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Piperine protects cisplatin-induced apoptosis via heme oxygenase-1 induction in auditory cells☆

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Abstract

Piperine is a major component of black pepper, *Piper nigrum* Linn, used widely in traditional medicine. In this study, we examined whether piperine could protect House Ear Institute-Organ of Corti 1 (HEI-OC1) cells against cisplatin-induced apoptosis through the induction of heme oxygenase (HO)-1 expression. Piperine (10–100 μM) induced the expression of HO-1 in dose- and time-dependent manners. Piperine also induced antioxidant response element-luciferase and translocated nuclear factor-E2-related factor-2 (Nrf2) to nucleus. Piperine activated the c-Jun N-terminal kinase (JNK), extracellular signal-regulated kinase and p38 mitogen-activated protein kinase (MAPK) pathways, and the JNK pathway played an important role in piperine-induced HO-1 expression. Piperine protected the cells against cisplatin-induced apoptosis. The protective effect of piperine was abrogated by zinc protoporphyrin IX, an HO inhibitor, and antisense oligodeoxynucleotides against HO-1 gene. These results demonstrate that the expression of HO-1 by piperine is mediated by both JNK pathway and Nrf2, and the expression inhibits cisplatin-induced apoptosis in HEI-OC1 cells.

Keywords: Cisplatin; Heme oxygenase; MAPK; Nrf2; Piperine

1. Introduction

Piperine is a major plant alkaloid presented in pepper, which is one popular spice worldwide [1]. It is known to possess several pharmacological actions, such as antimicrobial, antipyretic and anti-inflammatory effects [2]. It has been reported to show antioxidant activity in several experimental conditions [3]. It also inhibits the productions of nitric oxide and tumor necrosis factor-α [4] and the expressions of several proinflammatory cytokines and matrix metalloproteinases [5].

A cellular defensive mechanism against electrophiles and oxidants relies on detoxification by phase II-detoxifying enzymes, antioxidants enzymes and stress response proteins [6–8]. The expressions of these genes, including the glutathione (GSH) *S*-transferase, NAD(P)H:quinone oxidoreductase, and heme oxygenase (HO)-1 are regulated at the transcriptional level through a *cis*-acting enhancer sequence known as antioxidant response element (ARE) [1,9]. The ARE-mediated gene expression has been implicated as a pivotal protection mechanism against various stimuli such as oxidative damage and carcinogenic intermediates [10]. Nuclear factor-E2-related factor-2 (Nrf2), a member of the basic-leucine zipper NF-E2 family, is known to play a key role in the ARE-mediated gene expression [9,11]. Recent studies with Nrf2$^{-/-}$ mice demonstrate that Nrf2 regulates the expressions of variety of genes, including chaperones, antioxidant genes, and genes regulating protein degradation [11–13]. Nrf2$^{-/-}$ mice are more susceptible to toxic chemicals and stresses [12].

HO is the rate-limiting enzyme in heme degradation, catalyzing the cleavage of the heme ring to form ferrous iron, carbon monoxide (CO) and biliverdin. Three mammalian HO isoforms have been identified, one of which, HO-1, is a stress-responsive protein induced by various stimuli [14]. Bilirubin generated by HO-1 is an antioxidant capable of scavenging peroxy radicals and inhibiting lipid peroxidation [15], and

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another product, CO, is known as anti-inflammatory and antiapoptotic effects [16,17]. The release of Fe^{2+} is known to mitigate any antioxidant actions of HO-1 and may explain a rather narrow threshold of HO-1 overexpression to confer protection because Fe^{2+} catalyzes the formation of reactive oxygen species in the Fenton reaction [18]. However, Fe^{2+} has very recently been reported to have cytoprotective effect via nuclear factor κB activation [19]. Although intensively pursued, it still remains unclear how iron controls life and death of cell.

In recent years, several groups have investigated the functional significance of HO-1 induction, usually by observing the ability of cells to resist different stress insults when HO-1 is under- or overexpressed [20–22]. These studies have supported the designation of an important cellular defense role for HO-1 against oxidant injury. The expression of the HO-1 gene is primarily regulated at the transcriptional level, although there are interspecies variations in the regulation of HO-1 [23,24]. The transcription factor Nrf2 plays a key role in HO-1 gene activation [11]. Most studies have focused on the activation of the mitogen activated protein kinases (MAPKs), including extracellular signal-regulated kinase (ERK), c-Jun N-terminal kinase (JNK) and p38 related to cell growth and various stress responses. All three pathways appear to be involved to some extent in the up-regulation of HO-1 expression in response to diverse stimuli.

