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Thiol antioxidant and thiol-reducing agents attenuate 15-deoxy-$\Delta^{12,14}$-prostaglandin J_2-induced heme oxygenase-1 expression

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Abstract

Heme oxygenase-1 (HO-1) is induced as a beneficial and adaptive response in cells and tissues exposed to oxidative stress. Herein we examined how various eicosanoids affect the induction of HO-1, and the possible mechanism underlying 15-deoxy-$\Delta^{12,14}$-prostaglandin J_2 (15d-PGJ_2)-induced HO-1 expression. PGH_2, PGD_2 and its metabolites of the PGJ_2 series, and PGA_1 markedly induced the protein expression of HO-1. Arachidonic acid (AA), docosahexaenoic acid (DHA), PGE_2, $PGF_{2\alpha}$, and thromboxane B_2 (TXB_2) were shown to have no effect on the induction of HO-1. 15d-PGJ_2 was the most potent activator achieving significance at 5 μM. Although 15d-PGJ_2 significantly activated the MAPKs of JNK and ERK, the activation of JNK and ERK did not contribute to the induction of HO-1 as determined using transfection of dominant-negative plasmids and MAPKs inhibitors. Additional experiment indicated that 15d-PGJ_2 induced HO-1 expression through peroxisome proliferator-activated receptor (PPAR)-independent pathway. 15d-PGJ_2 significantly decreased the intracellular level of reduced glutathione; and the thiol antioxidant, N-acetyl-L-cysteine (NAC), and the thiol-reducing agent, dithiothreitol (DTT), inhibited the induction of HO-1 by 15d-PGJ_2. Finally, NAC and DTT exhibited significant inhibition of HO-1 mRNA and HO-1 promoter reporter activity induced by 15d-PGJ_2. These results suggest that thiol antioxidant and reducing agents attenuate the expression

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of HO-1 induced by 15d-PGJ_2, and that the cellular thiol-disulfide redox status may be linked to HO-1 activation.
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Introduction

Heme oxygenase (HO, EC 1.14.99.3) is a microsomal enzyme that catalyzes the initial and rate-limiting reaction in heme catabolism, which converts heme to biliverdin and releases equimolar amounts of carbon monoxide (CO) and free iron (Maines, 1997; Ponka, 1999). Three isoforms transcribed from separate genes have been characterized. HO-1 is an inducible form found in large guantities in the liver and spleen, and HO-2 is a constitutively active form found mainly in the brain and testes (McCoubrey and Maines, 1994). Another HO isoform, HO-3, has only recently been described with properties similar to those of HO-2 (McCoubrey et al., 1997). Poss and Tonegawa (1997a,b) showed that HO-1-deficient mice are hypersensitive to cytotoxicity when given additional hydrogen peroxide, indicating that HO-1 plays an important role in the cytoprotective defense response against oxidative stress (Choi and Alam, 1996). HO-1 gene expression is upregulated by both inflammatory mediators and anti-inflammatory cytokines, such as LPS, IL-1β, and IL-10 (Yet et al., 1997; Lee and Chau, 2002). However, much evidence has suggested that upregulation of HO-1 contributes to the anti-inflammatory action of cells and tissues. Minamino et al. (2001) demonstrated that HO-1 transgenic mice are protected from pulmonary inflammation and vessel wall hypertrophy induced by hypoxia. CO, a product of HO, inhibits the expression of LPS-induced proinflammatory cytokines (Muller et al., 1987), and the anti-inflammatory interleukin-10 mediates the induction of HO-1 (Lee and Chau, 2002).

Arachidonic acid is first converted to PGH_2 by cyclooxygenase and subsequently converted to one of several related products, including PGD_2, PGE_2, $PGF_{2\alpha}$, PGI_2, and thromboxane A_2, through the action of specific PG synthases. 15d-PGJ_2 is derived from PGD_2 and involves the sequential conversion of PGD_2, PGI_2, Δ^{12}-PGJ_2, and 15d-PGJ_2 (Fukushima, 1992). Several proteins have been identified which are induced by cyclopentenone PGs, such as heat shock proteins (Santoro et al., 1989), γ-glutamylcysteine synthetase (Ohno et al., 1990), collagen (Tasaki et al., 1991), gadd 45 (Ohtani-Fujita et al., 1998), and heme oxygenase (Koizumi et al., 1995). Although their intracellular receptor has not been described, 15d-PGJ_2 was shown to be a high-affinity ligand for peroxisome proliferator-activated receptor γ (PPARγ) (Kliewer et al., 1995). Recently, PPARγ has been considered to have anti-inflammatory actions through activation by arachidonic acid metabolites, such as 15d-PGJ_2 (Jiang et al., 1998; Ricote et al., 1998, 1999). 15d-PGJ_2 represses several genes related to inflammation, including the inducible nitric oxide synthase (iNOS) and tumor necrosis factor α (TNF_α) genes in activated macrophages (Ricote et al., 1998). However, the repression is partly dependent on PPARγ expression (Chawla et al., 2001), and receptor-independent biological actions of 15d-PGJ_2 and other cyclopentenone PGs have been proposed (Rossi et al., 2000; Straus et al., 2000). Other studies have indicated that some cyclopentonene PGs induce the synthesis of HO-1 in cells of the mice and rat, but the mechanism underlying 15d-PGJ_2-induced HO-1 protein synthesis in human cells has been largely unexplored. We report herein that PGD_2, PGA_1, and the J series of PGs markedly induced HO-1 protein synthesis in human HepG2 hepatoma cells, and that the induction of HO-1 by 15d-PGJ_2 may be mediated by modulation of the cellular thiol-disulfide redox status.

