Journal of Life Science 2008 Vol. 18. No. 11. 1485~1492 ©JLS / ISSN 1225-9918

Prostaglandin A_2-induced Apoptosis is Not Inhibited by Heme Oygenase-1 in U2OS Cells

Kyoung-Won Ko, Sun-Young Lee, Ji-Hyun Ahn[1], Jaetaek Kim[1], In-Kyung Kim and Ho-Shik Kim*

Department of Biochemistry, College of Medicine, The Catholic University of Korea, Socho-gu, Banpo-dong, Seoul 137-701, Korea
[1]Division of Endocrinology and Metabolism, Department of Internal Medicine, College of Medicine, Chung-Ang University, 224-1 Heuk Seok-dong, Dongjak-ku, Seoul 156-755, Korea

Received September 18, 2008 / Accepted October 29, 2008

Prostaglandin A_2 (PGA_2), one of cyclopentenone PGs, induced both apoptosis and *heme oxygenase (HO)-1* expression in U2OS cells. PGA_2-induced apoptosis was not perturbed by either over-expression or knock-down of *HO-1*, whereas H_2O_2-induced cell death was inversely modulated by the expression level of *HO-1*. In addition, N-acetyl-L-cysteine (NAC), a thiol antioxidant, blocked both apoptosis and *HO-1* expression induced by PGA_2. But, non-thiol antioxidants like butylated hydorxyanisole (BHA) and ascorbic acid did not block either apoptosis or *HO-1*-induction. Taken together, these results suggest that PGA_2 induces both apoptosis and *HO-1* expression, which are critically related to the thiol-reactivity of PGA_2, but not oxidative stress, and *HO-1* expression may be independent or functionally located downstream of apoptosis by PGA_2 without contribution to apoptosis progression.

Key words : Prostaglandin, cyclopentenone, apoptosis, heme oxygenase-1, oxidative stress

Introduction

Prostaglandin A_2 (PGA_2), produced from prostaglandin E_2 (PGE_2) by non-enzymatic dehydration has been reported to induce cell death in various cancer cell lines [8]. Although the molecular mechanism involved in PGA_2-induced cell death remains elusive, it was suggested that PGA_2 may activate either caspase-dependent or -independent apoptosis depending on cell types [10,11].

During the progression of apoptosis, PGA_2 has been shown to induce stress proteins such as GADD153, p21, c-Myc and HSP-70 which play important roles in many models of apoptosis [1,5-7]. So, many reports have been focused on the role of these stress proteins in PGA_2-induced cell death. In hepatocellular carcinoma cells like SK-HEP1, induction of *p53* mRNA and protein was related with the activation of cell death [15] (unpublished data). On the other hand, in cells without wild-type *p53* like Hep3B cells, c-Myc expression was a critical determinant of PGA_2-induced cell death, which was prevented by HSP70 over-expression [1].

PGA_2 belongs to cyclopentenone PGs which have α,β-unsaturated carbonyls. These α,β-unsaturated carbonyls are susceptible to nucleophilic addition reaction with thiol-containing compounds. It has been reported that cyclopentenone PGs are directly transported to nuclei, where it binds to nuclear proteins leading to altered expression of various genes [20,21]. So, the binding of cyclopentenone PGs with thiol-containing proteins seems to be closely related to the cell-death inducing activity. For example, intracellular thiol-containing molecules like reduced glutathione was able to modulate the cell-death activity of cyclopentenone PGs by preventing them from transporting or binding to proteins containing sulfhydryls, which initiates PGA_2-induced cell death [23,25].

Heme oxygenase (HO)-1 [EC 1:14.99.3] is a defense enzyme against oxidative stress by catalyzing the rate-limiting step in the heme catabolism, producing biliverdin, iron and carbon monoxide. These three metabolites of heme were reported to exert anti-inflammatory, anti-apoptotic and anti-proliferative effects in several animal models, thus explaining the biological effect of HO-1. Specifically defining, CO has anti-inflammatory effect, biliverdin has a strong anti-oxidant effect and ferritin suppresses the generation of free radicals [24]. HO-1 can be induced by a variety of stimuli such as hydrogen peroxide, heme, NO and growth factors [29]. To be consistent with its cyto-protective role, HO-1 was able to inhibit apoptosis induced by various stimuli including cisplatin, nitric oxide and hydrogen peroxide [9,18,27]. The cyto-protective role of HO-1 was also proved in animal models of *HO-1* transgenic and knock-out mice [17,26].

It has been reported that cyclopentenone PGs induce

***Corresponding author**
Tel : +82-2-590-1181, Fax : +82-2-596-4435
E-mail : hoshik@catholic.ac.kr

HO-1 at mRNA and protein levels in some cell lines [3,31]. However, there has not been a definitive report about the induction of *HO-1* by PGA_2, yet. Zhaung *et al* observed very slight elevation of HO-1 protein by PGA_2 in neuronal cells [32], but in lymphocytes PGA_2 induced *HO-1* expression only at the mRNA level, but not at protein level [3]. Moreover, no report about the *HO-1* induction in cancer cells by PGA_2 was until now.

