*J. Toxicol. Pub. Health*
Vol. 21, No. 1, pp. 77-85 (2005)

**Official Journal of Korean Society of Toxicology**

Available online at http://www.toxmut.or.kr

# Cadmium Induces Cell Cycle Arrest and Change in Expression of Cell Cycle Related Proteins in Breast Cancer Cell Lines

**Young Joo Lee[1,2], Tae Seok Kang[1], Tae Sung Kim[1], Hyun Ju Moon[1], Il Hyun Kang[1], Ji Young Oh[1], Hoonjeong Kwon[2] and Soon Young Han[1]**

*[1]Endocrine Toxicology Division, National Institute of Toxicological Research, Seoul*
*[2]Department of Food and Nutrition, and Research Institute of Human Ecology, Seoul National University, Seoul, Korea*

*Received February 17, 2005; Accepted March 11, 2005*

**ABSTRACT.** Cadmium is an environmental pollutant exposed from contaminated foods or cigarette smoking and known to cause oxidative damage in organs. We investigated the cadmium-induced apoptosis and cell arrest in human breast cancer cells, MCF-7 cells and MDA-MB-231 cells. Obvious apoptotic cell death was shown in $CdCl_2$ 100 μM treatment for 12 hr, which were determined by DAPI staining and flow cytometric analysis. In cell cycle analysis, MCF-7 cells and MDA-MB-231 cells were arrested in S phase and G2/M phase respectively. These could be explained by the induction of cell cycle inhibitory protein, $p21^{Waf1/Cip1}$ and $p27^{Kip1}$, expression and reduction of cyclin/Cdk complexes in both cell lines. The decreased expression of cyclin A and Cdk2 in MCF-7 cells and cyclin B1 and Cdc2 in MDA-MB-231 cells were consistent with the flow cytometric observation. p-ERK expression was increased dose-dependent manner in both cell lines. It suggests that ERK MAPK pathway are involved in cadmium-induced cell cycle arrest and apoptosis. Moreover, cotreatment of zinc (100 μM, 12 hr) recovered the cadmium-induced cell arrest in both cells, which shows cadmium-induced oxidative stress mediates apoptosis and cell cycle arrest in human breast cancer cells.

**Keywords:** Cadmium, Cell cycle arrest, Cell cycle regulatory protein, ERK, Antioxidant, Zinc.

## INTRODUCTION

Cadmium is a toxic heavy metal, which is introduced and accumulated in human from environment, contaminated foods and mainly cigarette smoking. Cadmium was identified as a category 1 carcinogen from international Agency for Research on Cancer (IARC, 1993) and known to induce tumors in lung, prostate and testes *in vitro* experiments (Koizumi and Yamada, 2003; Waalkes, 2000). Besides the carcinogenic potential of cadmium, it causes damages in liver, kidney, bone and central nerve system as well as in testis by acute or chronic administration (Stohs and Bagchi, 1995; Waalkes and Diwan, 1999). Generally, toxic heavy metals including cadmium have been suggested to affect on antioxidative system via producing hydroxyl radicals, superoxides and other reactive oxygen species and modulating the activities of antioxidation-related enzymes, such as superoxide dismutase (SOD) and catalase (Szuster-Ciesielska *et al.*, 2000). Although the mechanism of cadmium toxicity has not been completely proved, extensive researches have exhibited that cadmium-induced oxidative stress increases lipid peroxidation and DNA damage, and finally links to apoptosis and necrosis in organs (Stohs and Bagchi, 1995; Sarkar *et al.*, 1998).

Several reports have been suggested the association between apoptosis and cell cycle control. As shown in the reports, toxicants can arrest cells in a mitogenic phase to prevent from inaccurate division of damaged cells and to eliminate them through apoptosis (Pietenpol and Stewart, 2002). These cell cycle controls are tightly controlled by regulating the formation of cyclin/Cdk complexes or by binding of Cdk inhibitors such as

Correspondence to: Soon Young Han, Endocrine Toxicology Division, National Institute of Toxicological Research, Korea Food and Drug Administration, 5 Nokbun-dong Eunpyung-gu, Seoul 122-704, Korea
E-mail: soonyoungh@kfda.go.kr

**List of abbreviations**: ROS, reactive oxygen species; cdk, cyclin dependent kinase; MAPK, mitogen-activated protein kinase; ERK, extracellular signal regulated kinase

$p21^{Waf1/Cip1}$ and $p27^{Kip1}$ on the cyclin complexes. However, since the effect on cell cycle would differ in depends on the species of toxicants and cell lines, researches for cadmium should be implemented in various tissues and cell lines.