Materials and methods

Materials and cell culture

DHA, PGA_1, PGJ_2, 16,16-dimethyl PGD_2, and BRL49653 were purchased from Cayman Chemical (Ann Arbor, MI), while arachidonic acid, PGD_2, PGE_2, $PGF_{2\alpha}$, PGH_2, TXB_2, 15d-PGJ_2, PD98059, SB203580, 2′,5′-dideoxyadenosine, WY-14643, ciglitazone, and indomethacin were purchased from Biomol (Plymouth Meeting, PA). Vitamin C, vitamin E, allopurinol, NAC, wortmannin, and DTT were purchased from Sigma Chemical (St. Louis, MO). The human HepG2 hepatoma cell lines were cultured in MEM containing 10% heat-inactivated fetal bovine serum and 1 mM sodium pyruvate (Invitrogen, Carlsbad, CA).

Western blot analysis

Equal amounts of total cellular protein (50 μg) were resolved by SDS-polyacrylamide gel electrophoresis (PAGE), transferred onto an Immobilon-P membrane (Millipore, Bedford, MA), and blotted with anti-HO-1, anti-HO-2 (BD Biosciences, Franklin Lakes, NJ), anti-JNK1, anti-ERK1 (Santa Cruz Biotechnology, Santa Cruz, CA), or anti-PPARγ (Affinity BioReagents, Inc., Golden, CO) antiserum as described previously (Liang et al., 1999a).

Reverse-transcription polymerase chain reaction (RT-PCR)

Total RNA was isolated from both the control and tested cultured cells, and RT-PCR was performed as previously described (Liang et al., 1999b). Two sets of primers were used to amplify the mRNA of HO-1 and HO-2: for HO-1, they were 5′-TGATAGAAGAGGCCAAGA-3′ and 5′-TTTCCAGAGAGAGGGACA-3′; and for HO-2, they were 5′-TGGAGCGCAACAAGGACCAT-3′ and 5′-CCGGTAGAGCTGCTTGAACT-3′.

Kinase assay

Equal amounts of total cellular protein (200 μg) were immunoprecipitated with JNK1-, p38-, or ERK1-specific antibodies (Santa Cruz Biotechnology) and protein A/G-PLUS agarose for 12 h at 4°C. The kinase assay was performed in kinase buffer with Gst-c-Jun fusion protein (for JNK), Gst-ATF2 (for p38), or myelin basic protein (for ERK) as substrates as previously described (Liang et al., 1999b).

Plasmids and transition transfection

The dominant-negative (DN)-ERK1 expression plasmid was generously provided by Prof. Peter E. Shaw, The University of Nottingham (Robbins et al., 1993), and the DN-JNK expression plasmid was constructed from human JNK1 with a double-point mutation of Thr183 to Ala and Tyr185 to Phe (Lin et al., 2002). The PPARγ expression plasmid was generously provided by Professor Christopher K. Glass (University of California-San Diego).

The pGL2/hHO3.2-Luc reporter plasmid, containing a 3292-bp fragment, -3106 to +186 relative to the transcription start site of the human HO-1 gene, was amplified from the human BAC clone CTA-

286B10 (Kim et al., 1996) using the primers 5′-AGAGAACAGTTAGAAAAGAAAG-3′ and 5′-TACGGGCACAGGCAGGATCAGAA-3′. The PCR products were inserted into the pCR2.1-TOPO cloning vector (Invitrogen), and cut with Kpn I/Xba I such that the resulting PCR products contained the Kpn I/Xba I sites, and was ligated in-frame into the unique Kpn I/Nhe I sites present within the pGL2 plasmid (Promega, Madison, WI). Therefore, we obtained a pGL2/hHO3.2-Luc reporter construct containing about a 3.2-kb region of the human HO-1 promoter driving luciferase gene expression. Sequence identities were confirmed using an ABI PRISM 377 DNA analysis system (Perkin-Elmer Corp., Taipei, Taiwan).