In this study, we tried to observe the induction of *HO-1* in cancer cells by PGA_2 and if so, wanted to find the relationship between *HO-1* expression and PGA_2-induced cell death. Considering the anti-apoptotic function of HO-1, we assumed that HO-1 can prevent cells from undergoing apoptosis forming the negative feedback loop against PGA_2-induced cyto-toxicity. To address this issue, we analyzed the effect of *HO-1* expression using overexpression construct and small interfering RNA (siRNA) of *HO-1* on the PGA_2-induced cell death.

Materials and Methods

Cell culture

U2OS (Human osteosarcoma) cells were maintained in Dulbecco's Modified Eagle Medium (Invitrogen, Carlsbad, CA) supplemented with 10% heat-inactivated fetal bovine serum (Invitrogen) in a humidified atmosphere of 95% air and 5% CO_2.

Materials

PGA_2 was purchased from BIOMOL Research Laboratories, Inc. (Plymouth Meeting, PA). N-acetyl-L-cysteine (NAC) was from Sigma-Aldrich (St. Louis, MO). Unless otherwise specified, all materials were from Sigma-Aldrich.

Cell viability test

Cell viability was measured by the MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] reduction assay. U2OS cells were seeded at a density of 5×10^3 cells per well in 96-well plates and incubated with various concentrations of PGA_2. After incubation for indicated periods, MTT was added to each well. Three hr later, the medium was removed and 100 μl of isopropanol-HCl was added to each well. Optical density of each well was measured at 570 nm. Data was expressed as relative cell survival which represents percentage of optical density of cells treated with PGA_2 to that of non-treated cells.

Cell death assessment

U2OS cells were seeded at a density of 5×10^4 cells per well in 24-well plates and treated with various concentrations of PGA_2. After incubation for indicated periods, cells were stained with annexin V and propidium iodide (PI) using Annexin-V-FLUOS Staining kit (Roche Applied Science, Penzberg, Germany), according to the manufacturer's instruction. Stained cells were examined under fluorescence microscope.

Immunoblot analysis

Cell lysates prepared with RIPA (50 mM Tris-Cl, pH7.4, 1% NP-40, 0.25% Na-deoxycholate, 150 mM NaCl, 1 mM EDTA) were subjected to 10% SDS-PAGE. The proteins were then transferred onto a nitrocellulose transfer membrane (Whatman, Dassel, Germany). After transfer, membranes were blocked for 30 min in TTBS buffer (25 mM Tris, pH 7.4, 138 mM NaCl, 0.05% Tween-20) containing 5% skim milk and then incubated with mouse monoclonal antibody against HO-1 (BD Biosciences, Franklin Lakes, NJ). After four washes in TTBS the membranes were incubated with anti-mouse IgG-peroxidase conjugated. The membranes were then washed four times in TTBS, followed by detection by ECL Advance Western Blotting detection kit (Amersham, Piscataway, NJ).

Knock-down of *HO-1*

For knock-down of *HO-1*, On-TARGETplus SMARTpool siRNA against to human *HO-1* from Dharmacon RNA technologies (Lafayette, CO) were used. U2OS cells seeded at a density of 3×10^4 cells per well in 24-well plates were transfected with siHO-1 using Lipofectamine™ 2000 (Invitrogen), according to the manufacturer's instruction. The Silencer Negative Control siRNA (Dharmacon RNA Technologies) was used as a negative control.

Overexpression of *HO-1*

To generate HO-1 expression construct, HO-1 coding sequence (CDS) was amplified by PCR with first strand cDNA synthesized from RNA of PGA_2-treated U2OS cells. Primers for HO-1 CDS were as follows; forward Primer 5′-GAATTC ATGGAGCGTCCGCAACCC-3′ and reverse primer 5′-GAA TTC TCACATGGCATAAAGCCCTAC-3′. The PCR products were cloned into the pCR2.1-TOPO vector (Invitrogen) and sequenced. The right clone was then subcloned into the pcDNA3 vector (Invitrogen). To overexpress *HO-1*, U2OS

cells were seeded at a density of 3×10^4 cells per well in 24-well plates and transfected with pcDNA3/HO-1 using Genjet (SignaGen Laboratories, Gaithersburg, MD), following the manufacturer's insturction.

Real-time quantitative RT-PCR (QRT-PCR)

Total RNA extracted from cells treated as indicated was reversely transcribed to cDNA using random hexamer and Cyclescript reverse transcriptase (Bioneer, Inc., Daejon, Korea) and subjected to QRT-PCR. The QRT-PCR was performed with mixture containing cDNA, SYBR Green I, Ex Taq DNA polymerase (Takara Bio Inc., Otsu, Japan), ROX as a reference dye and primers using Mx3000P® QPCR System (Stratagene, La Jolla, CA). Primers of *HO-1* were as follows; forward primer 5′-ATTGCCAGTGCCACCAAGTTC AAG-3′ and reverse primer 5′-ACGCAGTCTTGGCCTCTTC TATCA-3′. *GAPDH* was used as an internal control. The PCR reaction was consisted of 10 sec at 95°C, followed by 40 cycles of 95°C for 5 sec and 60°C for 30 sec and finally melting reaction of 95°C for 1 min, 55°C for 30 sec, 95°C for 30 sec. Gene expression was calculated by ΔΔCt method [19]. All tests were carried out in triplicate and repeated three times.

