ANTIOXIDANTS & REDOX SIGNALING
Volume 9, Number 12, 2007

DOI: 10.1089/ars.2007.1828

Forum Original Research Communication

Nrf2-Mediated Heme Oxygenase-1 Induction Confers Adaptive Survival Response to Tetrahydropapaveroline-Induced Oxidative PC12 Cell Death

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ABSTRACT

Tetrahydropapaveroline (THP), a dopaminergic isoquinoline neurotoxin, has been reported to contribute to neurodegeneration in parkinsonism. As THP bears two catechol moieties, it undergoes autooxidation or enzymatic oxidation to produce reactive oxygen species (ROS), which may contribute to the THP-induced cell death. Although ROS are cytotoxic, the initial accumulation of ROS may provoke a survival response. In this study, treatment of PC12 cells with THP increased expression of heme oxygenase-1 (HO-1) as an adaptive survival response. Furthermore, THP-induced cytotoxicity was attenuated by the HO-1 inducer ($SnCl_2$) and exacerbated by the HO-1 inhibitor (ZnPP). To elucidate the molecular mechanisms underlying THP-mediated HO-1 expression, we examined the possible involvement of NF-E2–related factor 2 (Nrf2), which plays an important role in the transcriptional regulation of detoxifying/antioxidant genes. THP treatment elevated nuclear translocation of Nrf2 and subsequent binding to antioxidant response element (ARE). PC12 cells transfected with dominant-negative Nrf2 exhibited increased cytotoxicity and decreased HO-1 expression after THP treatment. Moreover, U0126 and LY294002, which are pharmacologic inhibitors of extracellular signal-regulated kinase1/2 and phosphoinositide 3-kinase, respectively, attenuated HO-1 expression as well as Nrf2-ARE binding activity. Taken together, these findings suggest that HO-1 induction *via* Nrf2 activation may confer a cellular adaptive response against THP-mediated cell death. *Antioxid. Redox Signal.* 9, 2075–2086.

INTRODUCTION

PARKINSON'S DISEASE is a common, slowly progressing neurodegenerative disorder characterized by tremor, muscle rigidity, and akinesia, which are attributed to the deficiency of dopamine as the result of excitotoxicity and apoptosis of nigrostriatal dopaminergic neurons (29). 1-Methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) is an exogenous neurotoxin suspected to cause parkinsonism in humans and experimental animals. The monoamine oxidase (MAO) converts MPTP to 1-methyl-4-phenyl-pyridinium ion (MPP^+), which selectively damages the dopaminergic neurons (3). Therefore, MPTP has been generally considered a potential causative factor of Parkinson disease (21). A group of naturally occurring neurotoxic tetrahydroisoquinoline alkaloids, structurally related to MPTP, have been detected in certain regions of mammalian brains (37). Tetrahydropapaveroline, 6,7-dihydroxy-1-(3′,4′-dihydroxybenzyl)-1,2,3,4-tetrahydroisoquinoline (THP), is one such dopaminergic isoquinoline neurotoxin. It has been considered to be synthesized by a nonenzymatic Pictet–Spengler condensation of dopamine with 3,4-dihydroxyphenylacetaldehyde (dopaldehyde, a metabolite of dopamine) produced by MAO (37). Ironically, THP has also been detected at relatively high concentrations in the urine and brain of parkinsonian patients

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undergoing 3,4-dihydroxyphenylalanine (L-DOPA) therapy (4, 26, 37).

Recently, it was reported that THP treatment increases substantially the intracellular reactive oxygen species (ROS) generation, suggesting the involvement of oxidative stress in THP-induced apoptosis (22, 43, 44, 45). Oxidative stress is a primary pathogenic cause of nigral dopaminergic cell death in Parkinson disease. Our previous studies demonstrated that THP induced apoptosis in PC12 (43) and C6 glioma (44) cells by generating ROS. Interestingly, THP-induced DNA damage and cell death were augmented in the presence of metals, such as copper and iron (20, 44). ROS can damage various biomolecules including DNA, lipids and proteins, and they can alter gene-transcription profiles by acting as important intracellular messengers that target transcription factors and upstream kinases. In this context, it is interesting to note that ROS induce expression of diverse detoxifying/antioxidant enzymes through the activation of signaling molecules. Among them, heme oxygenase-1 (HO-1) is a representative antioxidant defense enzyme that catalyzes the cleavage of the heme to biliverdin, carbon monoxide, and iron. The induction of HO-1 by a vast variety of stimuli such as oxidative stress, heavy metals, UV irradiation, and thiol-reactive substances, suggests that this antioxidant enzyme plays a critical cytoprotective role. Thus, it has been recognized that upregulation of HO-1 represents cellular adaptation to stressful conditions (30, 35). NF-E2–related factor (Nrf2) has been recognized as a redox-sensitive transcription factor that binds antioxidant response element (ARE) or electrophile response element (EpRE), located in the promoter regions of many detoxifying/antioxidant genes including HO-1 (1, 24). Recently, it has been reported that the induction of HO-1 through Nrf2 activation confers protection against oxidative stress (41). Moreover, a common function of ARE/EpRE is to regulate the induction of those genes that encode proteins involved in controlling the cellular redox status, catalyzing the detoxification reactions and protecting cells against oxidative damage (1, 24). In the present work, we investigated the role of HO-1 upregulation *via* Nrf2 activation in adaptive survival response to THP-induced oxidative stress in PC12 cells.

MATERIALS AND METHODS

Materials

THP, poly-D-lysine, MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide], hemin, $SnCl_2$ (stannous chloride), *N*-acetyl-L-cysteine (NAC), and NADPH were purchased from Sigma Chemical Company (St. Louis, MO). Dulbecco's modified Eagle's medium (DMEM), fetal bovine serum, horse serum, and F-12 and N-2 supplement were products of GIBCO (Invitrogen, Carlsbad, CA). Tetramethylrhodamine ethyl ester perchlorate (TMRE) was obtained from Molecular Probes (Invitrogen). The *in situ* cell-death detection kit was supplied by Roche Diagnostics GmbH (Mannheim, Germany). [γ-^{32}P]ATP was the product of NEN Life Science (Boston, MA). Antibodies against Akt and phospho-Akt were purchased from Cell Signaling Technology (Beverly, MA). Antibodies against Nrf2, extracellular signal-regulated kinase 1/2 (ERK1/2) and phospho-ERK1/2 were products of Santa Cruz Biotechnology, Inc. (Santa Cruz, CA). An antibody against HO-1 was purchased from Stessgen Biotechnologies Corp. (Victoria, BC, Canada). Secondary antibodies were obtained from ZYMED (Invitrogen). Zinc-protoporphyrin (ZnPP) was obtained from Alexis (Carlsbad, CA). LY294002 and U0126 were purchased from Calbiochem (San Diego, CA) and TOCRIS (Ellisville, MO), respectively. Dominant-negative Nrf2 (DN-Nrf2) and the control pEF plasmids were kindly provided by Dr. Jeffrey A. Johnson (University of Wisconsin–Madison, Madison, WI).

Cell culture and treatment

PC12 cells (passage number ranging from 20 to 30) were cultured in poly-D-lysine–coated plates at 37°C in a humidified atmosphere of 10% CO_2/90% air. Growth medium consisted of 85% DMEM supplemented with 10% heat-inactivated horse serum, 5% fetal bovine serum, and 1% penicillin–streptomycin–fungizone mixture (Cambrex, East Rutherford, NJ). The medium was changed every other day, and cells were plated at an appropriate density according to each experimental scale. After 12-h incubation, cells were switched to serum-free N-2–defined medium for treatment.

Measurement of cell viability

PC12 cells were plated at a density of 4×10^4 cells/300 μl in 48-well plates, and the cell viability was determined by the conventional MTT reduction assay. In this assay, viable cells convert MTT to insoluble blue formazan crystals by the mitochondrial respiratory chain enzyme succinate dehydrogenase. After incubation, cells were treated with the MTT solution (final concentration, 1 mg/ml) for 2 h at 37°C. The dark blue formazan crystals formed in intact cells were solubilized with dimethylsulfoxide (DMSO), and absorbance at 570 nm was measured with a microplate reader (TECAN GmbH, Salzburg, Austria).

Transient transfection and preparation of HO-1 siRNA

A day before transfection, cells were subcultured at a density of 1×10^6 cells/60-mm dish to maintain an ~60% confluency. Transient transfections were performed using the *N*-[1-(2,3-dioleolloxy)propyl]-*N,N,N*-trimethylammonium methylsulfate (DOTAP) liposomal transfection reagents (Roche Diagnostics GmbH) according to the instructions supplied by the manufacturer. Diluted oligonucleotide (200 pmol in 50 μl HEPES-buffered saline) and diluted DOTAP (30 μl in 100 μl HEPES-buffered saline) were gently mixed and incubated for 15 min at 15–25°C. Cell-culture medium was replaced by fresh N2 medium containing the DOTAP-oligonucleotide mixture. After the 12-h transfection, cells were treated with THP. An oligonucleotide sequence for HO-1 siRNA was selected to knockdown HO-1 expression using the siRNA target-finder software at www.invitrogen.com. The rat HO-1–specific siRNA (5′-CCG UGG CAG UGG GAA UUU AUG CCA U-3′) and the nonspecific siRNA (5′-CCG ACG GUG AGG UUA UAU CGU GCA U-3′) were provided by Invitrogen.