HepG2 cells were seeded in 60-mm dishes and either mock-treated or transfected with DN-JNK, DN-ERK, or PPARγ plasmid using LipofectAMINE™ 2000 (Gibco) for 48 h (Liang et al., 2001). After transfection, cells were then treated with 15d-PGJ_2, and the cell lysate was collected for the kinase assay or Western blot analysis. For the reporter plasmid assay, HepG2 cells were seeded in 6-well plates for 24 h. Then cells were transfected with the pGL2e-hHO3.2 reporter plasmid, and phRL-TK (Promega) as an internal control using LipofectAMINE 2000™ (Invitrogen). After 12 h of transfection, the medium was replaced with complete medium and incubated for another 24 h. Transfected cells were then treated with drugs for the luciferase activity assay. Each well was washed twice with cold PBS and harvested in 100 μl of lysis buffer (0.5 M Hepes, pH 7.8, 0.5% Triton N-101, 1 mM $CaCl_2$, and 1 mM $MgCl_2$). Then 75 μl of cell lysate was placed into a 96-well white plate, and the luciferase activity was determined using a FireLite™ luciferase reporter gene assay kit (Packard Instrument Co., Meriden, CT). Luciferase activity was measured on a TopCount microplate scintillation and luminescence counter (Packard 9912V1) in the single-photon counting mode for 3 s/well. Luciferase activities of reporter plasmids were normalized to luciferase activities of the internal control plasmid.

Glutathione assay

Cells were washed twice with PBS, extracted with a 25% (w/v) metaphosphoric acid solution containing 5 mM EDTA, and centrifuged at 12,000 rpm for 10 min to precipitate the proteins. The supernatant was incubated with 5 mM EDTA and 100 μg *O*-phthalaldehyde, and then the fluorescence intensity was determined by excitation at 350 nm and emission at 420 nm (Kim et al., 2001).

Statistical analysis

Data are presented as the mean ± S.E. for the indicated number of independently performed experiments. Statistical analysis was done using one-way Student's *t* test.

Results

Prostaglandins as potential HO-1 activators

To identify the endogenous activator of HO-1 expression, we screened a large number of lipophilic chemicals, including DHA, arachidonic acid, and its metabolites, and found that HO-1 expression was potently induced by some of the PG derivatives. As shown in Fig. 1A, the protein expression of HO-1 in HepG2 cells was significantly induced by PGA_1, PGH_2 metabolites including PGH_2 itself, PGD_2, PGJ_2,

