be exposed to excess NO and undergo cell death directly.

In this study, we showed that NOC 18 slightly reduced the levels of Bcl-2 followed by Bax in HL-60 cells. Delia *et al.* reported the down-regulation of Bcl-2 during TPA-induced monocytic differentiation of HL-60 cells.[19] The down-regulation of both Bcl-2 and Bax might be part of the differentiation process. The fact that the down-regulation of Bcl-2 occurred earlier than that of Bax might contribute to the susceptibility of the cells to the induction of apoptosis. We postulate that when cells with a low ratio of Bcl-2 to Bax are modulated by additional unknown actions of NO, they are obliged to undergo apoptosis. Very recently, Meβmer *et al.* demonstrated that NO induces p53 accumulation followed by up-regulation of Bax in a mouse macrophage cell line, and that overexpression of Bcl-2 suppresses NO-mediated apoptosis.[7,39] However, since HL-60 cells have been shown to lack the p53 gene,[40] there must be p53-independent pathways for the induction of apoptosis by NO such as that coupled to cell differentiation.

In addition, we demonstrated that NOC 18 strongly induced the activity of CPP32 but not ICE in HL-60 cells. Recently, Meβmer *et al.* reported that NO induces cleavage of poly(ADP-ribose) polymerase (PARP), one of the substrates for CPP32, in a mouse macrophage cell line.[41] Therefore, CPP32-like proteases would be implicated in NOC 18-induced apoptosis of HL-60 cells. It now seems clear that CPP32 is more biologically relevant to apoptosis than ICE. For example, selective inhibitors of CPP32 inhibit apoptosis in several mammalian systems whereas comparable concentrations of ICE-selective inhibitors are much less effective.[42]

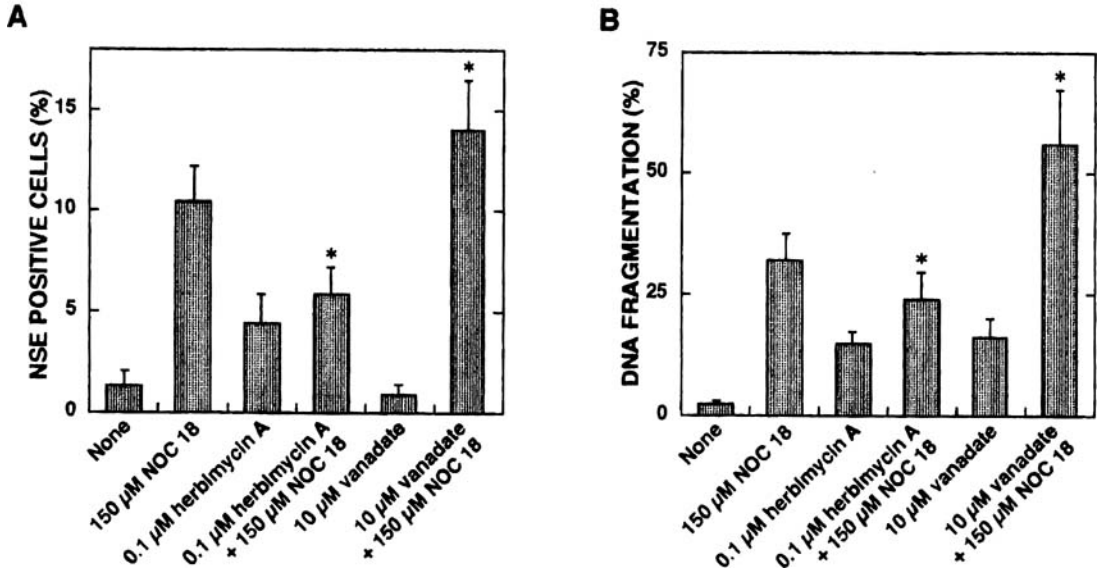


FIGURE 6 Effect of herbimycin A and vanadate on NSE activity and DNA fragmentation in HL-60 cells induced by NOC 18. The cells, at an initial density of 1.5×10^5 cells/ml, were pretreated for 30 min with or without 0.1 μM herbimycin A or 10 μM vanadate, followed by treatment with 150 μM NOC 18. **(A)** After 72 h, the cells were fixed and stained for NSE activity. **(B)** After 24 h, the percentages of fragmented DNA were determined by the diphenylamine method. Each value represents the mean ± S.D. of 3–4 independent experiments. *, statistically different from the cells treated with 150 μM NOC 18 ($p < 0.05$).

We further suggested that protein tyrosine phosphorylation might be involved in apoptosis as well as monocytic differentiation of HL-60 cells induced by NO. Since Katagiri *et al.* reported that the *src* family of PTKs play essential roles in TPA-induced monocytic differentiation of HL-60 cells,[43,44] the same PTKs may also play a similar role in the monocytic differentiation of HL-60 cells induced by NO. With reference to NO-mediated apoptosis, to our knowledge, this is the first report on the involvement of tyrosine phosphorylation in the process. NO has been shown to modulate cellular functions through redox mechanisms such as nitrosylation of protein sulfhydryl groups.[45] Recent sequence analysis of PTP isoforms from several sources revealed the presence of an essential cysteine residue in a conserved catalytic domain.[46] Therefore, NO may modulate PTP activity by S-nitrosylation to elevate phosphorylation levels of tyrosine residues in certain proteins regulating apoptosis and differentiation of HL-60 cells.

In summary, we showed that NOC 18 can induce apoptosis during monocytic differentiation of HL-60 cells. In addition, we also showed that NOC 18 slightly down-regulates the levels of Bcl-2 earlier than the levels of Bax and strongly induces the CPP32-like activity but not the ICE-like activity, possibly responsible for the induction of apoptosis. We further suggested the possible involvement of tyrosine phosphorylation in the pathway. Further work will be required to reveal a possible correlation of tyrosine phosphorylation with apoptotic mediators including the caspase family.

Acknowledgments

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