Terminal deoxynucleotidyl transferase-mediated dUTP nick end-labeling assay (TUNEL staining)

This assay was conducted by following the instructions for the *in situ* apoptosis-detection kit. The technique relies on the specific labeling of the 3′-OH end of DNA breaks with modified nucleotides by terminal deoxynucleotidyl transferase. PC12 cells were plated at a density of 1×10^5 cells/600 μl in chamber slides and fixed for 30 min in 10% neutral buffered formalin solution at room temperature. Endogenous peroxidase was blocked by incubation with 0.3% hydrogen peroxide in methanol for 30 min at room temperature. The cells were permeabilized with 0.1% sodium citrate and 0.1% Triton X-100 for 2 min at 4°C. Thereafter, cells were stained with peroxidase-conjugated anti-fluorescein anti-goat antibody (Fab fragment) for an additional 30 min. Finally, cells were rinsed with phosphate-buffered saline (PBS), mounted with 50% glycerol, and examined under a confocal microscope (Leica Microsystems Heidelberg GmbH, Heidelberg, Germany) with the fluorescence excitation at 488 nm and emission at 525 nm. At least four fields containing ~400 cells were analyzed, and one typical image from three independent experiments was shown.

Measurement of mitochondrial transmembrane potential ($\Delta\Psi_m$)

To measure the mitochondrial transmembrane potential, the lipophilic cationic probe TMRE was used. PC12 cells were plated at a density of 1×10^5 cells/600 μl in four-well chamber slides. After treatment with THP for 24 h at 37°C, cells were washed twice with PBS and TMRE (150 n*M*) was loaded. After 30-min incubation at 37°C, cells were examined under a confocal microscope with the fluorescence excitation at 544 nm and emission at 590 nm. At least four fields containing ~400 cells were analyzed, and one typical image from three independent experiments was shown.

Immunocytochemistry for HO-1

To demonstrate the activated HO-1 localization, we adopted the immunocytochemistry method that uses a monoclonal antibody recognizing HO-1. Cells (1×10^5 cells/600 μl in four-well chamber slides) were fixed in 10% neutral-buffered formalin solution for 30 min at room temperature. After a rinse with PBS, cells were blocked for 1 h at room temperature in fresh blocking buffer (0.5% Tween 20 in PBS, pH 7.4, containing 10% normal goat serum). Dilutions (1:100) of primary anti-HO-1 antibody were made in PBS with 1% bovine serum albumin, and cells were incubated overnight at 4°C. After three washes with PBST (PBS containing 0.1% Tween 20), the cells were incubated with FITC-goat anti-rabbit IgG secondary antibody in PBST with 3% bovine serum albumin for 1 h at room temperature. Cells were rinsed with PBS, and stained cells were analyzed under a confocal microscope and photographed.

Western blot analysis

After treatment, cells were collected by scraping and centrifugation (1,000 *g* for 5 min), and cell pellets were washed with PBS. After centrifugation, cell lysis was carried out at 4°C by vigorous vortexing for 15 min in RIPA buffer [150 m*M* NaCl, 1% Nonidet P-40, 0.5% sodium deoxycholate, 0.1% SDS, 50 m*M* Tris-HCl (pH 7.4), 50 m*M* glycerophosphate, 20 m*M* EGTA, 20 m*M* NaF, 1 m*M* dithiothreitol (DTT), 1 m*M* Na_3PO_4, and protease inhibitor]. The cell lysates were centrifuged at 14,000 *g* for 15 min. Supernatant was stored at −70°C for Western blot analysis, and the protein concentration was determined by the BCA protein assay (Pierce, Rockford, IL). After addition of sample loading buffer, protein samples were electrophoresed on a SDS–polyacrylamide gel. Proteins were transferred to polyvinylidene difluoride (PVDF) blots at 300 mA for 5 h. The blots were blocked for 1 h at room temperature in fresh blocking buffer (PBST containing 5% nonfat dried milk). Dilutions (1:1,000) of primary antibodies were made in PBS with 3% nonfat dried milk, and blots were incubated overnight at 4°C. After three washes with PBST, the blots were incubated with horseradish peroxidase–conjugated secondary antibody in PBS with 3% nonfat dried milk for 1 h. The blots were washed again 3 times in PBST, and transferred proteins were incubated with ECL substrate solution (Amersham Pharmacia Biotech, Piscataway, NJ) for 1 min, according to the manufacturer's instructions, and visualized with an x-ray film.

Reverse transcription–polymerase chain reaction

Total RNA was isolated from PC12 cells using TRIzol (Invitrogen) by following the manufacturer's instructions. Reverse transcription–polymerase chain reaction (RT-PCR) was per-

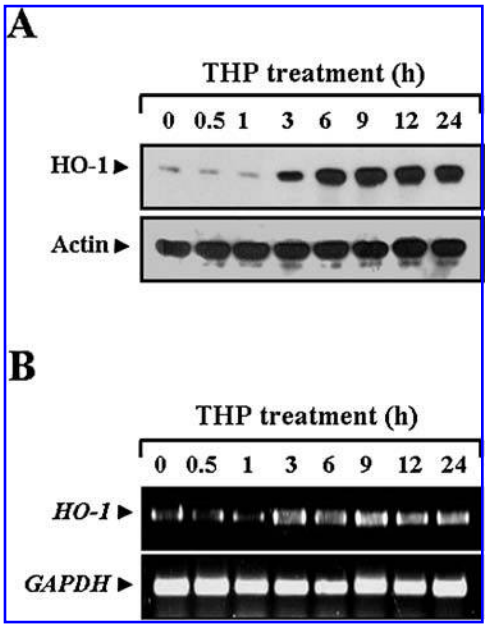

FIG. 1. Kinetics of THP-induced HO-1 expression. PC12 cells were treated with 30 μ*M* THP for indicated periods. Western blot analysis (**A**) and RT-PCR (**B**) were conducted to measure the levels of HO-1 protein and its mRNA transcript, respectively. Actin and GADPH, respectively, were measured for the confirmation of equal amounts of protein and mRNA loaded.

formed according to standard techniques. The PCR primers used in this study were as follows: HO-1, 5′-ACT TTC AGA AGG GTC AGG TGT CC-3′ (sense) and 5′-TTG AGC AGG AAG GCG GTC TTA G -3′ (antisense); and glyceraldehyde-3-phosphate dehydrogenase (GAPDH), 5′-AGT GTA GCC CAG GAT GCC CTT-3′ (sense) and 5′-GCC AAG GTC ATC CAT GAC AAC-3′ (antisense). The PCR conditions for determination of mRNA levels of HO-1 and GAPDH are as follows: initiation at 95°C for 5 min followed by 28 cycles of 94°C for 30 sec, 58°C (annealing temperature of HO-1 and GAPDH) for 1 min, and 72°C for 1 min, and final extension at 72°C for 5 min. Amplification products were resolved by 1.0% agarose gel electrophoresis, stained with ethidium bromide, and photographed under an ultraviolet light. All primers were purchased from Bionics (Seoul, Korea).

Preparation of nuclear proteins

After treatment with THP, PC12 cells (1×10^7 cells/7 ml in 100-mm dish) were washed with PBS, centrifuged, and resuspended in ice-cold isotonic buffer A [10 m*M* Hepes (pH 7.9), 1.5 m*M* $MgCl_2$, 10 m*M* KCl, 0.5 m*M* DTT and 0.2 m*M* phenylmethylsulfonyl fluoride (PMSF)]. After 10-min incubation in an ice bath, cells were centrifuged again and resuspended in ice-cold buffer C [20 m*M* Hepes (pH 7.9), 20% glycerol, 420 m*M* NaCl, 1.5 m*M* $MgCl_2$, 0.2 m*M* EDTA, 0.5 m*M* DTT, and 0.2 m*M* PMSF] followed by incubation at 0°C for 20 min. After vortex mixing, the resulting suspension was centrifuged, and the supernatant was stored at −70°C.

Electrophoretic mobility shift assay (EMSA) for determining the ARE binding activity

A synthetic double-strand oligonucleotide containing the ARE binding domain was labeled with [γ-^{32}P]ATP using T4 polynucleotide kinase and separated from unincorporated [γ-^{32}P]ATP by gel filtration using a nick-spin column (Amersham Pharmacia Biotech, Piscataway, NJ). The sequences of oligonucleotides in double strands used in the present study were 5′-TTT TCT GCT GAG TCA AGG TCC G-3′ and 3′-AAA AGA