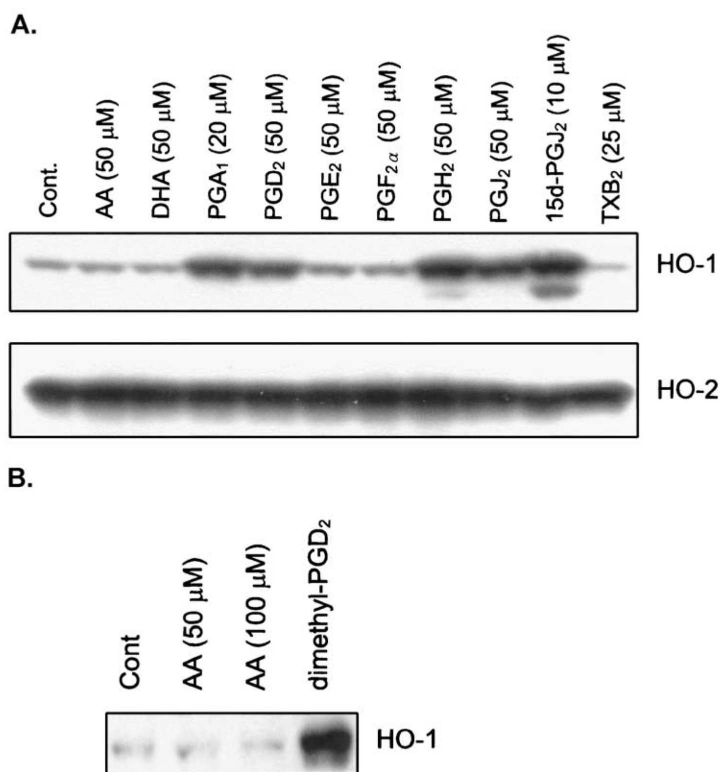


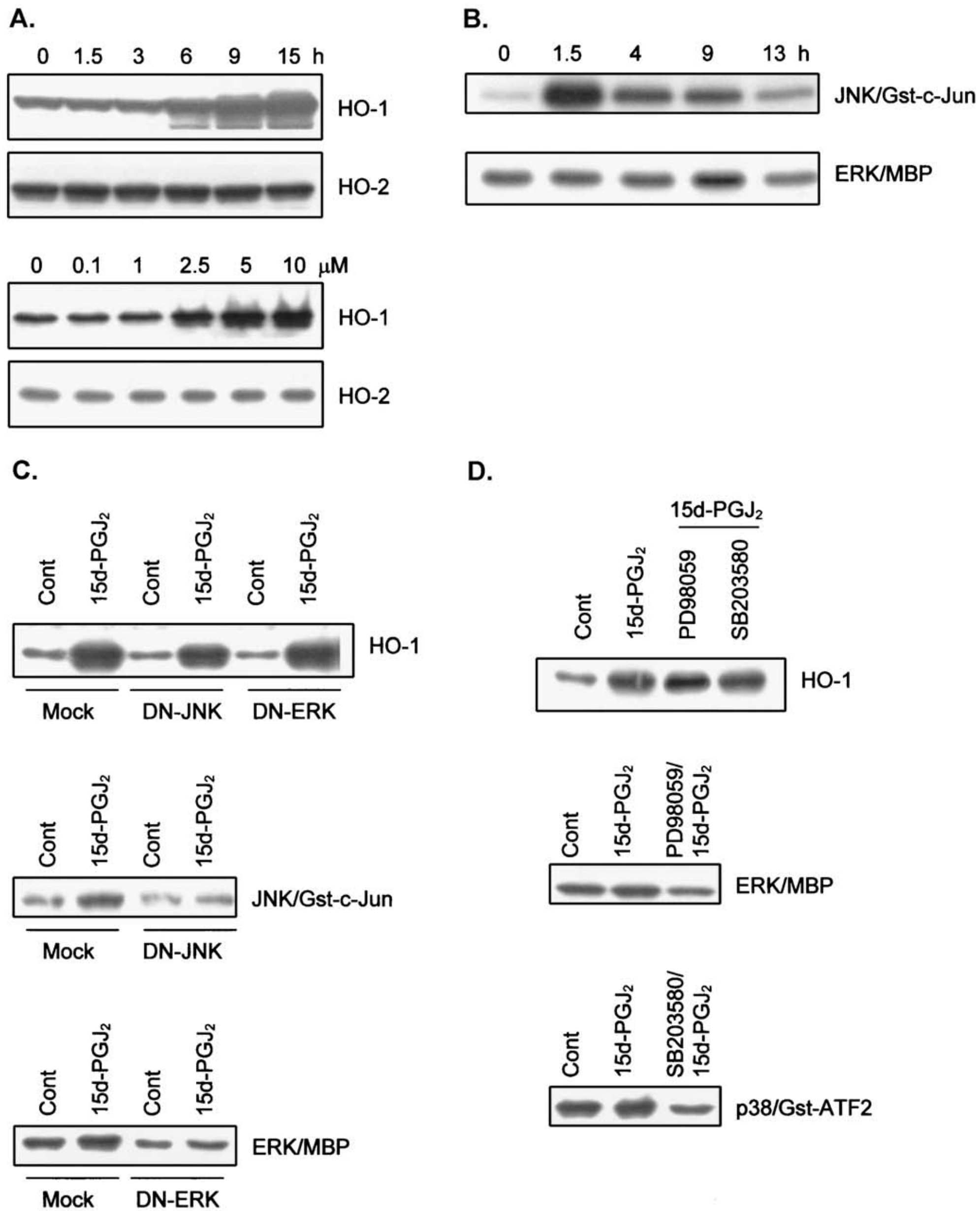
Fig. 1. Effect of arachidonic acid, docosahexaenoic acid, and various eicosanoids on the protein expression of HO-1 in HepG2 cells. (A) Cells were treated with various compounds or (B) with arachidonic acid and 50 μM 16,16-dimethyl-PGD_2 for 15 h; HO-1 and HO-2 proteins were detected by Western blotting. 15d-PGJ_2, 15-deoxy-$\Delta^{12,14}$-PGJ_2; AA, arachidonic acid; TXB_2, thromboxane B_2.

and 15d-PGJ_2. The induction potency was 15d-PGJ_2 > PGA_1 PGJ_2 ≒ PGD_2 ≒ PGH_2, with 15d-PGJ_2 being the most potent activator. Arachidonic acid, DHA, PGE_2, $PGF_{2\alpha}$, and TXB_2 had no significant effects on the induction of HO-1 expression. We examined the induction of HO-1 by PGD_2 through its metabolites, such as PGJ_2 and 15d-PGJ_2, in HepG2 cells. We used 16,16-dimethyl PGD_2, a metabolically stable synthetic analog of PGD_2, to examine the induction of HO-1. As shown in Fig. 1B, 16,16-dimethyl PGD_2 exhibited significant induction of HO-1 in HepG2 cells. The PG precursor, arachidonic acid, failed to induce HO-1 expression up to a concentration of 100 μM. These results suggest that PGD_2 is not dependent on its metabolites, such as the J series of PGs, to induce the expression of HO-1, and that HepG2 cells may lack the conversion enzymes which catalyze the metabolites of arachidonic acid to PGH_2 and PGD_2.