Cisplatin (*cis*-diamminedichloroplatinum II) is a highly effective chemotherapeutic agent, which is used to treat several types of solid tumors [25]. However, reversible and irreversible side effects, including ototoxicity, may limit its utility and therapeutic profile. Therefore, many researchers have tried to ameliorate the ototoxic side effect of cisplatin. Several studies indicate that antioxidants are effective in the preventions of drug-induced hearing loss [26,27]. Indeed, antioxidants have shown efficacies in the attenuation of noise-induced hearing loss, significantly protecting auditory outer hair cells and electrophysiological responsiveness [27]. In this study, we examined whether piperine protects ototoxic effect of cisplatin in House Ear Institute-Organ of Corti 1 (HEI-OC1) cells. The cell, an auditory cell, is derived from cochlear organ of corti in mice. Our results demonstrated that piperine induced the expression of HO-1, and the induction played and important role in the protective effects of piperine on cisplatin-induced apoptosis.

2. Materials and methods

2.1. Materials

Piperine and cisplatin were purchased from Sigma-Aldrich (St. Louis, MO, USA). Zinc protoporphyrin IX (ZnPP IX), an inhibitor of heme oxygenase activity, was from Porphyrin Products (Logan, UT, USA). Genomic DNA purification kit was obtained from Promega (Madison, WI, USA). PD98059, SB203580, SP600125 and anti-HO-1 antibody were purchased from Calbiochem (San Diego, CA, USA). Antibodies against Nrf2, Lamin B and β-actin were purchased from Santa Cruz Biotechonology (Santa Cruz, CA, USA). Anti-phospho-JNK, ERK and p38 antibodies were purchased from Cell Signaling Technology (Beverly, MA, USA). Unless indicated otherwise, all other chemicals were obtained from Sigma-Aldrich.

2.2. Cell culture

The establishment and characterization of the conditionally immortalized HEI-OC1 cells were described by Kalinec et al. [28]. HEI-OC1 cells have been recently established and characterized from long-term cultures of immorto-mouse cochlea. Expression of outer hair cell (OHC) specific markers, including Math1 and Myosin 7a, suggests that HEI cells may represent OHC precursors [29]. Cells were maintained in high-glucose Dulbecco's modified Eagle's medium (GIBCO BRL, Grand Island, NY, USA) supplemented with 10% fetal bovine serum (GIBCO BRL) at 33°C in a humidified incubator with 5% CO_2.

2.3. MTS assay for cell viability

Cells were subcultured in 96-well plates at a density of 5×10^4 cells per well. Cells were treated with cisplatin in the presence or absence of piperine or ZnPP. The MTS assay

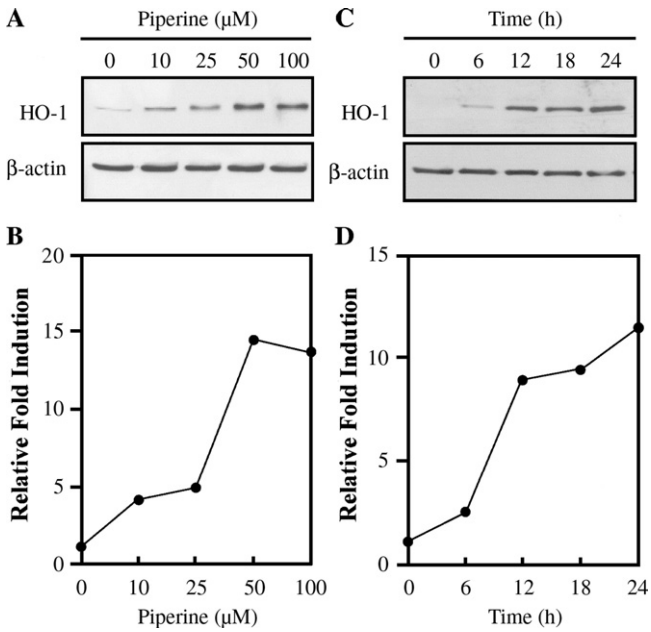

Fig. 1. Induction of HO-1 expression by piperine in HEI-OC1 cells. (A and B) HO-1 expression was measured in HEI-OC1 cells at 24 h after treatment with various concentrations of piperine. (C and D) Cells were treated with 50 μM piperine, and HO-1 expression was measured at various time points indicated in the figure. Total cellular proteins were isolated from cells treated with piperine, and Western blot analysis was performed using specific antibodies for HO-1 and β-actin. Relative fold induction of HO-1 protein levels was quantified as described under Materials and methods.

was performed with the CellTiter 96 Aqueous nonradioactive cell proliferation assay kit (Promega), according to manufacturer's instructions. The absorbance was read at 490 nm on an enzyme-linked immunosorbent assay reader, and the percentage of cell survival was obtained.