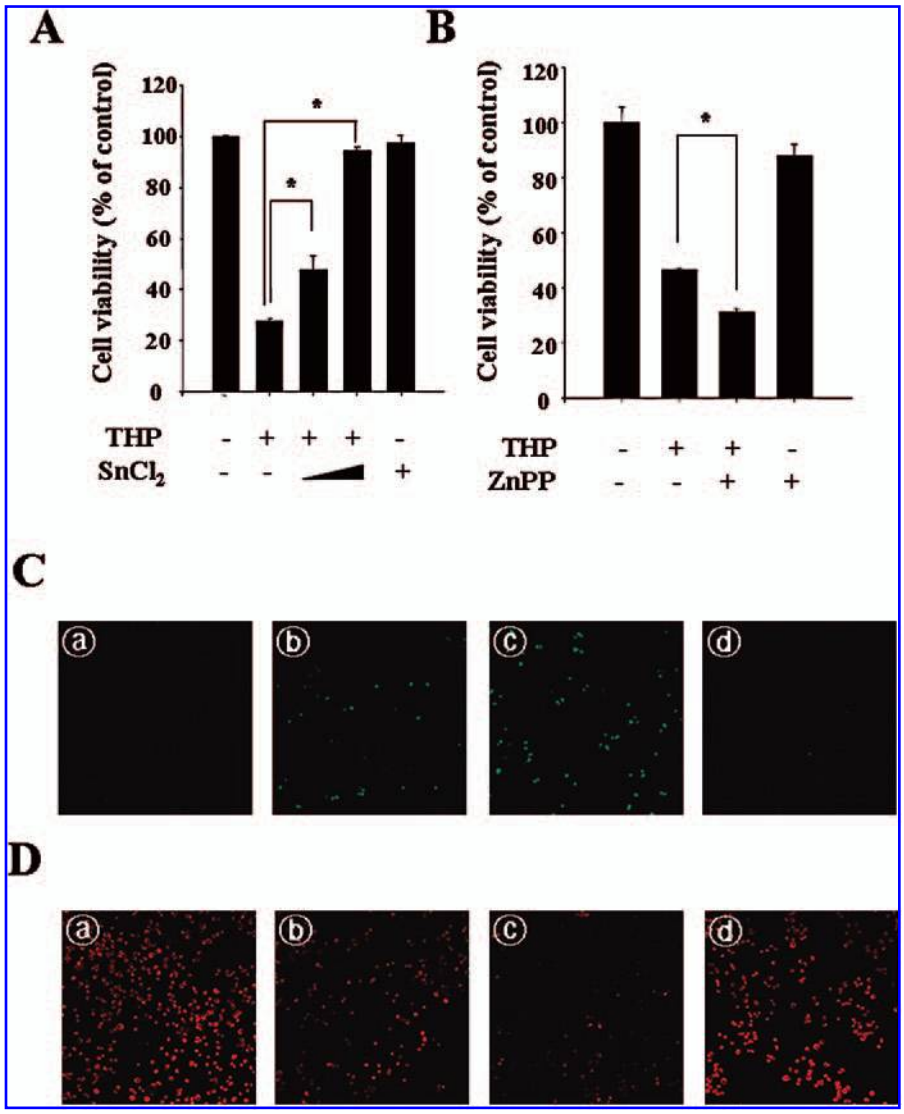

FIG. 2. Effects of HO-1 inducer/inhibitor on THP-induced apoptosis. (**A**) PC12 cells were treated with 30 μM THP and $SnCl_2$ (10 or 25 μM). Cytotoxicity was assessed by the MTT reduction assay at 24 h. (**B**) PC12 cells were preincubated with 0.2 μM ZnPP for 30 min, followed by exposure to 30 μM THP. Cytotoxicity was assessed by the MTT reduction assay at 24 h. The values are expressed as mean ± SD ($n = 3$). *Significantly different from THP alone ($p < 0.01$). (**C**) THP-induced internucleosomal DNA fragmentation was determined by *in situ* terminal nick-end labeling (TUNEL): (a) control; (b) cells exposed to 30 μM THP alone for 24 h; (c) cells treated with 30 μM THP for 24 h after 30-min preincubation with 0.2 μM ZnPP; (d) cells exposed to 0.2 μM ZnPP alone for 24 h. (**D**) Mitochondrial depolarization was assessed by changes in the TMRE fluorescence as described under Materials and Methods: (a) control; (b) cells exposed to 30 μM THP alone for 24 h; (c) cells treated with 30 μM THP for 24 h after 30-min preincubation with 0.2 μM ZnPP; (d) cells exposed to 0.2 μM ZnPP alone for 24 h.

CGA CTC AGT TCC AGG C-5′. The oligonucleotide was synthesized by Bionics (Seoul, Korea). Before addition of the [γ-^{32}P]-labeled oligonucleotide (100,000 cpm), 10 μg of the nuclear extract was mixed in an ice bath for 15 min with gel-shift binding buffer [20% glycerol, 5 m*M* $MgCl_2$, 2.5 m*M* EDTA, 2.5 m*M* DTT, 250 m*M* NaCl, and 50 m*M* Tris-HCl, pH 7.5 with 0.25 μg/ml poly(dI-dC)]. The DNA–protein complexes were resolved by 6% nondenaturating polyacrylamide gel electrophoresis at 145 V for 2 h followed by autoradiography.

Statistical analysis

All data from Western blot analysis, RT-PCR, and EMSA were derived from at least three independent experiments. The data from the MTT dye-reduction assay were expressed as mean ± SD ($n = 3$), and statistical analysis for multiple comparisons was performed with one-way ANOVA followed by the Student–Newman–Keuls test using SPSS software (SPSS 12.0KO for Windows). The criterion for statistical significance was $p < 0.05$.

RESULTS

THP induced the expression of HO-1 in PC12 cells

As HO-1 is one of the major antioxidant defense enzymes that are readily induced in response to oxidative stress, it has been suggested that upregulation of HO-1 expression and increased HO activity play a key role in cytoprotection against oxidative damage. PC12 cells were treated with 30 μ*M* THP for the indicated period, and the induction of HO-1 protein and its mRNA transcript was examined by Western blot analysis and RT-PCR, respectively. The elevated protein level of HO-1 was detected at 3 h after THP treatment, which steadily increased up to 24 h (Fig. 1A). Consistent with this finding, the expression of HO-1 mRNA was evident in 3 h after THP treatment, which was sustained up to 24 h (Fig. 1B).

HO-1 inducer/inhibitor modulated THP-induced apoptosis

In an attempt to determine whether the THP-induced HO-1 expression could confer cytoprotection against oxidative cell death, we treated PC12 cells with an inducer or an inhibitor of HO-1 in combination with THP. As shown in Fig. 2A, THP-mediated cytotoxicity was attenuated in the presence of 10 μ*M* and 25 μ*M* $SnCl_2$. We verified the induction of HO-1 by $SnCl_2$ at these concentrations (data not shown). $SnCl_2$ alone elicited no apparent cytotoxicity. In contrast, the blockage of HO-1 activity by zinc protoporphyrin IX (ZnPP) exacerbated the cytotoxicity induced by THP (Fig. 2B). In another experiment, PC12 cells treated with 30 μ*M* THP underwent apoptosis, as determined by positive TUNEL staining. In this analysis, the intensely stained nuclei represent the terminal incorporation of labeled dUTP into the 3′ end of fragmented DNA derived from apoptotic nuclei. ZnPP pretreatment further increased the proportion of TUNEL-positive cells (Fig. 2C). In addition to the nuclear DNA fragmentation, mitochondrial dysfunction often occurs during apoptosis as a general event. Mitochon-

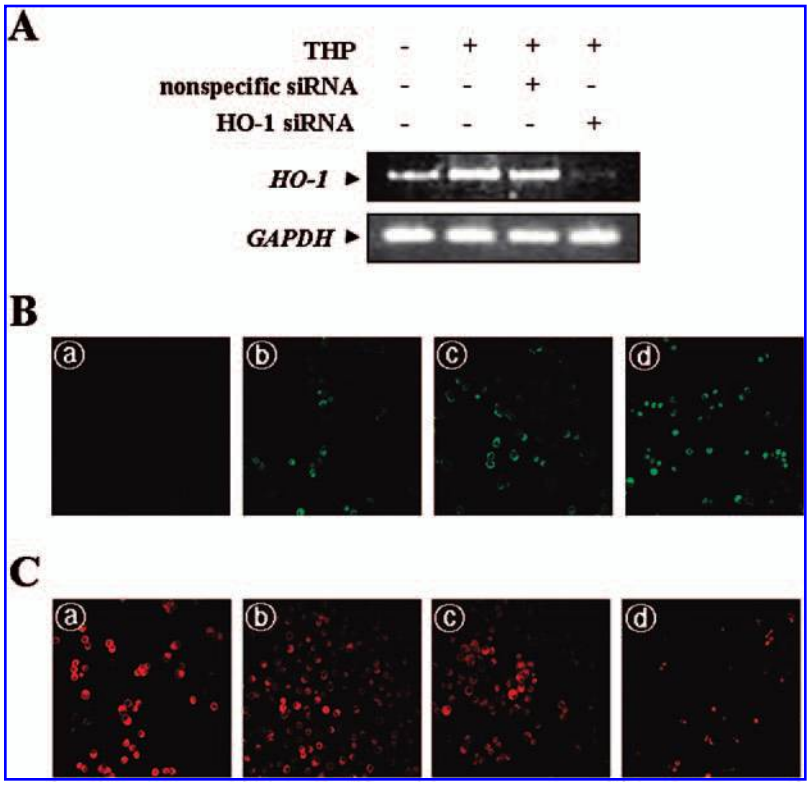

FIG. 3. Enhancement of THP-induced apoptosis by siRNA knockdown of *HO-1* gene. (**A**) PC12 cells were transfected with nonspecific siRNA or HO-1 siRNA using DOTAP transfection reagent. After 12-h transfection, cells were treated with 30 μ*M* THP for an additional 9 h. The total RNA samples were analyzed by RT-PCR for the mRNA transcripts of HO-1. (**B**) After transfection, cells were treated with 30 μ*M* THP for an additional 24 h. THP-induced apoptosis was assessed by TUNEL staining: (a) control; (b) cells exposed to 30 μ*M* THP alone for 24 h; (c) cells treated with 30 μ*M* THP for 24 h after transfection with nonspecific siRNA; (d) cells treated with 30 μ*M* THP for 24 h after transfection with HO-1 siRNA. (**C**) THP-induced dissipation of mitochondrial transmembrane potential was exacerbated by transfection with HO-1 siRNA: (a) control; (b) cells exposed to 30 μ*M* THP alone for 24 h; (c) cells treated with 30 μ*M* THP for 24 h after transfection with nonspecific siRNA; (d) cells treated with 30 μ*M* THP for 24 h after transfection with HO-1 siRNA.

dria undergo major changes in membrane integrity before classic signs of cell death become manifest. Abnormalities occur in the inner as well as the outer mitochondrial membranes, leading to the dissipation of the transmembrane potential and permeability changes that finally release soluble intermembrane proteins. When PC12 cells were exposed to THP, the mitochondrial transmembrane potential was reduced, as evidenced by the decrease in red fluorescence with a voltage-sensitive dye, TMRE (Fig. 2D). THP-induced dissipation of transmembrane potential was significantly augmented by the pretreatment with ZnPP.