Induction of HO-1 by 15d-PGJ_2 does not mediate the MAPK and PPAR pathways

It has been demonstrated that activation of the MAPKs pathway contributes to the induction of HO-1 by cadmium and arsenite (Elbirt et al., 1998; Alam et al., 2000). To examine whether 15d-PGJ_2 induces

the expression of HO-1 through the MAPKs pathway, we performed the following experiments. 15d-PGJ_2 induced the expression of HO-1 in time- and dose-dependent manners (Fig. 2A). In the time-dependent experiment, we found that 15d-PGJ_2 was sufficient to activate JNK after 1.5 h. On the other hand, 15d-PGJ_2 slightly stimulated ERK activity at 6 h after drug treatment (Fig. 2B). To examine whether MAPKs, including ERK, JNK, and p38, could influence HO-1 expression, we transfected cells



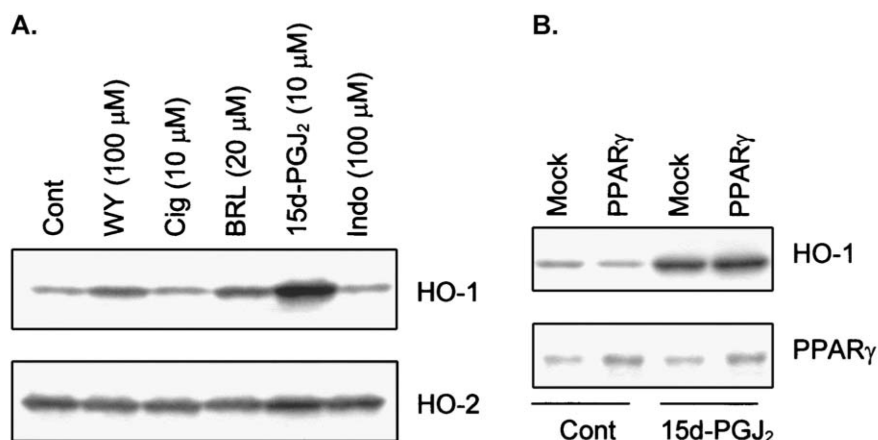


Fig. 3. Effect of PPAR agonists and overexpression of PPARγ on the induction of HO-1 expression. (A). Cells were treated with various PPAR agonists for 15 h, and the HO-1 and HO-2 protein expression were detected by Western blotting. WY, WY-14643; Cig, ciglitazone; BRL, BRL49653; Indo, indomethacin. (B). Transfected cells were treated with or without 10 μM 15d-PGJ_2 for 15 h, and the HO-1 and PPARγ protein expression were detected by Western blotting.

with dominant-negative (DN) mutants of the JNK or ERK expression plasmid. As shown in Fig. 2C (top), HO-1 levels were not significantly diminished by overexpression of the DN-JNK or DN-ERK. To verify that overexpression of dominant-negative JNK or ERK efficiently decreased endogenous JNK or ERK activities, we determined the total JNK and ERK activities by immunocomplex kinase assay. As shown in Fig. 2C (middle and bottom), overexpression of the DN-JNK or DN-ERK significantly decreased the JNK or ERK activity in the cells with or without 15d-PGJ_2. In addition, the MEK inhibitor, PD98059, and the p38 kinase inhibitor, SB203580, also exhibited an inefficient inhibition of HO-1 induced by 15d-PGJ_2. The immunocomplex kinase assay showed that ERK and p38 kinases activities were significantly inhibited by their inhibitors PD98059 and SB203580, respectively (Fig. 2D, middle and bottom). These data suggest that the induction of HO-1 by 15d-PGJ_2 might not be mediated by activation of the MAPKs pathway, and that there are clear differences between the activation pathways of 15d-PGJ_2 and the other treatments tested (cadmium and aresenite).

Fig. 2. Effect of MAPK pathways on the expression of HO-1 induced by 15d-PGJ_2 in HepG2 cells. (A) Cells were treated with 10 μM 15d-PGJ_2 for various times as indicated (top), or treated with various concentrations of 15d-PGJ_2 for 15 h (bottom). The total cell lysates was used to detect HO-1 and HO-2 proteins by Western blot. (B) Cells were treated with 10 μM 15d-PGJ_2 for various times as indicated, the total cell lysates was preformed the JNK (Gst-c-Jun as the substrate) and ERK (MBP as the substrate) kinases activity assays as described in ‘‘Materials and methods’’. (C) Cells were transfected with mock, dominant-negative (DN) JNK, or DN-ERK expression plasmid, and treated with 10 μM 15d-PGJ_2 for 15 h. Total cells lysates was used to detect the HO-1 protein was detected by Western blotting (top). Transfected cells were treated with 10 μM 15d-PGJ_2 for 1.5 h (middle) or 9 h (bottom), and determined the JNK (middle) and ERK (bottom) kinases activity assays as described in ‘‘Materials and methods’’. (D) Cells were treated with 50 μM PD98059 or 10 μM SB203580 and 10 μM 15d-PGJ_2 for 15 h, and the HO-1 protein was detected by Western blotting (top). Cells were treated with 50 μM PD98059 or 10 μM SB203580 and 10 μM 15d-PGJ_2 for 9 h, and determined the ERK (middle) and p38 (Gst-ATF2 as the substrate, bottom) kinase activity assays as described in ‘‘Materials and methods’’.