Recent study has been demonstrated cellular events involved in proliferation, differentiation as well as cell death are mediated by activation of mitogen-activated protein kinases (MAPKs), c-jun N-terminal kinase (JNK), p38 and extracellular signal regulated kinase (ERK) (Chuang *et al.*, 2000). Among the MAPKs, ERK has critical roles in proliferation, growth arrest or apoptosis (Chao and Yang, 2001; Son *et al.*, 2001). We have determined the activated ERK, which are responsible for the extracellular stress signal and activation of other subsequent MAPKs.

Although many approaches have been done to identify the toxic mechanism of cadmium in cell cycle arrest and apoptosis, it was not enough to make it understood the cell line specific effects (Garcia-Morales *et al.*, 1994). Current study was objected to investigate cadmium effects on cell cycle progression and expression of cell cycle regulator protein in human breast cancer cell lines, MCF-7 cells (ERα positive cells) and MDA-MB-231 cells (ERα negative cells). In addition, we examined whether cadmium induced cell cycle arrest and cell damage can prevented by treating antioxidant, zinc, to make use of these finding to reducing cadmium toxicity.

## MATERIAL AND METHOD

### Chemicals and reagents

Chemicals used in this study, cadmium chloride ($CdCl_2$), zinc chloride ($ZnCl_2$), MTT, 4',6'-diamidino-2-phenylindole (DAPI) and propidium iodide (PI), trypan blue solution and other reagents were obtained from Sigma (St. Louis, MO, USA).

### Cell cultures

MCF-7 cell line, estrogen receptor positive human breast cancer cell line, and MDA-MB-231 cell lines, estrogen receptor α negative human breast cancer cell lines, were provided by American Type Culture Collection (ATCC) and Korean cell line bank (KCLB) respectively. Both cells were maintained in RPMI 1640 medium supplemented with 10% heat inactivated fetal bovine serum (FBS) and 1% penicillin/streptomycine, and incubated at 37°C in 5% $CO_2$ condition. All media constituents were obtained from Gibco BRL (Grand Island, NY).

### Viability test

Cell viability was determined by MTT assay. For treatment of cadmium with or without antioxidants, cells were counted and plated to be same initial density ($10^5$/ml) in 96 well plates. After 24 hr incubation, $CdCl_2$ dissolved in DMSO was added to medium to contain less than 0.5% DMSO and to be final concentration (10 μM~250 μM). $ZnCl_2$ was cotreated in a range of concentration with cadmium. After 12 hr of treatment, 15 μl of 5 mg/ml MTT solution was added in each well and incubated for 4 hr. Medium were carefully removed and the absorbance of fomazan dissolved in 100 μl of DMSO was measured using ultra microplate reader (Bio-Tech Instruments, Inc., USA) in 570 nm. Viability was calculated using absorbances in each dose, which were compared with that in vehicle control.

### Trypan blue staining

To evaluate apoptotic cell death, the alteration of membrane permeability was examined by trypan blue staining. Cells were plated in 6 well plate ($10^5$/ml) and treated with cadmium for 12 hr as described above. Cells harvested by trypsinization were suspended in 100 μl of PBS and stained with 20 μl of trypan blue solution (0.4% w/v, Sigma). Both stained and intact cells which were not stained, were counted using hemacytometer (Bright-Line, USA).

### Nuclear staining with DAPI (determination of apoptotic cell death)

After cadmium treatment for 12 hr in 6 well plate, cells ($2.5 \times 10^4$/ml) were washed with ice-cold PBS, fixed with 4% paraformaldehyde for 3 hr and stained with 4',6'-diamidino-2-phenylindole (DAPI). Apoptotic changes were observed under fluorescence microscope (Olympus, Japan).