Small interference RNA knockdown of the HO-1 gene exacerbated THP-treated PC12 cell death

HO-1 siRNA constructs were used to investigate the effect of HO-1 expression on THP-mediated cell death in PC12 cells. First, we verified that HO-1 mRNA levels were reduced in the PC12 cells harboring HO-1 siRNA (Fig. 3A). Transfection with a nonspecific siRNA construct failed to block HO-1 expression in PC12 cells. The PC12 cells transfected with HO-1 siRNA increased the proportion of TUNEL-positive cells after THP treatment (Fig. 3B) whereas the extent of apoptosis in nonspecific siRNA-transfected PC12 cells was similar to that observed in normal cells. Moreover, when PC12 cells transfected with HO-1 siRNA were exposed to THP, the mitochondrial transmembrane potential was rapidly reduced, as shown by the decrease in TMRE dye (Fig. 3C).

THP treatment increased the HO-1–specific ARE-binding activity of Nrf2

Most of the genes encoding detoxifying/antioxidant enzymes have an enhancer region named ARE. Recently, it has been reported that Nrf2 is the major transcription factor that binds to ARE and increases the ARE-driven expression of target genes including HO-1. Therefore, we attempted to examine the nuclear translocation of Nrf2 in the THP-stimulated PC12 cells. Nuclear extracts from PC12 cells were prepared after treatment with THP for indicated periods. Protein from cell lysates was analyzed with Western blot using an Nrf2-specific antibody. As illustrated in Fig. 4A, the nuclear accumulation of Nrf2 increased transiently after THP treatment. To clarify the role of Nrf2 in the transcriptional activation of ARE of *HO-1* gene, EMSA was performed using the [γ-^{32}P]-labeled oligonucleotide harboring the HO-1–specific ARE sequence. Treatment of PC12 cells with THP led to increased Nrf2-ARE binding activity (Fig. 4B).

Dominant-negative Nrf2 (DN-Nrf2) mutation in the PC12 cells aggravated the cytotoxicity of THP

Further to elucidate the role of Nrf2 in cytoprotection against THP-induced apoptosis, we used the dominant-negative mutant form of the *Nrf2* gene construct with truncated N-terminal (DN-Nrf2). We verified the decreased Nrf2-ARE binding as well as nuclear translocation of Nrf2 in DN-Nrf2–overexpressing cells (data not shown). Because of knockout of the functional *Nrf2* gene, PC12 cells harboring DN-Nrf2 were expected to be more susceptible to the THP-induced cytotoxicity. Thus, PC12 cells transfected with DN-Nrf2 exhibited the reduced ARE-binding activity of Nrf2, compared with the control cells transfected with the blank vector (pEF) (Fig. 5A), and more susceptible to THP-induced apoptosis, as revealed by the increased proportion of TUNEL-positive apoptotic cells (Fig. 5B). Moreover, the THP-induced mitochondrial transmembrane perturbation was substantially augmented by functional inactivation of the *Nrf2* gene (Fig. 5C).

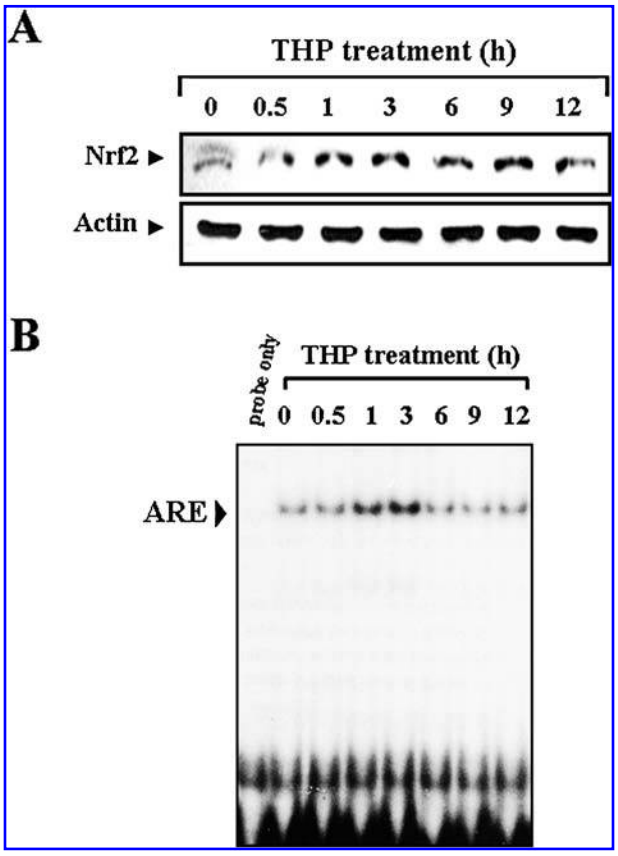

FIG. 4. THP-induced activation of Nrf2. Nuclear extracts from PC12 cells were prepared after treatment with 30 μM THP for indicated periods. (**A**) Protein from cell lysates was analyzed with Western blot using an Nrf2-specific antibody. (**B**) Nuclear extracts were incubated with [γ-^{32}P]-labeled oligonucleotides harboring an ARE consensus sequence. Nrf2-ARE binding activity was assessed with EMSA, as described under Materials and Methods.

DN-Nrf2 mutation negated THP-induced HO-1 expression in PC12 cells

To determine whether THP-stimulated Nrf2 activation is indispensable for HO-1 induction, a *DN-Nrf2* gene was used. The PC12 cells transfected with DN-Nrf2 failed to induce the HO-1 expression after THP treatment (Fig. 6A). We also compared the expression of HO-1 by the immunocytochemical

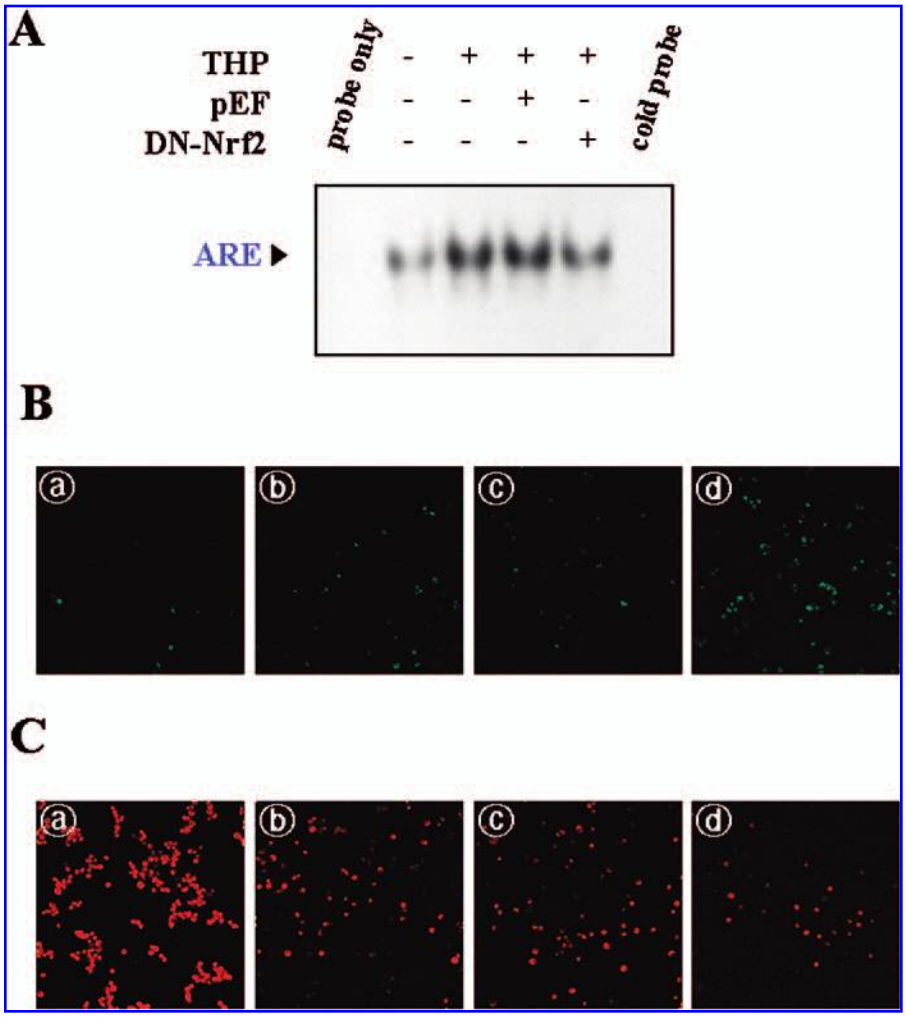

FIG. 5. Effects of dominant-negative mutant Nrf2 on THP-induced cell death. PC12 cells were transfected with pEF (blank vector) or Nrf2 dominant-negative plasmid (DN-Nrf2) using DOTAP transfection reagent. After 12-h transfection, cells were treated with 30 μM THP for an additional 3 h. (**A**) Nrf2-ARE binding activity was assessed by EMSA. (**B**) THP-induced internucleosomal DNA fragmentation was determined by TUNEL staining: (a) control; (b) cells exposed to 30 μM THP alone for 24 h; (c) cells treated with 30 μM THP for 24 h after transfection with pEF; (d) cells treated with 30 μM THP for 24 h after transfection with DN-Nrf2. (**C**) Mitochondrial depolarization was assessed by changes in the TMRE fluorescence: (a) control; (b) cells exposed to 30 μM THP alone for 24 h; (c) cells treated with 30 μM THP for 24 h after transfection with pEF; (d) cells treated with 30 μM THP for 24 h after transfection with DN-Nrf2.