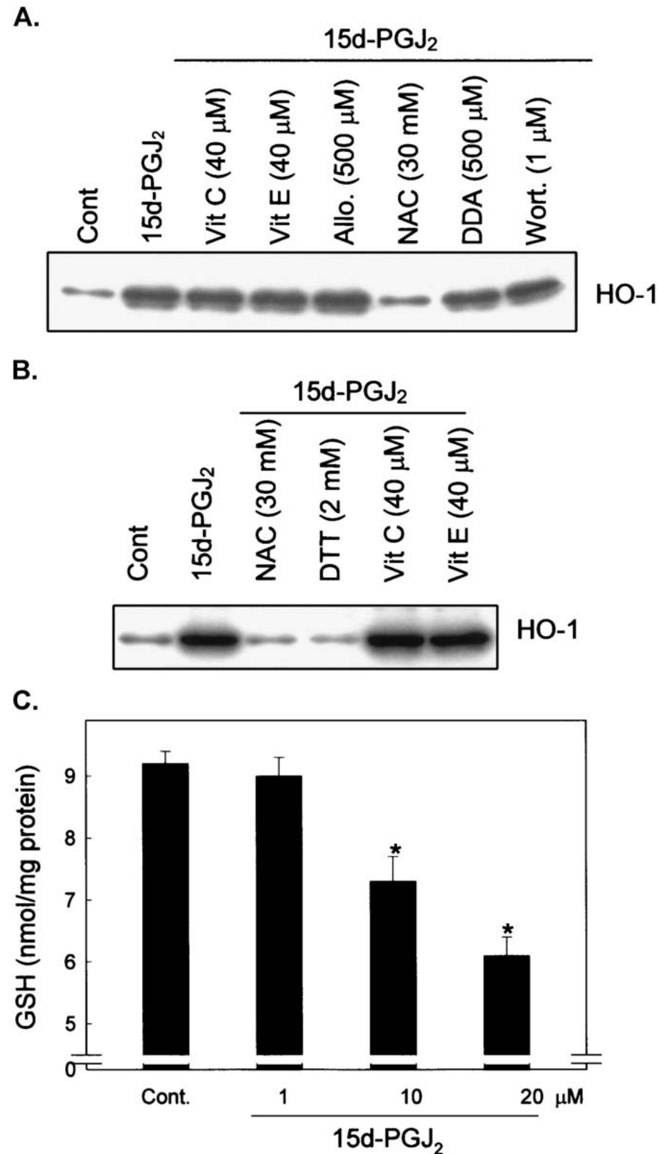


Fig. 4. Effect of various inhibitors on the protein expression of HO-1 induced by 15d-PGJ_2 in HepG2 cells. (A) Cells were pretreated with various drugs or (B) with NAC or DTT for 2 h, and 10 μM 15d-PGJ_2 was added for another 15 h. The HO-1 protein was detected by Western blotting NAC, N-acetyl-L-cysteine; DTT, dithiothreitol; Vit C, vitamin C; Vit E, vitamin E; Allo, allopurinol; NAC, N-acetyl-L-cysteine; DDA, 2′,5′-dideoxyadenosine; Wort, wortmannin. (C) Effect of 15d-PGJ_2 on glutathione level in HepG2 cells. Cells were treated with different concentrations of 15d-PGJ_2 for 3 h and assayed for GSH as described in ‘‘Materials and methods’’. The values were expressed as the mean $\pm$ S.E. of triplicate tests. *, $p < 0.05$ vs. the control.

15d-PGJ_2 as a potent agonist of $PPAR_\gamma$. To examine whether 15d-PGJ_2 induced HO-1 expression through activation of PPAR, cells were also treated with the other PPAR agonists, including WY-14643, ciglitazone, BRL49653, and indomethacin. As shown in the Fig. 3A, 15d-PGJ_2 strongly increased the HO-1 expression, and WY-14643 and BRL49653 slightly induced the HO-1 expression. On the contrary, ciglitazone and indomethacin had no effect on the HO-1 expression. To further examine the possibility of $PPAR_\gamma$ involved in the induction of HO-1, cells were transfected with $PPAR_\gamma$ expression plasmid and treated with 15d-PGJ_2. Western blot showed that $PPAR_\gamma$ was increase about two to three-fold in the cells with $PPAR_\gamma$ overexpression plasmid (Fig. 3B, lanes 2 and 4). However, no additional