Statistical analysis

Statistical significances were evaluated by one-way ANOVA test. In each case, a p value of less than 0.05 was determined as statistically significant. Data were expressed as mean±SEM of three independent experiments performed in triplicate.

Results

Induction of apoptosis and *HO-1* in U2OS cells by PGA_2

When U2OS cells were treated with PGA_2, their growth was reduced in a PGA_2 dose-dependent manner (Fig. 1A). To observe if U2OS cells treated with PGA_2 died and to identify the mode of cell death, we performed annexin V staining analysis and observed the nuclear morphology in PGA_2-treated cells. As shown in Fig. 1B, cytoplasmc membranes of PGA_2-treated cells were stained by annexin-V, a phosphatidylserine binding protein, indicating the externalization of phosphatidylserine in cytoplasmic membranes. And the nuclei of PGA_2-treated cells appeared to be condensed and fragmented (Fig. 1C). According to the increase of

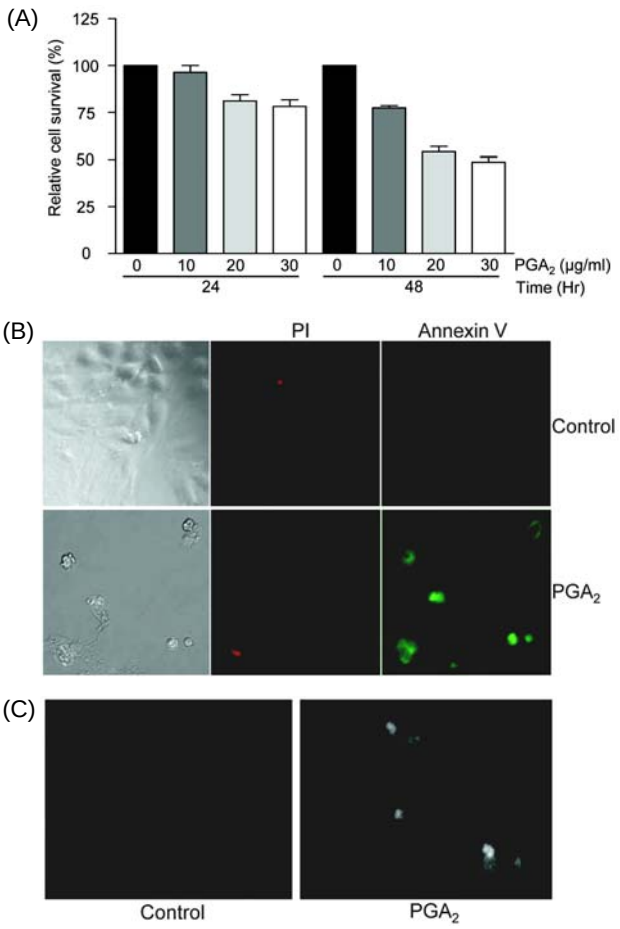

Fig. 1. Induction of apoptosis by PGA_2 in U2OS cells. (A) U2OS cells were seeded at a density of 5×10^3 cells per well in 96-well plates and treated with PGA_2. At the indicated times, MTT cell viability assay was performed and relative cell survival was calculated as described in Materials and Methods. Data represent mean±SEM of three independent experiments performed in triplicate. (B) U2OS cells cultured in the absence or presence of PGA_2 (20 ug/ml) for 24 hr were stained by annexin V and propidium iodide (PI) and observed under fluorescence microscope. (C) U2OS cells treated as described above were incubated for 15 min in the presence of Hoechst33342 and nuclear morphology was examined under fluorescence microscope.

PGA_2 concentrations, the levels of both mRNA (Fig. 2A) and protein (Fig. 2B) of *HO-1* were elevated in PGA_2-treated cells. These findings suggest that PGA_2 inhibit the growth of U2OS cells by activating apoptosis and *HO-1* is induced during this apoptosis.

Effect of *HO-1* expression on PGA_2-induced apoptosis

We, then, checked the effect of *HO-1* knock-down on the PGA_2-induced apoptosis using an inhibitor of HO-1 enzyme

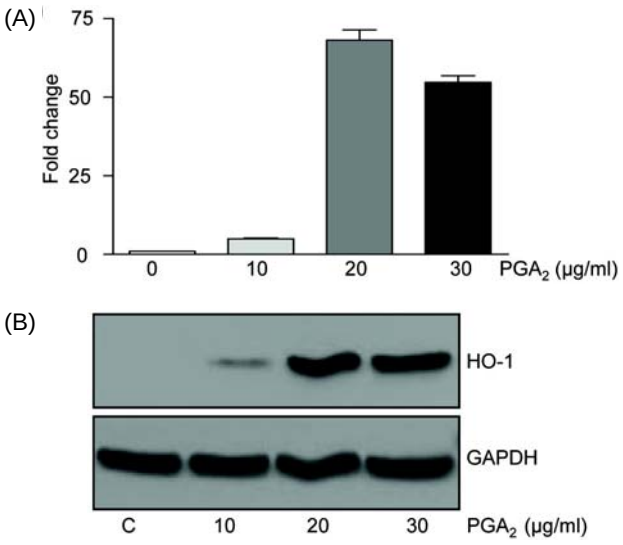

Fig. 2. Induction of *HO-1* expression by PGA_2. (A) RNA was extracted from U2OS cells treated with vehicle or PGA_2 for 24 hr and subjected to QRT-PCR. The expression level of *HO-1* was calculated by ΔΔCt method using *GAPDH* as the internal control. Data represent mean± SEM of three independent experiments performed in triplicate. (B) Cell lysates of U2OS cells treated as described above were subjected to immunoblot analysis against HO-1 protein. GAPDH was used for equal amount of proteins.