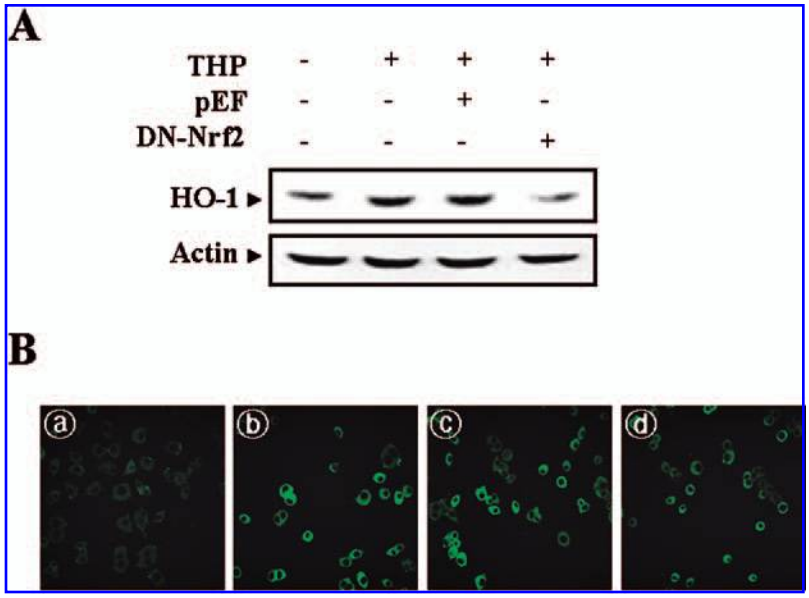

FIG. 6. Effects of dominant-negative mutant Nrf2 on THP-induced HO-1 expression. Cells were transfected with pEF (blank vector) or dominant-negative mutant Nrf2 (DN-Nrf2) construct using DOTAP transfection reagent. After 12-h transfection, cells were treated with 30 μM THP for an additional 9 h. (**A**) Protein from cell lysates was analyzed with Western blot using HO-1–specific antibody. (**B**) Immunocytochemical analysis of HO-1 expression in PC12 cells after treatment with 30 μM THP for 9 h, as described under Materials and Methods: (a) control; (b) cells exposed to 30 μM THP alone for 9 h; (c) cells treated with 30 μM THP for 9 h after transfection with pEF; (d) cells treated with 30 μM THP for 9 h after transfection with DN-Nrf2.

method with a monoclonal antibody recognizing this enzyme (Fig. 6B). Again PC12 cells harboring DN-Nrf2 accumulated lower levels of HO-1 than did those cells with functionally active Nrf2. Taken together, these findings support that the upregulation of HO-1 induced by THP is mediated through the Nrf2 activation.

The pharmacologic inhibitors of MEK1/2 and PI3K blocked THP-induced Nrf2-ARE binding and HO-1 expression

To elucidate the molecular mechanisms underlying THP-mediated HO-1 induction *via* Nrf2 activation, we examined the possible involvement of ERK1/2 and Akt/protein kinase B (PKB) using their pharmacologic inhibitors. In our previous study, we reported that the phosphorylation of ERK1/2 and Akt/PKB occurred relatively early after THP treatment (43). In this study, THP-mediated induction of ARE-binding activity of Nrf2 was attenuated by 25 μM U0126 (the inhibitor of MEK1/2, upstream of ERK1/2), as well as by the same concentration of LY294002 (the inhibitor of PI3K, upstream of Akt/PKB) (Fig. 7A and B). In addition, pretreatment with these inhibitors effectively suppressed the THP-induced HO-1 expression (Fig. 7C and D). These results suggest that the THP-induced Nrf2 activation and subsequent HO-1 expression are likely to be mediated through activation of ERK1/2 and Akt/PKB signaling pathways.

N-Acetylcysteine (NAC) treatment suppressed THP-induced HO-1 expression and Nrf2-ARE binding activity

To determine whether ROS are involved in HO-1 expression as well as Nrf2 activation in THP-treated PC12 cells, we used the ROS scavenger, NAC. The elevated Nrf2-ARE binding activity resulting from THP treatment was abolished when NAC was added to the medium (Fig. 8A), indicative of the possible involvement of ROS in Nrf2 activation. In a subsequent experiment, we also verified the inhibition of THP-induced HO-1 expression in the presence of NAC (Fig. 8B).

DISCUSSION

THP is an endogenous 1-benzyltetrahydroisoquinoline detected in some human tissues. This compound has been suspected to be responsible for the degeneration of dopaminergic neurons implicated in Parkinson disease and alcohol addiction (10, 28, 37). The concentrations of THP in the brains of parkinsonian patients were 0.12–0.22 pmol/g wet tissue (38). The plasma levels of THP in L-DOPA–treated parkinsonian patients were elevated to 0.11–0.34 nM (22). For some *in vitro* and *in vivo* experiments, 5–100 μM and 0.1–10 mg/kg (i.p.) of THP were used, respectively (5, 11, 20, 22). THP bears two catechol moieties attached to the tetrahydroisoquinoline backbone (Fig. 9). This catechol can undergo redox cycling, producing large quantities of superoxide anion, which, in turn, can be transformed into hydrogen peroxide by superoxide dismutase (11, 17, 22). In addition to ROS, catechols, after conversion to reactive quinoids, can directly modify the critical thiol residues in structural proteins and enzymes *via* nucleophilic addition, which has been suggested as a principal mechanism responsible for the cytotoxicity elicited by THP (11, 27, 46). High levels of ROS can cause the oxidative stress implicated in many human diseases, including neurodegenerative disorders, cancer, diabetes mellitus, ischemia, and failures in immunity and endocrine functions (14). Nevertheless, moderate amounts of ROS may mediate the intra-

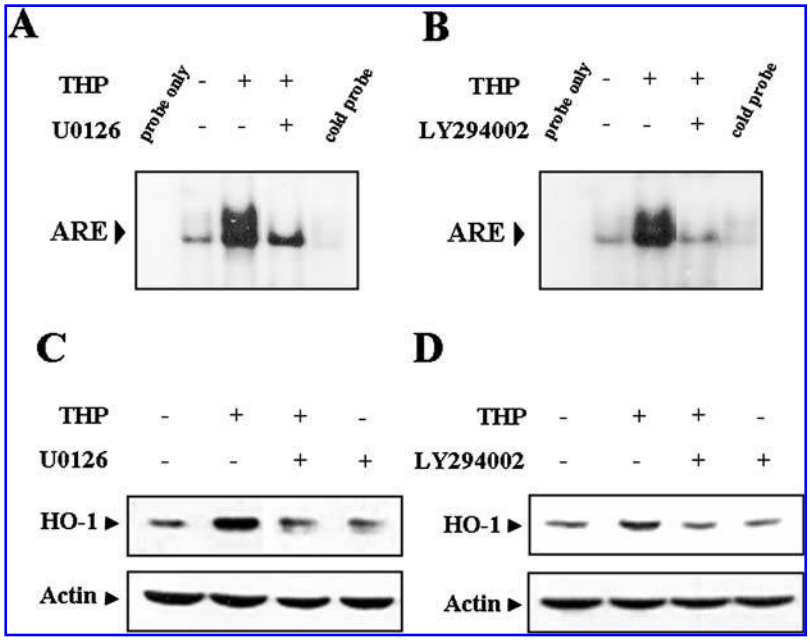

FIG. 7. Effects of pharmacologic inhibitors of MEK1/2 and PI3K on THP-induced Nrf2 activation and HO-1 expression. (**A, B**) Effects of U0126 (25 μM) and LY294002 (25 μM) on THP-mediated Nrf2-ARE binding activity. Nuclear extracts were incubated with [γ-^{32}P]-labeled oligonucleotides harboring the ARE consensus sequence and subjected to EMSA. (**C, D**) Effects of U0126 and LY294002 on THP-induced HO-1 expression assessed with Western blot.

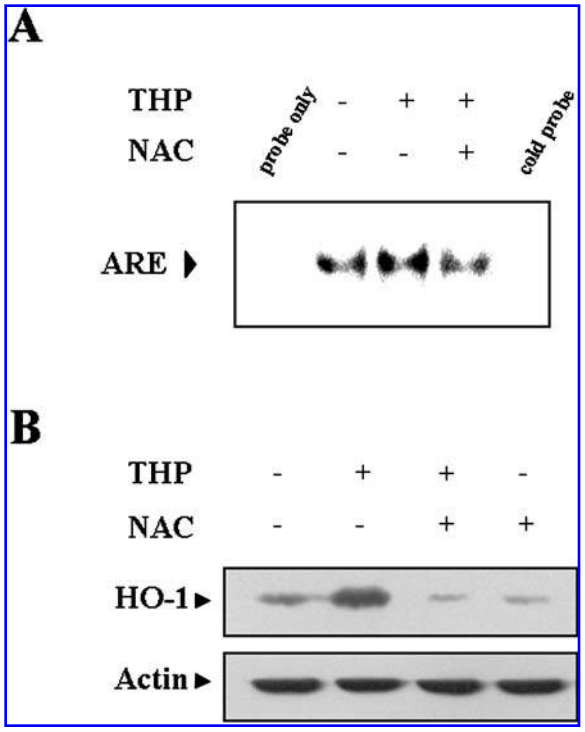

FIG. 8. Effect of NAC on THP-induced Nrf2-ARE binding activity and HO-1 expression. (**A**) PC12 cells were treated with 30 μM THP and 0.5 mM NAC for 3 h. Nuclear extracts were incubated with [γ-^{32}P]-labeled oligonucleotides harboring the ARE consensus sequence. (**B**) PC12 cells were treated with 30 μM THP and 0.5 mM NAC for 9 h and analyzed with Western blot using an HO-1–specific antibody.

cellular signal transduction, leading to transcriptional activation of the appropriate genes (12, 20). In agreement with this notion, our present study demonstrates that alterations in the cellular redox status induced by THP-generated ROS immediately turn on the cellular signaling cascades in such a way that they activate Nrf2 to rescue the PC12 cells from subsequent oxidative stress. This can be achieved by upregulation of the early-response genes encoding antiapoptotic or antioxidative proteins or both and enzymes including HO-1.