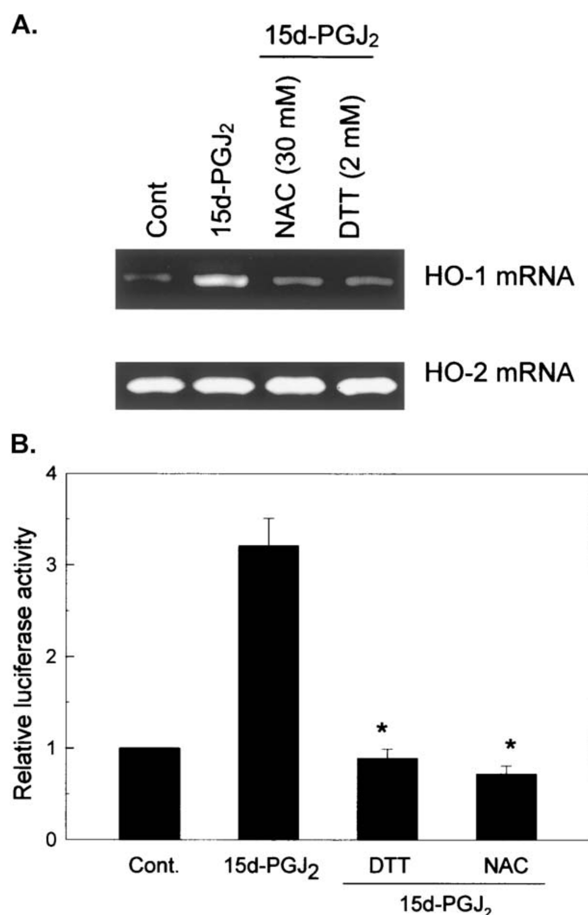


Fig. 5. Effect of NAC and DTT on mRNA levels and promoter activity of HO-1 induced by 15d-PGJ_2 in HepG2 cells. (A) Cells were pretreated with NAC or DTT for 2 h, 10 μM 15d-PGJ_2 was added for another 4 h, and HO-1 and HO-2 mRNA were detected by RT-PCR. (B) Cells were transfected with the pGL2/hHO3.2-Luc reporter plasmid and the phRL-TK internal control plasmid for 48 h, and 10 μM 15d-PGJ_2 was added for another 9 h. Luciferase activity was measured as described in "Materials and methods". The values are expressed as the mean ± S.E. of triplicate tests. *, $p < 0.05$ vs. 15d-PGJ_2 treatment.

induction of HO-1 was found in the $PPAR_\gamma$ overexpression cells with 15d-PGJ_2. These results suggested that induction of HO-1 expression might mediate PPAR-independent pathway in HepG2 cells with 15d-PGJ_2.

Thiol antioxidant and thiol-reducing agent attenuate 15d-PGJ_2-induced HO-1 expression

There is evidence which indicates that cyclopentenone PGs, such as the J series of PGs, are potential inducers of intracellular oxidative stress in human neuroblastoma cells (Kondo et al., 2001). To examine whether 15d-PGJ_2 induced HO-1 expression through acting as a pro-oxidant or as a signal transducer, we treated cells with the antioxidants, vitamins C and E, with the xanthine oxidase inhibitor, allopurinol, glutathione precursor-N-acetyl-L-cysteine (NAC), and adenylate cyclase inhibitor-2′,5′-dideoxyadenosine, and with the phosphatidylinositol 3-kinase (PI_3K) inhibitor, wortmannin, with 15d-PGJ_2. As shown in Fig. 4A, 15d-PGJ_2-induced HO-1 expression was significantly inhibited by NAC, whereas it was not significantly inhibited by the other antioxidants or by the inhibitors of signal transduction, such as vitamins C and E, allopurinol, 2′,5′-dideoxyadenosine, and wortmannin. In addition, dithiothreitol (DTT), a reducing thiol agent, also exhibited inhibitory effects on HO-1 expression induced by 15d-PGJ_2 (Fig. 4B). Also to investigate whether 15d-PGJ_2 could influence the cellular thiol-disulfide redox status, we measured intracellular glutathione (GSH) levels. As shown in Fig. 4C, GSH levels were significantly diminished by treatment with 10 and 20 μM 15d-PGJ_2. To examine whether DTT and NAC inhibited protein expression through transcriptional regulation, cells were pretreated with DTT or NAC for 2 h, 15d-PGJ_2 was added for 4 h, and the mRNA levels of HO-1 were detected by RT-PCR. As shown in Fig. 5A, DTT and NAC significantly inhibited the mRNA levels of HO-1 induced by 15d-PGJ_2. In addition, DTT and NAC also significantly inhibited the promoter activity of HO-1 induced by 15d-PGJ_2 (Fig. 5B).

Discussion

PGD_2 is known to be sequentially metabolized to PGJ_2, Δ^{12}-PGJ_2, and 15d-PGJ_2. A comparison of the PG biosynthetic pathway with HO-1 inducer profiles reveals that induction of HO-1 might be mediated mainly by the metabolites of PGD_2, the most active of which is the terminal metabolite, 15d-PGJ_2 (Fig. 1A). However, 16,16-dimethyl prostaglandin D_2 also markedly induced the expression of HO-1 (Fig. 1B). These results indicate that induction of HO-1 pathway potency is not dependent on the catabolism of PGD_2.