2.4. Morphological assessment of apoptosis

HEI-OC1 cells were washed twice with phosphate-buffered saline (PBS) and fixed with 3.7% formaldehyde in PBS for 10 min at room temperature. Fixed cells were washed twice with PBS. DAPI was added and the suspension was incubated for 5 min at room temperature. The cells were washed two more times with PBS and analyzed by fluorescence microscopy using a Zeiss microscope.

2.5. Genomic DNA isolation and DNA-laddering assay

Genomic DNA was isolated from cultured cell with the Wizard Genomic DNA purification kit (Promega). Briefly, cells were lysed with lysis buffer, followed by 1 h incubation with RNase A. The cell lysates were precipitated for proteins and spun at 15,000 rpm for 20 min. The supernatant was precipitated with isopropanol for isolation of DNA. After washing with 70% ethanol, DNA was hydrated and quantified. Equal amounts (10 μg) of DNA were electrophoresed on a 1.5% agarose gel (with incorporated ethidium bromide). The gel was then photographed under ultraviolet luminescence.

2.6. Preparation of cytosolic and nuclear extracts

Cytosolic and nuclear fractions were prepared as previously described [30]. Briefly, cells were washed three times with cold PBS and centrifuged at 1100 rpm for 10 min. The pellet was carefully resuspended in 3 pellet volumes of cold buffer containing 20 mM HEPES (pH 7.0), 0.15 mM EGTA, 10 mM KCl, 1% Nonidet-40, 1 μg/ml leupeptin, 1 mM phenylmethylsulfonyl fluoride, 20 mM NaF, 1 mM sodium pyrophosphate and 1 mM Na_3VO_4. The homogenate was then centrifuged at 500 g for 20 min, and the nuclear pellet was washed in 5 pellet volumes of cold PBS. After centrifugation at 500*g* for 20 min, nuclei were resuspended in 2 pellet volumes of hypertonic cold buffer containing 10 mM HEPES (pH 8.0), 25% glycerol, 0.4 M NaCl, 0.1 mM EDTA, 1 μg/ml leupeptin, 1 mM phenylmethylsulfonyl fluoride, 20 mM NaF, 1 mM sodium pyrophosphate and 1 mM Na_3VO_4 and incubated for 30 min at 4°C on a rotating wheel. Nuclear debris was removed by centrifugation at 900*g* for 20 min at 4°C. The supernatant was resolved by sodium docecyl sulfate (SDS)–polyacrylamide gel electrophoresis and submitted to Western blot using anti-Nrf2 and anti-Lamin B antibodies.