### Flow cytometric analysis

Changes in cell cycle distribution caused by cadmium treatment were measured using FACS analysis. Treated cells ($10^5$/ml) in 100 $mm^2$ dishes were harvested by trypsinization. Same quantity of cells ($10^6$/ml) in each time and concentration, were fixed with 70% ethanol and incubated with 400 μg/ml of propidium iodide (PI) and 1 mg/ml of RNase A in 37°C for 30 min. Cell cycle was analyzed using FACS (BeckmanCoulter, USA) with EPICS system II software (Ver.3.0).

### Western blot analysis

Cellular proteins in cadmium and zinc treated cells for 12 hr, which were plated in 100 $mm^2$ dishes ($10^5$/ml), were isolated using lysis buffer [50 mM Tris-HCl, 150 mM NaCl, 1% NP-40, 1 mM EDTA, 1 mM NaF, 1 mM phenylmethylsulfonyl fluoride (PMSF), aprotinin, pH 7.4].

Protein concentrations of extracts were quantified by Bradford method using Bio Rad Protein Assay Kit (Hercules, CA, USA). Same quantity, 20 μg/μl, of protein were separated in SDS PAGE of 10~15% SDS polyacrylaminde gel and transferred to PVDF membrane (Bio Rad) in 35 V, 4°C. The blot was blocked with 5% skim milk (PBS, 0.1% Tween-20), and incubated with primary antibodies for p21, p27, cyclin A, cyclin B1, cyclin D, Cdk2, Cdc2, p-ERK, RB, pRB (Santa Cruz biotechnology) and β-actin (Sigma) in 3% skim milk (PBS, 0.1% Tween-20). Corresponding horseradish peroxidase-conjugated secondary antibody (Santa Cruz Biotechnology) was reincubated. Changes in protein expression were detected using ECL plus kit (Amarsham, UK).

## RESULTS

### Cadmium causes dose-dependent cell death in human breast cells

To evaluate the cytotoxic effect of cadmium on human breast cells, we investigated the viability in the two breast cell lines, MCF-7 and MDA-MB-231 using MTT assay and trypan blue exclusion assay. Cell viability was decreased concentration dependently in both cell lines after 12 hr treatment (Figs. 1A, 1B) and marked cell death was shown in both cell lines above 100 μM treatment. Interestingly, MCF-7 was more sensitive to cadmium than MDA-MB-231 cells and $IC_{50}$ values from MTT assay were approximately 100 μM for MCF-7 and 125 μM for MDA-MB-231. Trypan blue assay, which examine alteration of membrane permeability, was also showed comparable results (Figs. 1C, 1D).

To determine whether the apoptosis was responsible for the cadmium-induced cell death, we have observed the cell morphology of cadmium treated cells after staining the cell with DAPI and analyzed the apoptotic cells by FACS analysis. In both cell lines treated 100 μM of cadmium, typical features of apoptosis including shrinkage of cytoplasm and obvious nuclear condensation were found under fluorescent microscope (Fig. 2). In addition, apoptotic sub-G1 population in FACS analysis was increased in cadmium treated MCF-7 cells above 50 μM treatment for 12 hr. In 24 hr treatment, MCF-7 cells exhibited sub-G1 population from 25 μM treatment and the distribution curves were shifted to lower DNA content due to the extensive cell death (Fig. 3). However, MDA-MB-231 cells were more resistant to cadmium and only small sub-G1 population was shown in high concentration (Fig. 4).