activity and small interfering RNA against *HO-1* (siHO-1). Tin protoporphyrin (SnPP), an irreversible inhibitor of HO-1, exerted insignificant effect on growth inhibitory effect of PGA_2, whereas SnPP potentiated the growth inhibition by H_2O_2, another inducer of *HO-1* (Fig. 3A). Because it is possible that HO-1 could contribute to apoptosis by PGA_2 via mechanisms related to the protein itself other than enzyme activity, likely through protein-protein interaction, we used siHO-1 to knock-down *HO-1*. As shown in Fig. 3B, siHO-1 blocked the induction of *HO-1* transcription and hence no increase of HO-1 protein by PGA_2. PGA_2-induced cell death was taken place in *HO-1* knocked-down cells to the same extent as that in control siRNA (siCon)-treated cells, whereas H_2O_2-induced cell death was potentiated by *HO-1* knock-down (Fig. 3C). These findings imply that neither the enzyme activity nor other functions of HO-1 protein *per se* is connected with cell death by PGA_2.

The effect of *HO-1* over-expression on PGA_2-induced cell death

To confirm the effect of siHO-1 on PGA_2-induced cell death and rule out the effect of residual HO-1 protein

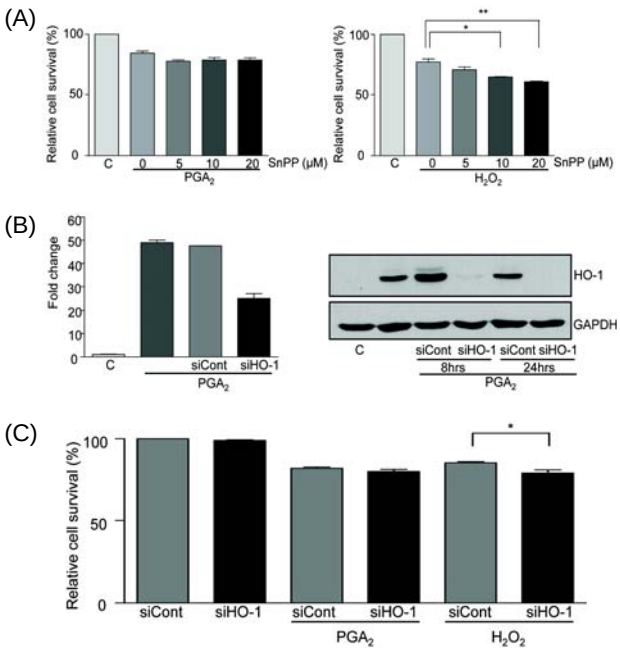

Fig. 3. The effect of SnPP and siHO-1 on PGA_2-induced cell death. (A) U2OS cells pretreated with SnPP for 1 hr were incubated in the presence of vehicle, PGA_2 or H_2O_2. At 24 hr after PGA_2 or H_2O_2 treatment MTT assay was performed. Data represent mean±SEM of three independent experiments performed in triplicate. *: $p<0.01$, **: $p<0.001$. (B) Three days after U2OS cells were transfected with siHO-1 (10 nM), total RNAs and cell lysates were prepared. Total RNAs and cell lysates were subjected to QRT-PCR and immunoblot analyses, respectively as described in Materials and Methods. (C) U2OS cells transfected with siHO-1 for 3 days were treated with PGA_2 or H_2O_2. One day later, MTT assay was performed and data represent as described above. *: $p<0.05$.

which is not completely knocked out by siHO-1, we tested the effect of *HO-1* overexpression on PGA_2-induced cell death. As shown in Fig. 4B, PGA_2 inhibited the growth of U2OS cells transfected transiently with *HO-1* expression construct to the same extent as in U2OS cells transfected with empty vector. But the growth inhibitory effect of H_2O_2 was recovered by HO-1 over-expression to be consistent with the effect of siHO-1 (Fig. 4C). These findings demonstrated once again that HO-1 induced by PGA_2 did not contribute to the cell death process initiated by PGA_2, confirming the effect of siHO-1.