Nrf2, a member of the Cap'n'Collar family of basic leucine transcription factors, has been found to interact with the 5′-flanking regions of the mouse and human HO-1 genes (24). Nrf2 forms an inactive complex with Keap1 in the cytoplasm (24). However, the activated Nrf2 is dissociated from Keap1, translocates to the nucleus, heterodimerizes with small Maf, and binds to ARE, eventually resulting in transcriptional regulation of target genes, such as glutamate cysteine ligase, glutathione (GSH) *S*-transferase, NAD(P)H:quinone oxidoreductase 1, and HO-1 (1, 24, 48). Because of the lack of such coordinated stress response, Nrf2 knockout (Nrf2$^{-/-}$) mice are prone to toxicity caused by butylated hydroxytoluene (6) or hyperoxia (8) and also to carcinogenesis induced by benzo[*a*]pyrene (32). Therefore, Nrf2 contributes to cellular protection against oxidative insults and chemical carcinogenesis *via* transcriptional upregulation of genes encoding antioxidant/detoxifying enzymes. In the present study, Nrf2 was found to be transiently activated in PC12 cells treated with THP. The translocation of Nrf2 into the nucleus after THP treatment was accompanied by an increased ARE binding of this transcription factor.

HO-1, also known as heat-shock protein 32 (HSP32), catalyzes the heme degradation, releasing biliverdin/bilirubin, carbon monoxide, and iron (35). However, the biologic significance of HO-1 induction, especially in neuronal cells, has been in debate. Schipper *et al.* (42) suggested that HO-1 overexpression contributes to iron deposition and mitochondrial damage observed in some aging-related neurodegenerative disorders. Conversely, it has been reported that neurons overexpressing HO-1 survive oxidative stress (7). Satoh *et al.* (39) also reported that HO-1–catalyzed production of biliverdin and bilirubin resulted in the inhibition of neuronal death induced by oxidative stress, because biliverdin and bilirubin as well as carbon monoxide possess substantial antioxidant properties. Our present study clearly demonstrates the critical role of HO-1 in adaptive cytoprotective response to THP-induced apoptosis.

The molecular mechanisms of HO-1 induction are not fully clarified, but it has been reported that the activated Nrf2 can induce HO-1 expression. Rushworth *et al.* (34) reported that lipopolysaccharide-induced HO-1 expression is mediated *via* the activation of Nrf2 in human monocytic cells. In addition, dominant-negative mutation of Nrf2 decreased the HO-1 mRNA level in response to heme, cadmium, zinc, arsenite, and *tert*-butyl-

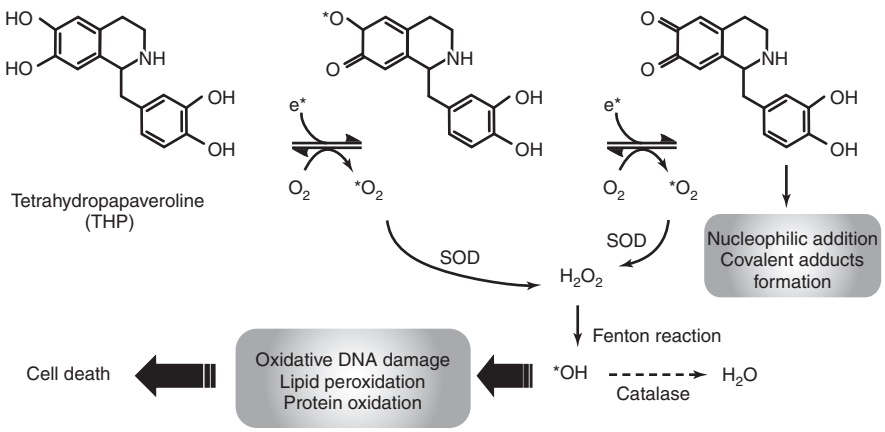

FIG. 9. A proposed scheme for redox cycling of THP, which leads to oxidative cell death *via* intracellular accumulation of ROS.

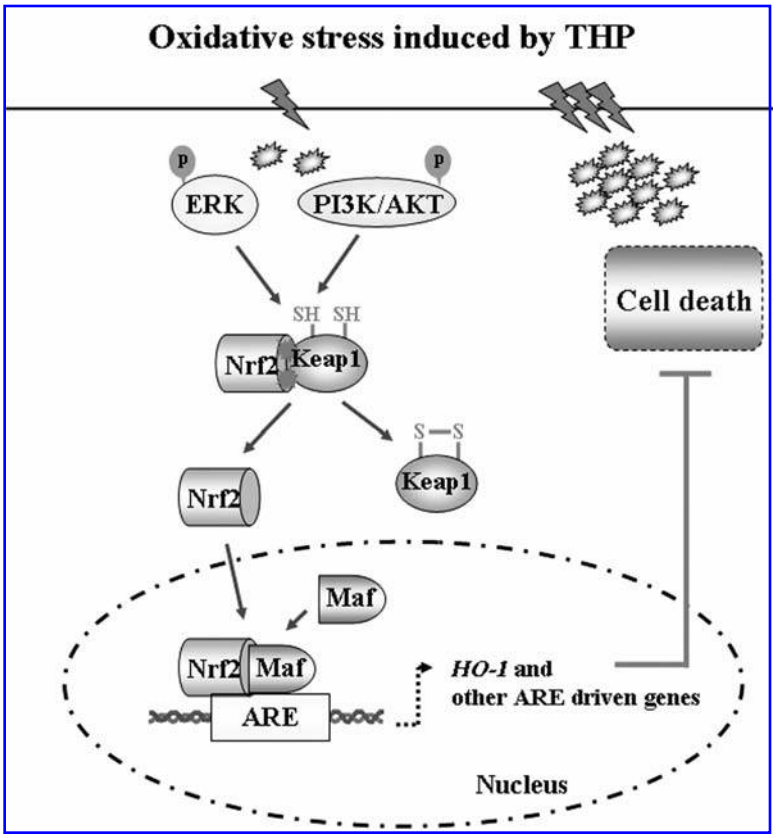

FIG. 10. A proposed pathway for THP-induced Nrf2 activation and HO-1 induction, which confers cytoprotection against oxidative stress on PC12 cells.

hydroquinone (1). In our study, HO-1 expression was induced through the activation of Nrf2 after THP treatment. However, the PC12 cells transfected with DN-Nrf2 failed to induce the HO-1 expression after THP treatment. Although Nrf2-ARE binding is essential for HO-1 induction, the expression of HO-1 is also regulated through the functional heat-shock elements. In rats, hypothermia-elevated *HO-1* mRNA transcription was accompanied by an increased binding of nuclear factors to the heat-shock element in the promoter region of *HO-1* (33). Therefore, we cannot rule out the possible involvement of heat-shock elements in the THP-mediated transcriptional activation of HO-1.

In this work, we note that THP-induced Nrf2 activation in PC12 cells is mediated through ERK1/2 or Akt/PKB phosphorylation, which may represent another plausible mechanism. Although Nrf2 can be phosphorylated by members of the protein kinase C (PKC) family, other upstream kinases may also be responsible for directly phosphorylating Nrf2. PKC was shown to stimulate dissociation of Nrf2 from Keap1 and subsequent nuclear accumulation (15). However, phosphorylation of Nrf2 at Ser and Thr residues by other kinases such as PI3K, c-Jun NH2-terminal kinase (JNK), and ERK1/2 is assumed likewise to mediate the release of Nrf2 from Keap1 and nuclear translocation (24). ERK1/2, in particular, plays an important role in both growth-factor and stress signaling (16). The ERK1/2 pathway, in general, is responsive to environmental stress, and its activation is considered to be crucial in cell survival or adaptation. ERK1/2 activation induces the phosphorylation of downstream cytosolic and nuclear factors that control a wide variety of intracellular signaling processes (47). Another kinase, Akt/PKB, is a serine/threonine protein kinase that mediates cell-survival signals from growth factors and cytokines (13). Akt/PKB is fully activated by phosphorylation at Thr 308 and Ser 473 in response to a vast variety of extracellular signals. In response to oxidative stress, the activation of signaling cascades mediated by PI3K results in depolymerization of actin microfilaments, thereby facilitating Nrf2 translocation to the nucleus (19). The PI3K cascade has also been implicated in the regulation of ARE-dependent genes in H4II rat hepatoma cells (18), as well as in IMR-32 human neuroblastoma cells (23). The role of PI3K in upregulation of HO-1 in response to diverse stimuli has been well documented (9, 25, 31, 36). Our previous studies also demonstrated that the robust phosphorylation of ERK1/2 and Akt/PKB occurred after THP treatment (43). In this study, THP-stimulated Nrf2-ARE binding activity was attenuated by the pharmacologic inhibition of the aforementioned kinases. Similarly, THP-induced HO-1 expression was suppressed by the same treatment. Therefore, we suggest that THP-induced HO-1 expression and Nrf2 activation are likely to be mediated through activation of ERK1/2 and Akt/PKB.