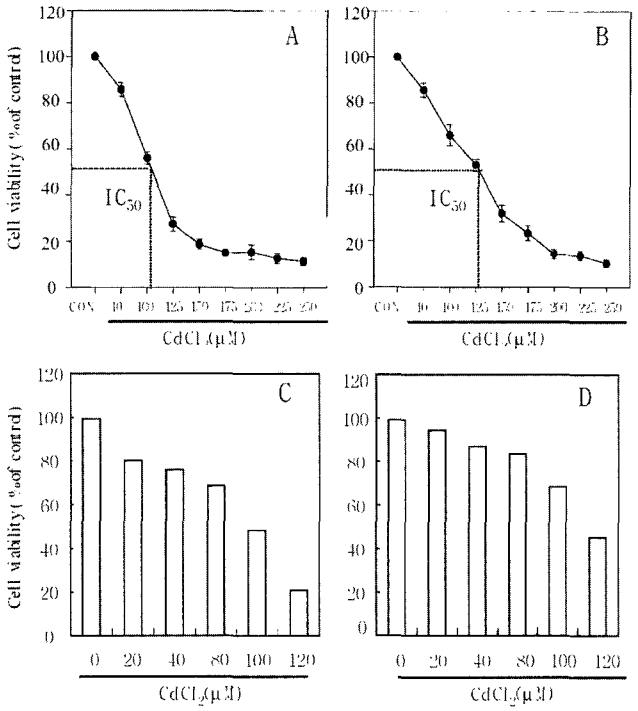


**Fig. 1.** The viability of MCF-7 cells (A, C) and MDA-MB-231 cells (B, D). Upper panel was determined by MTT assay (n = 6) and lower panel was done by trypan blue exclusion assay after 12 hr exposure to cadmium. The $IC_{50}$ lies in the center of the inflection of the curve.

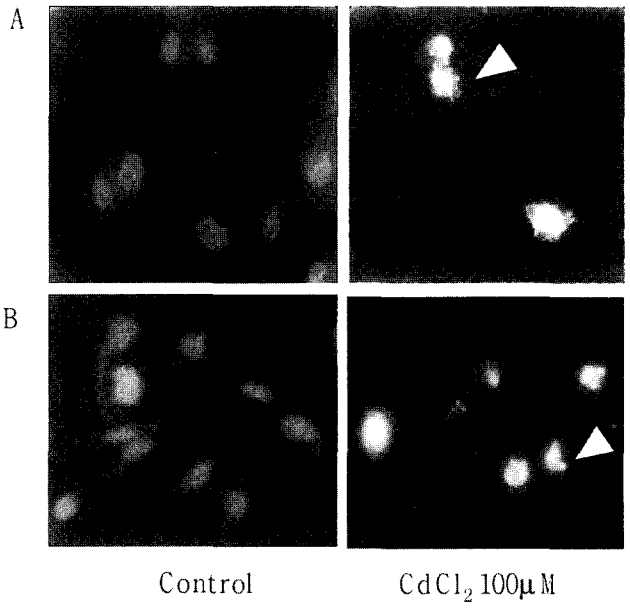


**Fig. 2.** Morphological evaluation of cadmium induced apoptosis. MCF-7 cells (A) and MDA-MB-231 cells (B) were incubated for 12 hr without or with cadmium 100 μM and quantified as normal or apoptotic by staining with DAPI under fluorescence microscope (×400). Nuclear fragmentation, apoptotic bodies and presenting condensation are indicated by arrow.