The effect of N-acetyl-L-cysteine (NAC) on the induction of apoptosis and *HO-1* by PGA_2

It has been already known that cyclopentenone PGs-

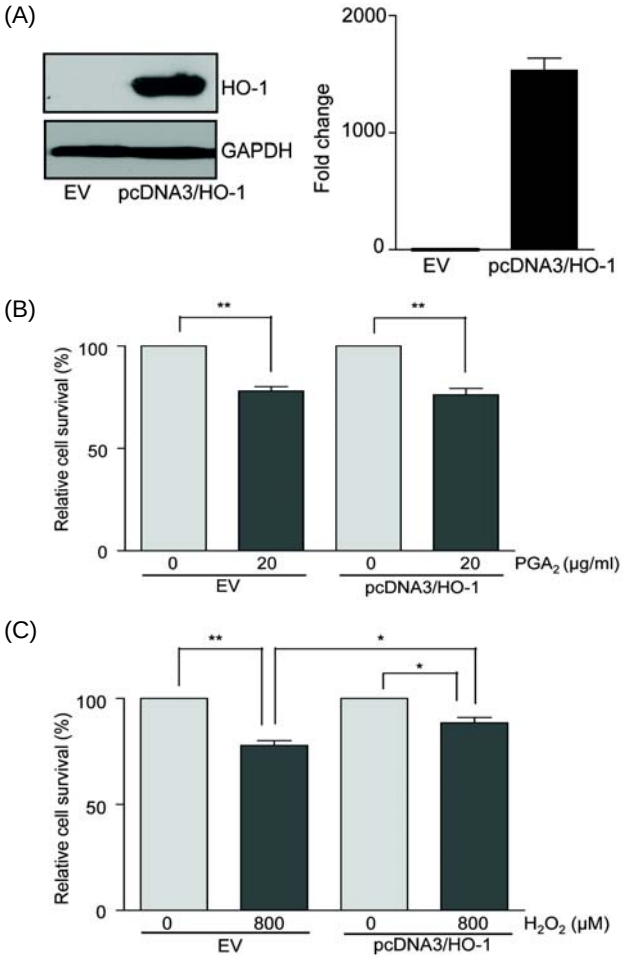

Fig. 4. The effect of *HO-1* overexpression on PGA_2-induced cell death. (A) U2OS cells were transfected with pcDNA3 empty vector or pcDNA3/HO-1. After 48 hr, total cellular proteins and total RNAs were prepared, and subjected to immunoblot analysis and QRT-PCR against *HO-1*, respectively, as described in Materials and Methods. (B,C) U2OS cells transfected with pcDNA3 empty vector or pcDNA3/HO-1 for 48 hr were treated with vehicle, PGA_2 (B) or H_2O_2 (C). After 24 hr MTT assay was performed. *: $p<0.01$, **: $p<0.001$.

induced apoptosis can be prevented by NAC, a thiol antioxidant [12]. We then tested the effect of NAC and other antioxidants on the apoptosis and *HO-1* induction by PGA_2. As shown in Fig. 5A, treatment of NAC before PGA_2 addition prevented cell death induced by PGA_2 in a dose dependent manner. Parallel to its prevention of cell death, NAC blocked *HO-1* induction at both mRNA and protein levels by PGA_2 (Fig. 5B). However, non-thiol antioxidants like ascorbic acid and butylated hydroxyanisole (BHA) could not inhibit the induction of *HO-1* (Fig. 5C) and cell death by PGA_2 (data not shown). These data imply that thiol-

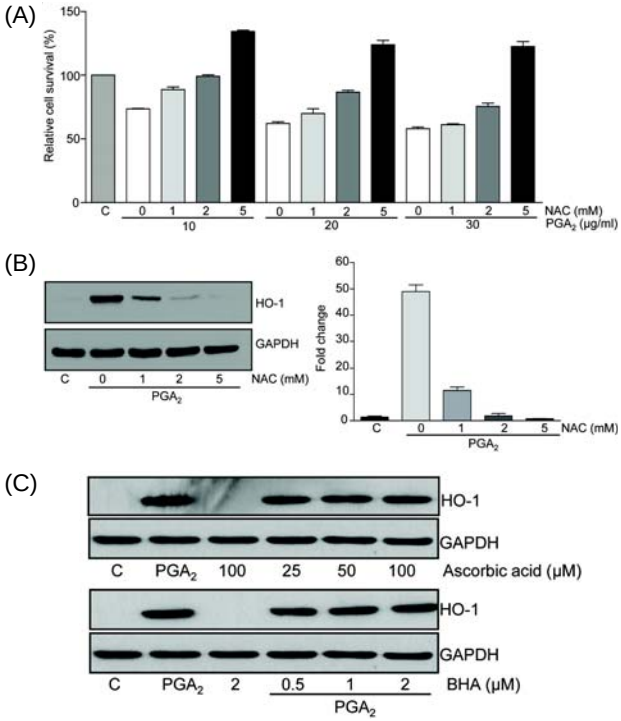

Fig. 5. The effect of antioxidants on the PGA_2-induced cell death and *HO-1* expression. (A) U2OS cells pretreated with NAC for 1 hour were treated with indicated concentrations of PGA_2. After 24 hr, MTT assay was performed. (B) Cell lysates and total RNA were prepared from U2OS cells treated as described above. Cell lysates were subjected to immunoblot analysis with anti-HO-1 and total RNA were subjected to QRT-PCR against HO-1 mRNA. Data represent mean±SD of three independent experiments in triplicate. (C) U2OS cells pretreated with indicated concentrations of ascorbic acid or BHA were incubated in the absence or presence of PGA_2. After 24 hr, cell lysates were prepared and subjected to immunoblot analysis against HO-1 protein.

reactivity of PGA_2, but not oxidative stress is commonly involved in induction of both apoptosis and *HO-1*, and HO-1 is functionally downstream or independent of PGA_2-induced apoptosis.