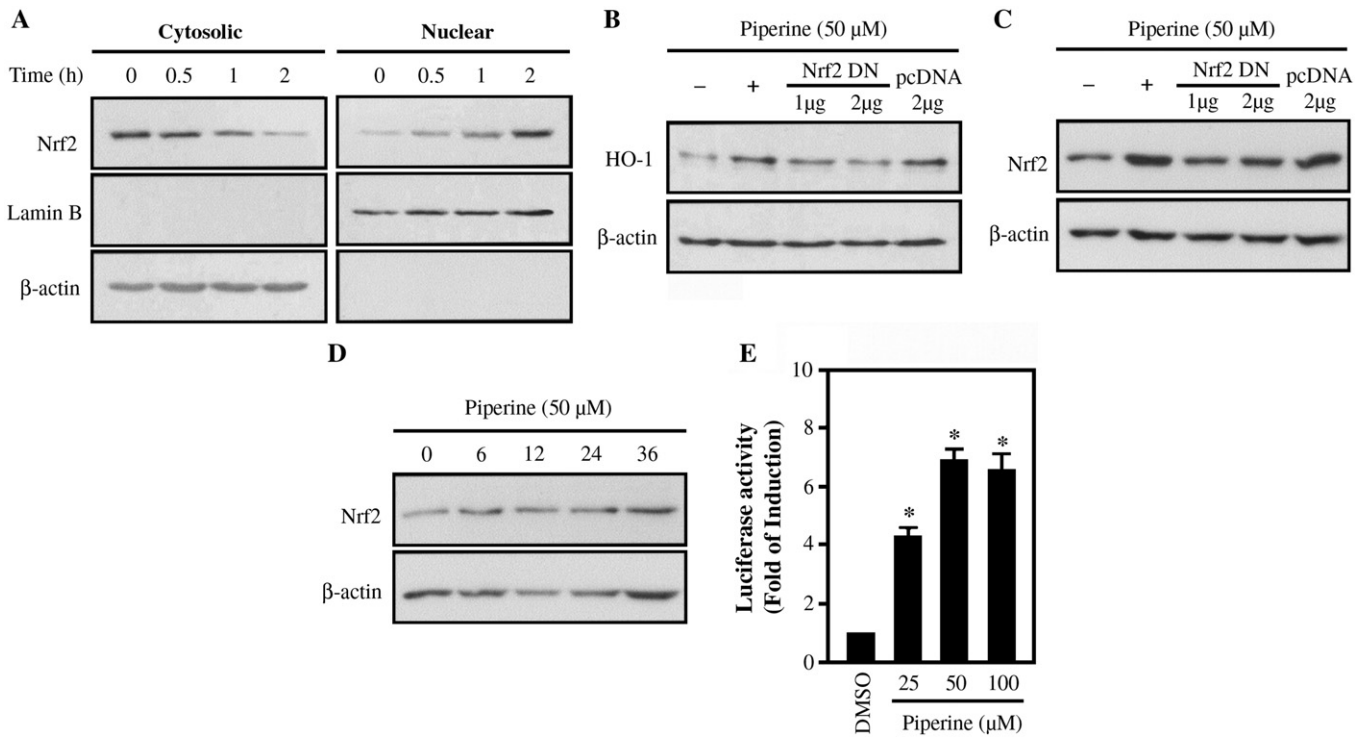

Fig. 2. Piperine increases Nrf2 nuclear translocation and ARE-luciferase activity. (A) HEI-OC1 cells were treated with 50 μM piperine for various time points indicated in the figure. Nrf2 protein in cytoplasm and nucleus were detected by Western blot. (B and C) HEI-OC1 cells were transfected with empty vector (pcDNA3) and expression vector for the Nrf2 DN. After 16 h of transfection, cells were kept in low serum medium and then stimulated with 50 μM piperine for 24 h. Cellular proteins were isolated, and Western blot analysis was performed using specific antibodies for HO-1, Nrf2 and β-actin. (D) HEI-OC1 cells were treated with 50 μM piperine for various time points indicated in the figure. Nrf2 protein in the homogenate was detected by Western blot. (E) Cells were transfected with an ARE-luciferase plasmid. After 16 h, cells were maintained in low serum medium and then stimulated with piperine for 24 h. Cells were lysed and analyzed for luciferase activities. The data represent the means±S.D. of three independent experiments. *P<.05 compared with treated DMSO.

2.7. Western blot analysis

Western blot analysis was performed as follows. Briefly, cells were harvested, washed twice with ice-cold PBS, and resuspended in 20 mM Tris-HCl buffer (pH 7.4) containing a protease inhibitor mixture (0.1 mM phenylmethylsulfonyl fluoride, 5 μg/ml aprotinin, 5 μg/ml pepstatin A and 1 μg/ml chymostatin). Protein concentration was determined with the Lowry protein assay kit. Samples were subjected to electrophoresis in a 12% SDS–polyacrylamide gel and then transferred to nitrocellulose. The membranes were incubated for 1 h in 5% (wt/vol) dried milk protein in Tris-buffered saline (TBS) containing 0.05% (vol/vol) Tween-20. The membranes were washed in TBS containing 0.05% (vol/vol) Tween-20 and incubated for 1 h in the presence of primary antibody. The membranes were washed extensively and then incubated for 1 h with antigoat IgG conjugated to horseradish peroxidase. The membranes were washed extensively again, and the protein bands were visualized using chemiluminescent reagents according to the manufacturer's instructions (Supersignal Substrate; Pierce). Autoradiographic signals were quantified by densitometric scanning (Molecular Dynamics, Sunnyvale, CA, USA). All densitometric values obtained for the HO-1 protein were normalized to values for β-actin obtained on the same blot. The HO-1 protein level in treated cells was expressed in densitometric absorbance units, normalized to control untreated samples and expressed as fold induction compared to controls.

2.8. Transient transfection and luciferase assay

A day before transfection, cells were subcultured at a density of 1×10^6 cells in 60-mm dish to maintain approximately 70–80% confluency. The cells were transiently transfected using lipofectamine with a plasmid-containing dominant-negative mutant of Nrf2 (Nrf2 DN) or ARE sequence, according to the instruction given by the manufacturer (GIBCO-BRL). After overnight transfection, cells were incubated for 1 h with PD98059, SB203580 or SP600125 before treatment with 50 μM piperine for 24 h, and protein was analyzed by Western blot. For luciferase assay, cells were washed twice with PBS and lysed with reporter lysis buffer (Promega). After 20 μl of the cell extract was mixed with 100 μl of the luciferase assay reagent at room temperature, the mixture was placed in a luminometer to measure the light produced.