The 5′-flanking region of the human HO-1 gene contains a number of DNA sequences of potential regulatory elements, such as AP-1 site, AP-2 like site, NF-κB site, STATx site, c-Rel site, HNF-1 site, HNF-4 site, HSE site, and GATA-X sites (Takahashi et al., 1999; Lavrovsky et al., 1994). In rat HO-1 promoter, the consensus E-box motif, CANNTG, seem to be essential for Δ^{12}-PGJ_2-induced the expression of rat HO-1 (Koizumi et al., 1995). However, we found several E-box like sequences in the 5′-flanking (−3106 to +186) of the human HO-1 gene. Further experiments are needed to determine which E-box like sequences is essential for 15d-PGJ_2-induced the expression of human HO-1 gene. Recently, another report indicated that the mouse HO-1 promoter contains a stress-response element (StRE), which is required for induction of HO-1 gene by 15d-PGJ_2 (Gong et al., 2002). We performed the computer-assisted identification of putative StRE (set at a cutoff score of >90) by MOTIF

(http://motif.genome.ad.jp) and TRANSFAC (http://transfac.gbf.de/TRANSFAC), and not found putative StRE in the 5′-flanking region (− 3106 to + 186) of human HO-1 gene. These results suggest that StRE sequences might not be important to mediate the induction of HO-1 gene by 15d-PGJ_2 in human.

In this study, we found that 15d-PGJ_2 activated the MAP kinases, ERK and JNK, in Hep3B cells, while dominant-negative components could not block the induction of HO-1 by 15d-PGJ_2 (Fig. 2B, C). Other studies have demonstrated that activation of MAP kinase mediates the induction of HO-1 by arsenite or cadmium. Elbirt et al. (1998) found that the MAP kinases, ERK and p38, are involved in the induction of HO-1 by arsenite. Another study indicated that the MAP kinase, p38, is involved in the induction of HO-1 by cadmium (Alam et al., 2000). The results from this study showed that there were clear differences between the activation pathways of 15d-PGJ_2 and the other treatments tested (cadmium and aresenite). However, we must mention that aresenite and cadmium are strong inducers of intracellular oxidative stress (Stohs and Bagchi, 1995; Lynn et al., 2000). It is possible that induction of HO-1 by cadmium and aresenite occurs through increased intracellular oxidative stress, and results in changes in GSH levels as does 15d-PGJ_2. We have shown that 15d-PGJ_2-induced HO-1 expression is significautly inhibited by the thiol antioxidant, NAC, and by the thiol reducing agent, DTT. In contrast to NAC, non-thiol antioxidants such as vitamins C and E, and allopurinol were unable to inhibit the induction of HO-1 by 15d-PGJ_2. We also confirm the decrease in intracellular GSH levels produced by 15d-PGJ_2. A characteristic of cyclopentenone PGs is that they contain α, β-unsaturated ketones, which are very susceptible to nucleophilic addition reactions with thiol, and are essential for the actions of the PGs (Atsmon et al., 1990a,b). Therefore, 15d-PGJ_2 is likely to induce HO-1 expression through oxidizing cellular thiols, GSH, and proteins. Upon treatment of 15d-PGJ_2, a decrease in the GSH level may facilitate conversion of protein thiol groups to disulphide bonds, and the subsequent accumulation of malfolded polypeptides, which in turn induces HO-1 expression (Fig. 6). However, determining which proteins participate in this induction of HO-1 requires further investigation.

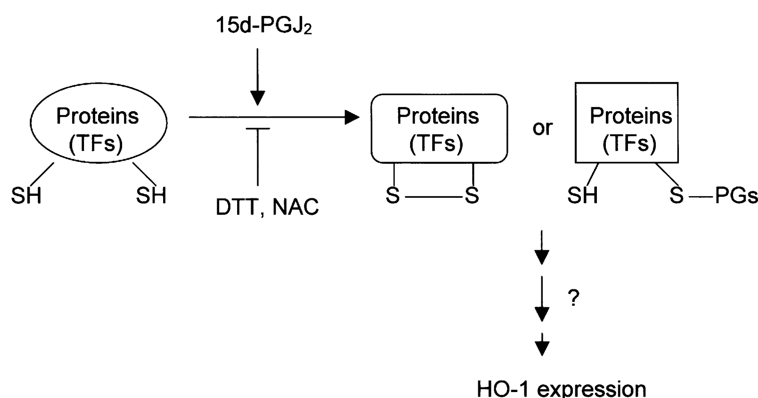


Fig. 6. Two possible mechanisms for the activation of HO-1 protein by 15d-PGJ_2. (i) 15d-PGJ_2 may form thiol conjugates with cysteine residue of proteins because of electrophilic carbons in their cyclopentenone ring (Atsmon et al., 1990a,b). Conjugation with proteins (including transcription factors) may lead to modification of protein functions, ultimately inducing HO-1 expression. (ii) 15d-PGJ_2 exerts a pro-oxidant effect, resulting in the conversion of a sulfhydryl group (-SH) into an oxidized disulfide (-S-S-) in cellular proteins (including transcription factors). This may lead to accumulation of denatured proteins, which in turn may activate HO-1.

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