Discussion

In this report, we showed that PGA_2 induced both expression of *HO-1* and cell death in U2OS cells. PGA_2-induced cell death was turned out to be apoptosis determined by annexin V and morphological changes like condensation and fragmentation of nuclei. Because HO-1 is a defense protein against apoptosis induced by oxidative stress and there have been some reports that reactive oxy-

gen species (ROS) is generated by cyclopentenone PGs [4,22], it was expected that HO-1 could play a role of negative regulator in PGA_2-induced apoptosis. However, elevated expression and knock-down of *HO-1* did not exert any modulator effect on it, whereas H_2O_2-induced cell death in which ROS is a causative agent of cell death, was potentiated and recovered by siHO-1 and HO-1-expression construct, respectively. Rather when apoptosis was blocked by NAC, the expression of HO-1 was gradually reduced parallel to the decrease of apoptosis. These results suggest that ROS may not contribute to PGA_2-induced apoptosis and HO-1 expression is functionally located downstream or an independent pathway of apoptosis.

PGA_2 belongs to the family of cyclopentenone PGs, which has uniquely α,β-unsaturated carbonyls in their cyclopentenone rings. Because these carbonyls are electrophile and thus susceptible to nucleophilic addition reaction, these PGs can readily conjugate with proteins containing sulfhydryls and other cellular thiols like glutathione. Several such proteins that have sulfhydryls like IκB (Inhibitor of κB) kinase and ATM (ataxia telangiectasia mutated) have been reported as target molecules of PGA_2 [14,28].

In this study, PGA_2 induced *HO-1* at mRNA level suggesting the involvement of transcriptional factor activation. For the transcription of *HO-1*, it was found that nuclear transcription factor erythroid 2p45 (NF-E2)-related factor 2 (Nrf2) plays a critical role. At the basal state, Nrf2 resides in the cytosol via binding to Kelch-like ECH-associated protein 1 (Keap1), preventing the nuclear translocation of Nrf2 and thus transcriptional activation of Nrf2-dependent genes including *HO-1* [2,16]. Therefore, it could be postulated that PGA_2 binds to sulfhydryls of Keap1 resulting in conformational change of Keap1 and, in turn, breakage of Nrf2 and Keap1 complex to allow the nuclear translocation of Nrf2, finally leading to transcription of *HO-1*. Actually the binding of 15d-PGJ_2, another cyclopentenone PG to thiol-containing proteins, which leads to inhibition of pro-inflammatory gene transcription was identified in renal mesangial cells [30]. In the same context, the effect of NAC could be explained. Because NAC also can readily conjugate with PGA_2, excessive NAC may interfere the binding between PGA_2 and sulfhydryls of Keap1, thus blocking the nuclear translocation of Nrf2 and other PGA_2-induced signaling pathways leading to *HO-1* transcription.

Regarding the effect of NAC, the involvement of ROS should be considered. Because cyclopentenone PGs including PGA_2 [4,24] (data not shown) can increase ROS generation, the effect of NAC can be due to its radical scavenging activity. But, we could not observe that antioxidants such as BHA, ascorbic acid and PDTC blocked the cell death and HO-1 induction by PGA_2 (Fig. 4C and data not shown), supporting strongly that thiol-reactivity rather than ROS generation may be a critical determinant of cell death and HO-1 induction by PGA_2 in this cell line.

The mechanism of PGA_2-induced cell death was different depending on the cell types. In terms of *de novo* protein synthesis requirement in cell death, PGA_2 induced either pathway requiring new protein synthesis or not. That is, L1210, HepG2 and Hep3B cells underwent apoptosis which is blocked by cycloheximide pretreatment whereas in HL-60 and SK-HEP1 cells, PGA_2 activate apoptosis without new protein synthesis [1,10,13]. In either case, it is known that PGA_2 should be transported to nucleus, where it binds molecules containing sulfhydryls to activate new protein synthesis-dependent or -independent apoptosis program. In U2OS cells, cycloheximide pretreatment could not change the progression of cell death by PGA_2 indicating that newly induced proteins may not be involved in the apoptosis by PGA_2 (data not shown). To be consistently, knock down of *HO-1*, one of newly induced proteins did not exert any effect on the PGA_2-induced apoptosis. However, siRNA of *HO-1* potentiated the H_2O_2-induced cell death, in which ROS plays a critical role. This contrasting effect of HO-1 siRNA between the cell death induced by PGA_2 and H_2O_2 still support the suggestion that ROS may not be involved in PGA_2-induced cell death. And the inhibitory effect of NAC on PGA_2-induced apoptosis should be explained by blocking PGA_2 transport to nucleus similarly to its effect on HO-1 induction by PGA_2.

Taken together, HO-1 induction was not involved in the cell death by PGA_2. So, although the role of HO-1 in this cell death is not identified in this work, it may contribute to other biological effects of PGA_2, which should be clarified in the future. And the signal transduction pathway leading to HO-1 induction by PGA_2 should also be characterized.

Acknowledgement

The authors wish to acknowledge the financial support of the Catholic Medical Center Research Foundation.