2.9. HO-1 antisense oligodeoxynucleotides treatment

The oligodeoxynucleotides (ODN) for HO-1 was directed against the flanking translation initiation codon in the human HO-1 mRNA. The sequences of the anti-sense and sense HO-1 ODN were 5′-CGCCTTCATGGTGCC-3′ and 5′-GGCACCATGAAGGCG-3′, respectively. ODN were phosphorothioated on the first three bases on the 3′ end. Before use, the ODN were encapsulated in cationic liposomes (1 μg oligodeoxynucleotide/1 μg liposome) prepared using *N*-[1-(2,3-dioleoyloxy)propyl]-*N*,*N*,*N*-trimethylammonium methylsulfate (Boehringer-Mannheim, Indianapolis, IN, USA).

2.10. Statistical analysis

Differences in the data among the groups were analyzed by one-way analysis of variance, and all values were

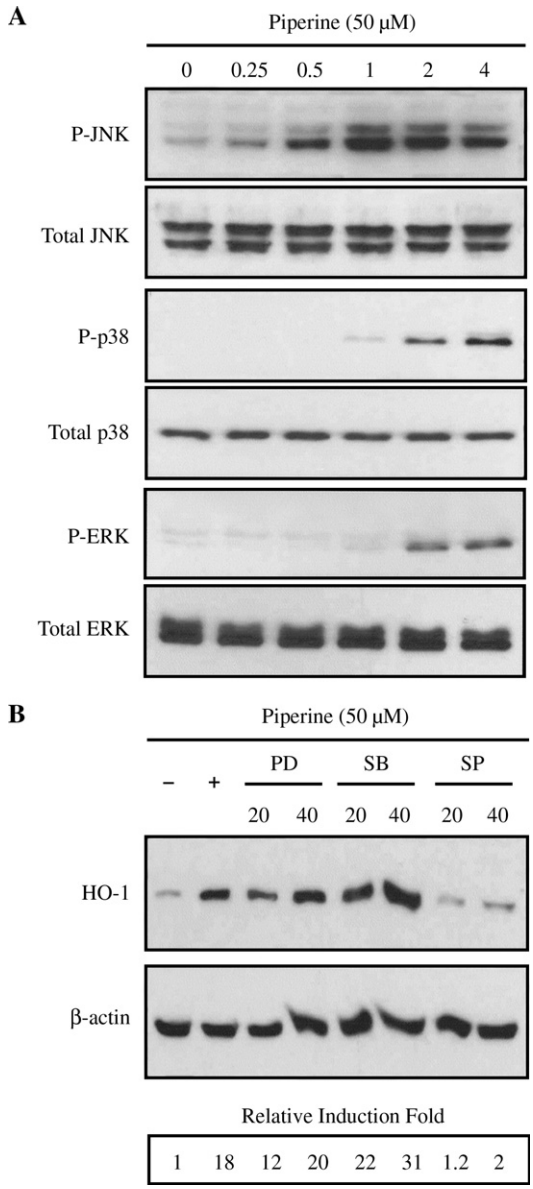

Fig. 3. Effects of piperine on phosphorylations of the MAPKs in HEI-OC1 cells. (A) The cells incubated with 50 μM piperine for the indicated times were subjected to Western blot analysis using anti-phospho JNK, ERK or p38 antibodies. As controls, the same cell lysates were subjected to Western blot analysis using total JNK, ERK or p38 antibodies. (B) Cells were pretreated with or without SB203580 (SB), PD98059 (PD) or SP600125 (SP) and then incubated in the absence or presence of 50 μM piperine for 24 h. Western blot analysis was performed using specific antibodies for HO-1 and β-actin. Relative fold induction of HO-1 protein levels was quantified as described under Materials and methods. The data show one of three independent experiments.

expressed as mean ± S.D. The differences between groups were considered to be significant at $P<.05$.

3. Results

3.1. Piperine induces HO-1 expression in HEI-OC1 cells

HEI-OC1 cells were treated with various concentrations of piperine (10–100 μM) for 24 h. The effect of piperine on HO-1 expression was shown in Fig. 1A. Piperine induced the expression of HO-1 in a dose-dependent manner (Fig. 1A). HO-1 protein levels were increased fourfold of control at 10 μM piperine and 15-fold at 50 μM after treatment with piperine (Fig. 1B). The induction was reached a peak at 50 μM piperine. Treatments of the cells with piperine resulted in a time-dependent increase in HO-1 protein expression (Fig. 1C). The expression of HO-1 was evident as early as 6 h (2.5-fold) and reached a maximum at 24 h (12-fold) after treatment with 50 μM piperine (Fig. 1D).