Besides phosphorylation of Nrf2 by upstream kinases, such as ERK1/2 and PI3K, which facilitates the nuclear translocation of Nrf2 oxidation and covalent modification of cysteine thiols present in its negative regulator. Keap1 represent another important mechanism responsible for activation of Nrf2, as such processes diminish affinity of Keap1 for Nrf2. One or both catechol moieties of THP can undergo oxidation *via* semiquinone to produce reactive quinoids that can also be reduced back to the parent molecule. During such redox cycling, ROS can also be generated. Our previous studies revealed the increased accumulation of ROS in THP-treated PC12 cells (43). Whereas ROS can oxidize critical

cysteine residues of Keap1, the reactive quinoid form(s) of THP may covalent modify the same residues. The THP-elevated Nrf2-ARE binding activity and the induction of HO-1 were effectively suppressed by the antioxidant NAC, lending support to the involvement of ROS in activation of Nrf2 signaling. However, NAC has a dual function in acting as an antioxidant. Although it can scavenge ROS by itself, the molecule can indirectly potentiate the cellular antioxidant capacity through *de novo* synthesis of GSH. Addition of NAC to the medium does lead to a concentration-dependent increase in the intracellular GSH levels in PC12 cells (40). As GSH can react with the electrophilic quinoids and NAC, as a precursor of GSH biosynthesis, blocked THP-induced Nrf2-ARE binding, it is plausible that covalent modification, as well as oxidation of cysteine thiol(s) in Keap1, contributes to Nrf2 activation in PC12 cells treated with THP. As mentioned earlier, THP-induced Nrf2 activation is under the control of ERK1/2 and PI3K Therefore, ROS derived from redox cycling of THP may activate one or both of these upstream kinases, thereby indirectly activating Nrf2, and additional work is needed to clarify this alternative mechanism.

In summary, THP produces ROS *via* redox cycling, which can induce the upregulation of HO-1 expression through activation of Nrf2, conferring adaptive survival response to THP-induced oxidative PC12 cell death. ERK1/2 and PI3K appear to play central roles in the Nrf2-mediated upregulation of HO-1 expression in THP-treated PC12 cells. Pharmacologic inhibitors of ERK1/2 or PI3K suppressed Nrf2 activation and subsequent HO-1 expression, suggesting the potential role of these kinases in HO-1 upregulation as well as Nrf2 activation (Fig. 10). However, the complete molecular mechanism that coordinates all these events must be clarified.

ACKNOWLEDGMENTS

This work was supported by a National Research Laboratory (NRL) grant from the Korea Science and Engineering Foundation, Republic of Korea.

ABBREVIATIONS

ARE, antioxidant response element; EpRE, electrophile response element; ERK1/2, extracellular signal-regulated kinase 1/2; GCL, γ-glutamylcysteine ligase; GSH, glutathione; GST, glutathione *S*-transferase; HO-1, heme oxygenase-1; MAO, monoamine oxidase; MPTP, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine; MPP^+, 1-methyl-4-phenylpyridinium ion; Nrf2, NF-E2–related factor 2; NQO1, NAD(P)H/quinine oxidoreductase 1; PKB, protein kinase B; PKC, protein kinase C; ROS, reactive oxygen species; THP, tetrahydropapaveroline.

REFERENCES

1. Alam J and Cook JL. Transcriptional regulation of the heme oxygenase-1 gene via the stress response element pathway. *Curr Pharm Des* 9: 2499–2511, 2003.
2. Alam J, Stewart D, Touchard C, Boinapally S, Choi AM, and Cook JL. Nrf2, a Cap'n'Collar transcription factor, regulates induction of the heme oxygenase-1 gene. *J Biol Chem* 274: 26071–26078, 1999.
3. Antkiewicz-Michaluk L. Endogenous risk factors in Parkinson's disease: dopamine and tetrahydroisoquinolines. *J Pharmacol* 54: 567–572, 2002.
4. Cashaw JL. Determination of tetrahydropapaveroline in the urine of parkinsonian patient receiving L-dopa-carbidopa (Sinemet) therapy by high-performance liquid chromatography. *J Chromatogr* 613: 267–273, 1993.
5. Cashaw JL and Geraghty CA. Tetrahydropapaveroline and the blood-brain barrier in rats. *Alcohol* 8: 317–319, 1991.
6. Chan K and Kan YW. Nrf2 is essential for protection against acute pulmonary injury in mice. *Proc Natl Acad Sci U S A* 96: 12731–12736, 1999.
7. Chen K, Gunter K, and Maines MD. Neurons overexpressing heme oxygenase-1 resist oxidative stress-mediated cell death. *J Neurochem* 75: 304–313, 2000.
8. Cho HY, Jedlicka AE, Reddy SP, Kensler TW, Yamamoto M, Zhang LY, and Kleeberger SR. Role of NRF2 in protection against hyperoxic lung injury in mice. *Am J Respir Cell Mol Biol* 26: 175–182, 2002.
9. Chung SW, Chen YH, and Perrella MA. Role of Etd-2 in the regulation of heme oxygenase-1 by endotoxin. *J Biol Chem* 280: 4578–4584, 2005.
10. Collins MA. Tetrahydropapaveroline in Parkinson's disease and alcoholism: a look back in honor of Merton Saldler. *Neurotoxicology* 25: 117–120, 2004.
11. De Marco F, Perluigi M, Marcante ML, Coccia R, Foppoli C, Blarzino C, and Rosei MA. Cytotoxicity of dopamine-derived tetrahydroisoquinolines on melanoma cells. *Biochem Pharmacol* 15: 1503–1512, 2002.
12. Droge W. Free radicals in the physiological control of cell function. *Physiol Rev* 82: 47–95, 2002.
13. Hatano E and Brenner D. Akt protects mouse hepatocytes from TNF-α and Fas-mediated apoptosis through NF-κB activation. *Am J Physiol Gastrointest Liver Physiol* 282: G1357–G1368, 2001.
14. Hensley K, Robinson KA, Gabbita SP, Salsman S, and Floyd RA. Reactive oxygen species, cell signaling, and cell injury. *Free Radic Biol Med* 28: 1456–1462, 2000.
15. Huang HC, Nguyen T, and Pickett CB. Phosphorylation of Nrf2 at Ser-40 by protein kinase C regulates antioxidant response element-mediated transcription. *J Biol Chem* 277: 42769–42774, 2002.
16. Ichijo H. From receptors to stress-activated MAP kinases. *Oncogene* 18: 6087–6093, 1999.
17. Jung YJ and Surh YJ. Oxidative DNA damage and cytotoxicity induced by copper-stimulated redox cycling of salsolinol, a neurotoxic tetrahydroisoquinoline alkaloid. *Free Radic Biol Med* 30: 1407–1417, 2001.
18. Kang KW, Cho MK, Lee CH, and Kim SG. Activation of phosphatidylinositol 3-kinase and Akt by *tert*-butylhydroquinone is responsible for antioxidant response element-mediated rGSTA2 induction in H4IIE cells. *Mol Pharmacol* 59: 1147–1156, 2001.
19. Kang KW, Lee SJ, Park JW, and Kim SG. Phosphatidylinositol 3-kinase regulates nuclear translocation of NF-E2-related factor 2 through actin rearrangement in response to oxidative stress. *Mol Pharmacol* 62: 1001–1010, 2002.
20. Kobayashi H, Oikawa S, and Kawanishi S. Mechanism of DNA damage and apoptosis induced by tetrahydropapaveroline, a metabolite of dopamine. *Neurochem Res* 31: 523–532, 2006.
21. Langston JW. Chronic parkinsonism in humans due to a product of meperidine-analog synthesis. *Science* 219: 979–980, 1983.
22. Lee JJ, Kim YM, Yin SY, Park HD, Kang MH, Hong JT, and Lee MK. Aggravation of L-DOPA-induced neurotoxicity by tetrahydropapaveroline in PC12 cells. *Biochem Pharmacol* 66: 1787–1795, 2003.
23. Lee JM, Hanson JM, Chu WA, and Johnson JA. Phosphatidylinositol 3-kinase, not extracellular signal-regulated kinase, regulates activation of the antioxidant-responsive element in IMR-32 human neuroblastoma cells. *J Biol Chem* 276: 20011–20016, 2001.
24. Lee JS and Surh YJ. Nrf2 as a novel molecular target for chemoprevention. *Cancer Lett* 224: 171–184, 2005.