References

1. Ahn, S. G., S. Y. Jeong, H. Rhim H and I. K. Kim. 1998. The role of c-Myc and heat shock protein 70 in human hepatocarcinoma Hep3B cells during apoptosis induced by prostaglandin A2/Delta12-prostaglandin J2. *Biochim. Biophys. Acta* **1448**, 115-125.
2. Alam, J. and J. L. Cook. 2003. Transcriptional regulation of the heme oxygenase-1 gene via the stress response element pathway. *Curr. Pharm. Des.* **9**, 2499-2511.
3. Alvarez-Maqueda, M., R. El Bekay, G. Alba, J. Monteseirín, P. Chacón, A. Vega, J. Martín-Nieto, F. J. Bedoya, E. Pintado and F. Sobrino. 2004. 15-deoxy-delta 12,14-prostaglandin J2 induces heme oxygenase-1 gene expression in a reactive oxygen species-dependent manner in human lymphocytes. *J. Biol. Chem.* **279**, 21929-21937.
4. Chen, Y. C., S. C. Shen and S. H. Tsai. 2005. Prostaglandin D(2) and J(2) induce apoptosis in human leukemia cells via activation of the caspase 3 cascade and production of reactive oxygen species. *Biochim. Biophys. Acta* **1743**, 291-304.
5. Choi, A. M., J. Fargnoli, S. G. Carlson and N. J. Holbrook. 1992. Cell growth inhibition by prostaglandin A2 results in elevated expression of gadd153 mRNA. *Exp. Cell. Res.* **199**, 85-89.
6. Gorospe, M., Y. Liu, Q. Xu, F. J. Chrest and N. J. Holbrook. 1996. Inhibition of G1 cyclin-dependent kinase activity during growth arrest of human breast carcinoma cells by prostaglandin A2. *Mol. Cell. Biol.* **16**, 762-770.
7. Holbrook, N. J., S. G. Carlson, A. M. Choi and J. Fargnoli. 1992. Induction of HSP70 gene expression by the antiproliferative prostaglandin PGA2: a growth-dependent response mediated by activation of heat shock transcription factor. *Mol. Cell. Biol.* **12**, 1528-1534.
8. Hubich, A. I. and M. V. Sholukh. 2006. Biochemistry of prostaglandins A. *Biochemistry (Mosc)* **71**, 229-238.
9. Kim, H. R., S. Kim, E. J. Kim, J. H. Park, S. H. Yang, E. T. Jeong, C. Park, M. J. Youn, H. S. So and R. Park. 2008. Suppression of Nrf2-driven heme oxygenase-1 enhances the chemosensitivity of lung cancer A549 cells toward cisplatin. *Lung Cancer* **60**, 47-56.
10. Kim, H. S., H. Rhim, S. W. Jeong, J. W. Kim and I. K. Kim. 2002. Induction of apoptosis dependent on caspase activities and growth arrest in HL-60 cells by PGA2. *Prostaglandins Other Lipid Mediat.* **70**, 169-183.
11. Kim, H. S., J. C. Shim, J. Y. Choi, H. Rhim and I. K. Kim. 2005. Prostaglandin A2 induces caspase-independent apoptosis in hepatocellular carcinoma cells. *Korean J. Hepatol.* **11**, 72-79.
12. Kim, H. S., J. H. Lee and I. K. Kim. 1996. Intracellular glutathione level modulates the induction of apoptosis by delta 12-prostaglandin J2. *Prostaglandins* **51**, 413-425.
13. Kim, I. K., J. H. Lee, H. W. Sohn, H. S. Kim and S. H. Kim SH. 1993. Prostaglandin A2 and delta 12-prostaglandin J2 induce apoptosis in L1210 cells. *FEBS Lett.* **321**, 209-214.
14. Kobayashi, M., H. Ono, K. Mihara, H. Tauchi, K. Komatsu, T. Shibata, H. Shimizu, K. Uchida and K. Yamamoto. 2006. ATM activation by a sulfhydryl-reactive inflammatory cyclopentenone prostaglandin. *Genes Cells* **11**, 779-789.
15. Lee, J. H., H. S. Kim, S. Y. Jeong and I. K. Kim. 1995. Induction of p53 and apoptosis by delta 12-PGJ2 in human hepatocarcinoma SK-HEP-1 cells. *FEBS Lett.* **368**, 348-352.
16. Lee, J. S. and Y. J. Surh. 2005. Nrf2 as a novel molecular target for chemoprevention. *Cancer Lett.* **224**, 171-184.
17. Lee, P. J., J. Alam, G. W. Wiegand and A. M. Choi. 1996. Overexpression of heme oxygenase-1 in human pulmonary epithelial cells results in cell growth arrest and increased resistance to hyperoxia. *Proc. Natl. Acad. Sci. USA* **93**, 10393-10398.
18. Li Volti, G., V. Sorrenti, P. Murabito, F. Galvano, M. Veroux, A. Gullo, R. Acquaviva, A. Stacchiotti, F. Bonomini, L. Vanella and C. Di Giacomo. 2007. Pharmacological induction of heme oxygenase-1 inhibits iNOS and oxidative stress in renal ischemia-reperfusion injury. *Transplant. Proc.* **39**, 2986-2991.
19. Livak, K. J. and T. D. Schmittgen. 2001. Analysis of relative gene expression data using real-time quantitative PCR and the 2(-ΔΔC(T)) method. *Methods* **25**, 402-408.
20. Narumiya, S., K. Ohno, M. Fujiwara and M. Fukushima. 1986. Site and mechanism of growth inhibition by prostaglandins. II. Temperature-dependent transfer of a cyclopentenone prostaglandin to nuclei. *J. Pharmacol. Exp. Ther.* **239**, 506-511.
21. Narumiya, S., K. Ohno, M. Fukushima and M. Fujiwara. 1987. Site and mechanism of growth inhibition by prostaglandins. III. Distribution and binding of prostaglandin A2 and delta 12-prostaglandin J2 in nuclei. *J. Pharmacol. Exp. Ther.* **242**, 306-311.
22. Nencioni, A., K. Lauber, F. Grünebach, L. Van Parijs, C. Denzlinger, S. Wesselborg and P. Brossart. 2003. Cyclopentenone prostaglandins induce lymphocyte apoptosis by activating the mitochondrial apoptosis pathway independent of external death receptor signaling. *J. Immunol.* **171**, 5148-5156.
23. Ohno, K., M. Hirata, S. Narumiya and M. Fukushima. 1992. Effect of glutathione content on cellular uptake and growth inhibitory activity of prostaglandin A2 in L-1210 cells. *Eicosanoids* **5**, 81-85.
24. Otterbein, L. E., M. P. Soares, K. Yamashita and F. H. Bach. 2003. Heme oxygenase-1: unleashing the protective properties of heme. *Trends Immunol.* **24**, 449-455.
25. Parker, J. and H. Ankel. 1992 Formation of a prostaglandin A2-glutathione conjugate in L1210 mouse leukemia cells. *Biochem. Pharmacol.* **43**, 1053-1060.
26. Poss, K. D. and S. Tonegawa. 1997. Reduced stress defense in heme oxygenase 1-deficient cells. *Proc. Natl. Acad. Sci. U S A* **94**, 10925-10930.
27. Radhakrishnan, N., M. Bhaskaran and P. C. Singhal. 2005. Hepatocyte growth factor modulates H2O2-induced mesangial cell apoptosis through induction of heme oxygenase-1. *Nephron Physiol.* **101**, 92-98.
28. Rossi, A., P. Kapahi, G. Natoli, T. Takahashi, Y. Chen, M. Karin and M. G. Santoro. 2000. Anti-inflammatory cyclo-