3.2. Piperine increases Nrf2 nuclear translocation and ARE-luciferase activity

Several genes encoding antioxidant enzymes, including HO-1, have a specific sequence contained with their enhancer region, the ARE [9,11]. We therefore examined whether the treatment of piperine induces Nrf2 translocation to the nucleus in HEI-OC1 cells. The cells were treated with 50 μM piperine for 0.5, 1 and 2 h, and the level of Nrf2 protein was determined by Western blot. As shown in Fig. 2A, piperine induced a strong accumulation of Nrf2 in the nucleus, compared with untreated cells. To determine whether Nrf2 mediates HO-1 induction by piperine, HEI-OC1 cells were transfected with an N-terminal truncated Nrf2 DN expression plasmid and then treated with piperine. Nrf2 DN substantially attenuated piperine-mediated induction of HO-1 and Nrf2 proteins in HEI-OC1 cells (Fig. 2B and C). The treatment of piperine induced the protein expression of Nrf2 in the homogenate (Fig. 2D). Its inductive effect was obvious at 6 h of the treatment then kept its level until 36 h. In addition, the reporter assay in which ARE sequence was introduced into a luciferase vector showed that piperine-treated HEI-OC1 cells increased ARE-luciferase activity (Fig. 2E).

3.3. Involvement of the JNK pathway in piperine-induced HO-1 expression

Previous studies have demonstrated that the activation of the MAPK pathways contributes to the induction of HO-1 [31,32]. Therefore, we tested whether piperine-induced HO-1 expression occurs through MAPK pathway in HEI-OC1 cells. Cells were exposed to piperine, and then Western blots were performed using anti-phospho-JNK1/2, ERK1/2 and p38 antibodies. As shown in Fig. 3A, the phosphorylated JNK1/2, ERK1/2 and p38, indicating activation, were all increased by piperine. The same blots were probed with antibodies for total JNK1/2, ERK1/2 or p38 as protein loading controls. To address the role of individual MAPK pathway in HO-1 expression by piperine, we examined the effects of SP600125, PD98059 and SB203580 on piperine-induced HO-1 expression. The piperine-mediated increase in HO-1 expression was completely blocked by SP600125, a specific inhibitor of JNK, whereas similar concentrations of PD98059 and SB203580 had no significant effect (Fig. 3B). These results indicated that kinases of the JNK pathway might be involved in the expression of HO-1 by piperine.

3.4. HO-1 expression by piperine protects HEI-OC1 cells against cisplatin-induced apoptosis

We examined the effect of piperine on cisplatin-induced apoptosis in HEI-OC1 cells. As shown in Fig 4A, incubation of the cells with cisplatin (20 μM) for 36 h resulted in a marked reduction of cell viability by 43%. However, preincubation of the cells with piperine for 12 h diminished cisplatin-induced cell death in a dose-dependent manner (Fig. 4A). To further confirm

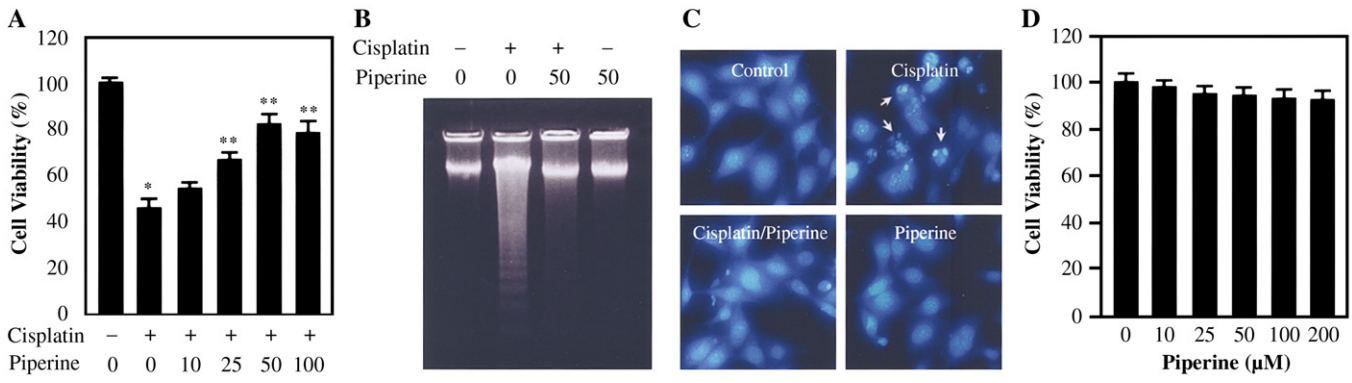

Fig. 4. The protective effect of piperine on cisplatin-induced apoptosis in HEI-OC1 cells. (A) Cells were pretreated with the indicated doses of piperine for 12 h and then incubated with 20 μM cisplatin for 36 h. (B) Cells were pretreated with or without 50 μM piperine for 12 h, added with 20 μM cisplatin for 36 h. After these treatments, genomic DNA was isolated from cells and separated on 1.5% agarose gel electrophoresis. (C) Cells were stained with DAPI and visualized under fluorescent microscope. (D) Cells were treated with indicated doses of piperine for 24 h. Cell viability was measured by MTS assay. The data represent the means ± S.D. of three independent experiments. *$P<.05$ compared with untreated control; **$P<.05$ compared with cisplatin.