25. Martin D, Rojo AI, Salinas M, Diaz R, Gallardo G, Alam J, Galarreta CM, and Cuadrado A. Regulation of heme oxygenase-1 expression through the phosphatidylinositol 3-kinase/Akt pathway and the Nrf2 transcription factor in response to the antioxidant phytochemical carnosol. *J Biol Chem* 279: 8919–8929, 2003.
26. Matsubara K, Fukushima S, Akane A, Kobayashi S, and Shiono H. Increased urinary morphine, codeine and tetrahydropapaveroline in parkinsonian patient undergoing L-3,4-dihydroxyphenylalanine therapy: a possible biosynthetic pathway of morphine from L-3,4-dihydroxyphenylalanine in humans. *J Pharmacol Exp Ther* 260: 974–978, 1992.
27. Montine TJ, Picklo MJ, Amarnath V, Whetsell WO Jr, and Graham DG. Neurotoxicity of endogenous cysteinylcatechols. *Exp Neurol* 148: 26–33, 1997.
28. Myers RD. Tetrahydroisoquinolines in the brain: the basis of an animal model of alcoholism. *Alcohol Clin Exp Res* 2: 145–154, 1978.
29. Nagatsu T. Isoquinoline neurotoxins in the brain and Parkinson's disease. *Neurosci Res* 29: 99–111, 1997.
30. Panahian N, Yoshiura M, and Maines MD. Overexpression of heme oxygenase-1 is neuroprotective in a model of permanent middle cerebral artery occlusion in transgenic mice. *J Neurochem* 72: 1187–1203, 1999.
31. Pischke SE, Zhou Z, Song R, Ning W, Alam J, Ryter SW, and Choi AM. Phosphatidylinositol 3-kinase/Akt pathway mediates heme oxygenase-1 regulation by lipopolysaccharide. *Cell Mol Biol* 51: 461–470, 2005.
32. Ramos-Gomez M, Kwak M, Dolan PM, Itoh K, Yamamoto M, Talalay P, and Kensler TW. Sensitivity to carcinogenesis is increased and chemoprotective efficacy of enzyme inducers is lost in *nrf2* transcription factor-deficient mice. *Proc Natl Acad Sci U S A* 92: 7445–7449, 2001.
33. Raju VS and Maines MD. Coordinated expression and mechanism of induction of HSP32 (heme oxygenase-1) mRNA by hyperthermia in rat organs. *Biochim Biophys Acta* 1217: 273–280, 1994.
34. Rushworth SA, Chen XL, Mackman N, Ogborne RM, and O'Connell MA. Lipopolysaccharide-induced heme oxygenase-1 expression in human monocytic cells is mediated via Nrf2 and protein kinase C. *J Immunol* 175: 4408–4415, 2005.
35. Ryter SW, Otterbein LE, Morse D, and Choi AM. Heme oxygenase/carbon monoxide signaling pathways: regulation and functional significance. *Mol Cell Biochem* 234: 234–235, 249–263, 2002.
36. Salina M, Diaz R, Abraham NG, Ruiz de Galarreta CM, and Cuadrado A. Nerve growth factor protects against 6-hydroxydopamine-induced oxidative stress by increasing expression of heme oxygenase-1 in a phosphatidylinositol 3-kinase-dependent manner. *J Biol Chem* 278: 13898–13904, 2003.
37. Sandler M, Carter SB, Hunter KR, and Stern GM. Tetrahydroisoquinoline alkaloids: in vivo metabolites of L-dopa in man. *Nature* 241: 439–443, 1973.
38. Sango K,Maruyama W, Matsubara K, Dostert P, Minami C, Kawai M, and Naoi M. Enantio-selective occurrence of (S)-tetrahydropapaveroline in human brain. *Neurosci Lett* 283: 224–226, 2000.
39. Satoh T, Baba M, Nakatsuka D, Ishikawa Y, Aburatani H, Furuta K, Ishikawa T, Hatanaka H, Suzuki M, and Watanabe Y. Role of heme oxygenase-1 protein in the neuroprotective effects of cyclopentenone prostaglandin derivatives under oxidative stress. *Eur J Neurosci* 17: 2249–2255, 2003.
40. Satoh E, Okada M, Takadera T, and Ohyashiki T. Glutathione depletion promotes aluminum-mediated cell death of PC12 cells. *Biol Pharm Bull* 28: 941–946, 2005.
41. Satoh T, Okamoto SI, Cui J, Watanabe Y, Furuta K, Suzuki M, Tohyama K, and Lipton SA. Activation of the Keap1/Nrf2 pathway for neuroprotection by electrophilic phase II inducers. *Proc Natl Acad Sci U S A* 103: 768–773, 2006.
42. Schipper HM. Heme oxygenase-1: role in brain aging and neurodegeneration. *Exp Gerontol* 35: 821–830, 2000.
43. Shin MH, Jang JH, and Surh YJ. Potential roles of NF-κB and ERK1/2 in cytoprotection against oxidative cell death induced by tetrahydropapaveroline. *Free Radic Biol Med* 36: 1185–1194, 2004.
44. Soh Y, Shin MH, Lee JS, Jang JH, Kim OH, Kang H, and Surh YJ. Oxidative DNA damage and glioma cell death induced by tetrahydropapaveroline. *Mutat Res* 544: 129–142, 2003.
45. Surh YJ. Tetrahydropapaveroline, a dopamine-derived isoquinoline alkaloid, undergoes oxidation: implications for DNA damage and neuronal cell death. *Eur J Clin Invest* 29: 650–651, 1999.
46. Wardman P. Bioreductive activation of quinones: redox properties and thiol reactivity. *Free Radic Res Commun* 8: 219–229, 1990.
47. Xu C, Yuan X, Pan Z, Shen G, Kim JH, Yu S, Khor TO, Li W, Ma J, and Kong AN. Mechanism of action of isothiocyanates: the induction of ARE-regulated genes is associated with activation of ERK and JNK and the phosphorylation and nuclear translocation of Nrf2. *Mol Cancer Ther* 5: 1918–1926, 2006.
48. Zhang Y, Gonzalez V, and Xu J. Expression and regulation of glutathione *S*-transferase P1-1 in cultured human epidermal cells. *J Dermatol Sci* 30: 205–214, 2002.

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Date of first submission to ARS Central, July 8, 2007; date of acceptance, July 8, 2007.

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2. Zhouguang Wang, Hongyu Zhang, Xinlong Xu, Hongxue Shi, Xichong Yu, Xiaojie Wang, Yongbo Yan, Xiaobing Fu, Houwen Hu, Xiaokun Li, Jian Xiao. 2012. bFGF inhibits ER stress induced by ischemic oxidative injury via activation of the PI3K/Akt and ERK1/2 pathways. *Toxicology Letters* **212**:2, 137-146. [CrossRef]
3. Inn-Goo Kyeong, Jung-Hoon Kang. 2012. Protective Effects of Carnosine and Anserine on Oxidative Modification of Neurofilament-L Induced by Catechol Neurotoxin, Tetrahydropapaveroline. *Bulletin of the Korean Chemical Society* **33**:2, 731-734. [CrossRef]
4. Yi-Chih Tsai, Yi-Hsiang Wang, Chih-Chiang Liou, Yu-Cun Lin, Haimei Huang, Yin-Chang Liu. 2011. Induction of Oxidative DNA Damage by Flavonoids of Propolis: Its Mechanism and Implication about Antioxidant Capacity. *Chemical Research in Toxicology* 111223152519003. [CrossRef]
5. Chan Lee, Gyu Hwan Park, Chang-Yul Kim, Jung-Hee Jang. 2011. [6]-Gingerol attenuates #-amyloid-induced oxidative cell death via fortifying cellular antioxidant defense system. *Food and Chemical Toxicology* **49**:6, 1261-1269. [CrossRef]
6. Fei Yin, Jian-hui Liu, Xu-xu Zheng, Li-xia Guo. 2010. GLP-1 receptor plays a critical role in geniposide-induced expression of heme oxygenase-1 in PC12 cells. *Acta Pharmacologica Sinica* **31**:5, 540-545. [CrossRef]
7. Zeng-Chun Ma, Qian Hong, Yu-Guang Wang, Hong-Ling Tan, Cheng-Rong Xiao, Qian-De Liang, Bo-Li Zhang, Yue Gao. 2010. Ferulic Acid Protects Human Umbilical Vein Endothelial Cells from Radiation Induced Oxidative Stress by Phosphatidylinositol 3-Kinase and Extracellular Signal-Regulated Kinase Pathways. *Biological & Pharmaceutical Bulletin* **33**:1, 29-34. [CrossRef]
8. Ho Kyun Lee, Soo Jin Na Choi. 2010. Effects of Heme Oxygenase-1 Expression in Mycophenolic Acid Induced Apoptosis of Jurkat Cell Lines. *Journal of the Korean Surgical Society* **78**:6, 343. [CrossRef]
9. Young-Joon Surh, Hyun-Jung Kim. 2010. Neurotoxic Effects of Tetrahydroisoquinolines and Underlying Mechanisms. *Experimental Neurobiology* **19**:2, 63. [CrossRef]
10. Jing-Ting Hong, Jui-Hung Yen, Lisu Wang, Ya-Hsuan Lo, Zong-Tsi Chen, Ming-Jiuan Wu. 2009. Regulation of heme oxygenase-1 expression and MAPK pathways in response to kaempferol and rhamnocitrin in PC12 cells. *Toxicology and Applied Pharmacology* **237**:1, 59-68. [CrossRef]
11. Cécile Mazière, Jean-Claude Mazière. 2009. Activation of transcription factors and gene expression by oxidized low-density lipoprotein. *Free Radical Biology and Medicine* **46**:2, 127-137. [CrossRef]
12. X HAN, J PAN, D REN, Y CHENG, P FAN, H LOU. 2008. Naringenin-7-O-glucoside protects against doxorubicin-induced toxicity in H9c2 cardiomyocytes by induction of endogenous antioxidant enzymes. *Food and Chemical Toxicology* **46**:9, 3140-3146. [CrossRef]
13. Jozef Dulak . 2007. Changing Faces of Heme Oxygenases. *Antioxidants & Redox Signaling* **9**:12, 2043-2048. [Citation] [Full Text PDF] [Full Text PDF with Links]