pentenone prostaglandins are direct inhibitors of IkappaB kinase. *Nature* **403**, 103-108.
29. Ryter, S. W., L. E. Otterbein, D. Morse and A. M. Choi. 2002. Heme oxygenase/carbon monoxide signaling pathways: regulation and functional significance. *Mol. Cell. Biochem.* **234-235**, 249-263.
30. Sánchez-Gómez, F. J., E. Cernuda-Morollón, K. Stamatakis and D. Pérez-Sala. 2004. Protein thiol modification by 15-deoxy-Delta12,14-prostaglandin J2 addition in mesangial cells: role in the inhibition of pro-inflammatory genes. *Mol. Pharmacol.* **66**, 1349-1358.
31. Straus, D. S. and C. K. Glass. 2001. Cyclopentenone prostaglandins: new insights on biological activities and cellular targets. *Med. Res. Rev.* **21**, 185-210.
32. Zhuang, H., S. Pin, X. Li and S. Doré. 2003. Regulation of heme oxygenase expression by cyclopentenone prostaglandins. *Exp. Biol. Med. (Maywood)* **228**, 499-505.

초록 : U2OS 세포에서 prostaglandin A_2에 의한 apoptosis는 heme oxygenase-1에 의하여 저해되지 않는다

고경원 · 이선영 · 안지현[1] · 김재택[1] · 김인경 · 김호식*
(가톨릭대학교 의과대학 생화학교실, [1]중앙대학교 의과대학 내과학교실)

Prostaglandin A_2 (PGA_2)는 사람 골육종 세포인 U2OS 세포주에서 apoptosis와 *heme oxygenase (HO)-1*의 발현을 함께 유도하였다. PGA_2에 의한 apoptosis는 *HO-1*의 과도한 발현이나 *HO-1*에 대한 small interfering RNA에 의한 발현저하에 의하여 변동되지 않았으나 H_2O_2에 의한 세포사망은 *HO-1*의 발현 수준에 반비례하여 변동되었다. 또한 thiol antioxidant인 N-acetyl-L-cysteine (NAC)은 PGA_2에 의한 세포사망과 *HO-1*의 발현 증가를 모두 차단하였지만, non-thiol antioxidant인 butylated hydroxyanisole (BHA)과 ascorbic acid는 세포사망과 *HO-1*의 발현 유도를 차단하지 않았다. 이와 같은 결과들은 PGA_2는 산화성 손상에 의해서가 아니라 PGA_2의 thiol-reactivity에 의하여 apoptosis와 *HO-1*의 발현을 유도하며, *HO-1*의 발현은 PGA_2에 의한 apoptosis와는 독립적인 현상이거나 기능적으로 apoptosis 유도의 하부에 위치하고 apoptosis의 진행에는 기여하지 않을 것이라는 것을 시사해 준다.