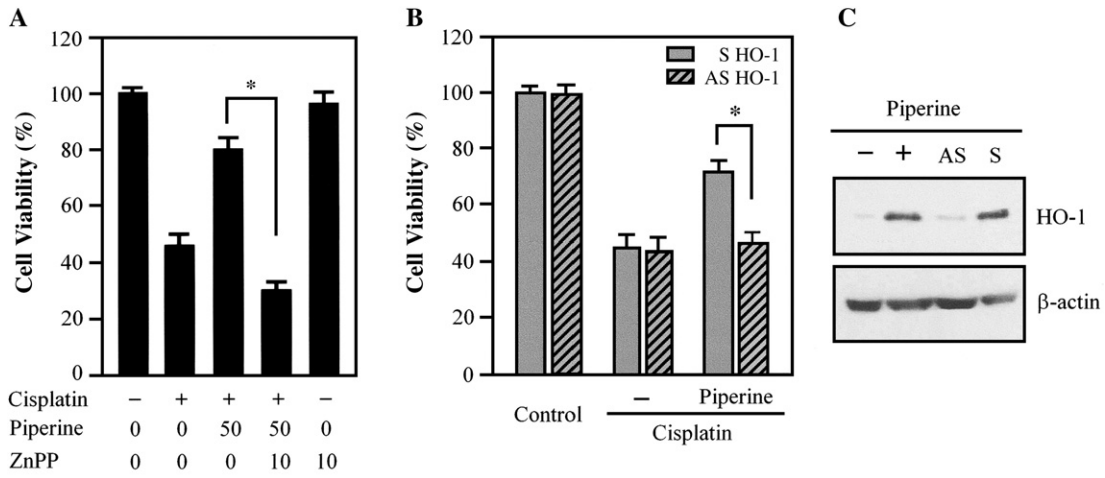

Fig. 5. HO-1 induced by piperine protects cisplatin-induced apoptosis. (A) Cells were pretreated with piperine for 12 h in the absence or presence of 10 μM ZnPP and then incubated with 20 μM cisplatin for 36 h. (B) Cells were pretreated with piperine for 12 h and then exposed to antisense or sense ODN against HO-1 gene (10 μg/ml) in the presence of cisplatin for 36 h. (C) Cells were pretreated with antisense or sense ODN against HO-1 gene (10 μg/ml) and then incubated with 50 μM piperine for 24 h. Cell viability was measured by MTS assay. The data represent the means±S.D. of three independent experiments. *$P<.05$. Western blot analysis was performed using specific antibodies for HO-1 and β-actin.

the protective effect of piperine on cisplatin-induced apoptosis, we examined biochemical and morphological changes accompanied in the cells treated with cisplatin in the absence or presence of piperine. As shown in Fig. 4B, the cells treated with cisplatin alone showed apparent DNA laddering, and the DNA laddering was blocked by pretreated piperine. DAPI staining revealed that piperine also abolished the apoptotic features of nuclei in cisplatin-treated cells (Fig. 4C). Meanwhile, piperine in the range of 10–200 μM had no significant effect on cell viability (Fig. 4D). We also examined whether HO-1 expression was responsible for the protection afforded by piperine against the apoptotic effect of cisplatin. The involvement of HO-1 in the protective effect of piperine was confirmed using an inhibitor of HO activity, ZnPP IX and antisense ODN against HO-1 gene. ZnPP IX blocked the protective effect of piperine on cisplatin-induced apoptosis (Fig. 5A). This protection was also abrogated by the antisense ODN against HO-1 gene (Fig. 5B). The antisense ODN inhibited HO-1 expression efficiently, whereas HO-1 sense ODN showed no inhibition (Fig. 5C). These results demonstrated that the observed protective effect of piperine on cisplatin-induced apoptosis was due to HO-1 expression.

4. Discussion

In this study, we tried to elucidate the cytoprotective effect of piperine against cisplatin-induced apoptosis in HEI-OC1 cells and the molecular mechanism of its cytoprotective action with focus on up-regulation of HO-1. HO-1 can be up-regualted by the constituents of several plants, including curcumin [33], catalposide [34] and resveratrol [35]. We have found that piperine induced HO-1 expression strongly in HEI-OC1 cells (Fig. 1). However, Liu et al. [36,37] has reported that piperine (CYP 2B1 inhibitor) prevented the induction of HO-1 induced by puromycin aminonucleoside in rat glomeruli and glomerular epithelial cells. When HEI-OC1 cells were exposed with piperine for 24 h, the level of induction at 10 μM piperine was approximately fourfold of control and 12-fold at 50 μM. However, piperine in the range of 10–200 μM had no significant effect on the cell viability under our experimental conditions (Fig. 4D). The plasma level of piperine in rat reached about 10 μM with 20 mg/kg of oral treatment [38]. Therefore, piperine seems to induce the expression of HO-1 efficiently in vivo system.

The transcription factor Nrf2 plays an essential role in the ARE-mediated expression of phase II-detoxifying detoxifying and antioxidant enzymes, and in the activation of other stress-inducible genes in response to oxidative stress [39]. Inducible proteins that require Nrf2 for their expression include GSH *S*-transferase, quinone reductase and HO-1 [11,40,41]. The current model of Nrf2 activation by electrophilies proposes that Nrf2 translocates to the nucleus following dissociation from the cytoplasmic factor Keap1 [42]. Although the expression of Nrf2 was induced significantly by piperine (Fig. 2D), we found that piperine translocated most of Nrf2 into the nucleus of HEI-OC1 cells, and the level of β-actin from the cytosol was independent on piperine treatment (Fig. 2A). Piperine supplementation increased the activities of detoxifying enzymes, and this may be attributed to their capacity for increasing of the antioxidant status [43]. Recently, piperine has been proved to inhibit the phase I enzymes, and there was a rise in GSH-metabolizing enzymes, which indicated an anticancer effect [44]. Previous results and our data (Fig. 2C) demonstrated that the translocation of Nrf2 to

the nucleus following piperine treatment was associated with increase in its ARE transcriptional activity [11,40]. Interestingly, Nrf2 DNs can block HO-1 protein expression by piperine (Fig. 2B). Therefore, our results suggest that Nrf2 may play a key role in piperine-induced HO-1 expression.

The roles of MAPKs have previously been demonstrated in various cell culture systems, and contradictory results on the regulatory role of different MAPK pathways for HO-1 gene expression were observed. For instance, in agreement with our data, the GSH depletory pherone and arsenite promote a JNK-dependent induction of HO-1 expression [45,46]. On the other hand, arsenite induces HO-1 expression via the ERK and p38 pathways in leghorn male hepatoma (LMH) chicken hepatoma cells [47], but inhibition of p38 has no effect on cadmium- and hemin-dependent HO-1 expression in HeLa cells [48]. One possible interpretation for these diverging observations may stem from the diverse assortment and intensity of the signaling pathways activated by different inducers in different cell types. Our results show that, although the JNK, ERK and p38 pathways are activated by piperine, they do not participate equally in the induction of HO-1 in HEI-OC1 cells. Piperine-induced HO-1 expression was directly related with JNK pathway because the inhibitor, SP600125, blocked the expression completely (Fig. 3). Our result was similar with those shown in the reported of Xu et al. and Keum et al. [49,50]. In their results, ARE-mediated phase II drug metabolism gene expressions was done via the JNK1- and Nrf2-dependent pathways [49,50].

HO-1 has been recently recognized as an important cellular defense mechanism against various stresses [14,17]. We found that the treatment of the cells with piperine resulted in high resistance to cisplatin-induced cell death (Fig. 4). The involvement of HO-1 in the cytoprotective action of piperine was examined using an inhibitor of HO, ZnPP IX and antisense ODN against HO-1 gene. ZnPP IX and HO-1 antisense ODN abrogated the protective effect of piperine on cisplatin-induced cell death (Fig. 5). So et al. and Kim et al. [51,52] demonstrated that cisplatin induced apoptotic death of auditory cells through ROS generation, and HO-1 attenuated the cisplatin-induced apoptosis of auditory cells through down-regulation of ROS generation. Therefore, these results demonstrate that the induction of HO-1 expression by piperine may serve as one of the important mechanisms for the protective effect of piperine on cisplatin-induced apoptosis.

In conclusion, the present results suggest that piperine induces HO-1 expression via Nrf2 and JNK pathway, and HO-1 expression by piperine could contribute to cellular defense mechanism against cisplatin-induced apoptosis. In this respect, the search for potent inducers of HO-1 from nontoxic food materials may be attributed to decrease the ototoxic side effect of cisplatin in human being. Further studies are extensively ongoing to define the in vivo effect of piperine to systemically administration of cisplatin.

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