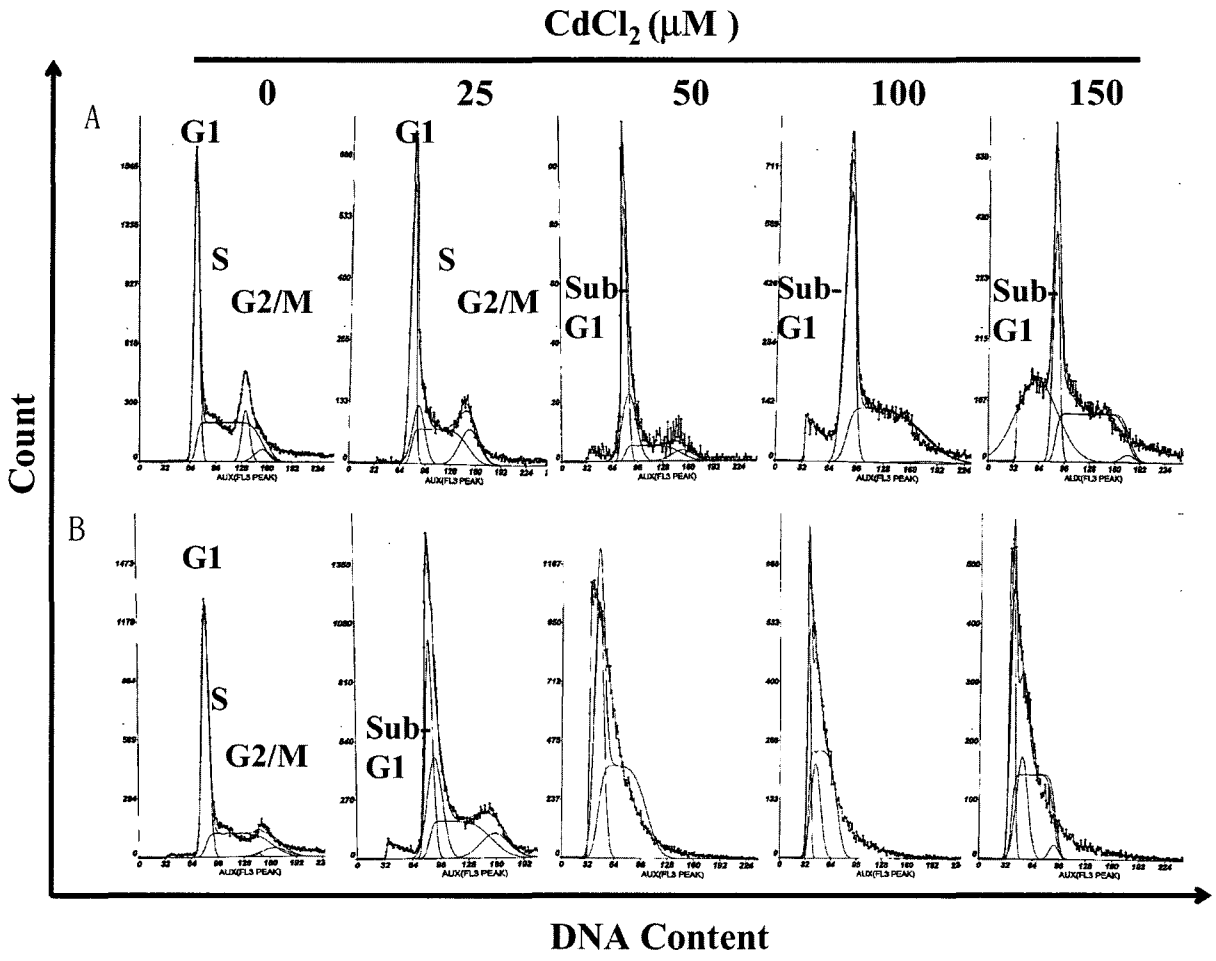


**Fig. 3.** Cell cycle was analyzed in untreated or cadmium (25 μM, 50 μM, 100 μM or 150 μM) treated MCF-7 cells for 12 hr (A) and 24 hr (B).

### Cadmium induces cell cycle arrest in human breast cancer cells

The effect of cadmium treatment on cell cycle distribution in MCF-7 and MDA-MB-231 cells was investigated. MCF-7 cells showed considerable reduction of G2/M phase in 12 hr and 24 hr treatment, but significant increase in S phase in 100 and 150 μM (Figs. 3, 5A). Whereas, MDA-MB-231 cells treated with 100 μM of cadmium were markedly increased in G2/M phase, that is, arrested in G2/M phase (Figs. 4, 5B).

### Cadmium induces changes in expression of cell cycle regulating proteins

To identify cell cycle regulators, which were affected by cadmium treatment and caused cell cycle arrest, the expression of regulatory protein was determined by Western blotting (Figs. 6, 7). The cell cycle inhibitory regulators, $p21^{Waf/Cip1}$ and $p27^{kip1}$, were concentration-dependently increased in both cell lines without cell specific differences. Cyclin D1 expression, which regulates G1 cell cycle, was not affected by cadmium in either cell lines. In agreement to the observation of cell cycle analysis, expressions of Cdk2 and cyclin A, which regulate progression of S phase, were concentration-dependently reduced in MCF-7 cells but not in MDA-MB-231 cells. In MDA-MB-231 cells arrested in G2/M phase, reductions in cyclin B1 and Cdc2 expression were observed at 100 and 150 μM cadmium treatment, while those proteins in MCF-7 cells were not affected. We also evaluate the level of pRb, which are needed in dissociation of E2F and initiation of transcriptional events in S phase. The pRb expression was increased and the Rb expression was conversely decreased in both cell lines.

### Antioxidant, zinc, can inhibit cadmium induced cell cycle arrest

To determine antioxidant, zinc, can protect cells from cadmium-induced cell cycle arrest, various concentra-

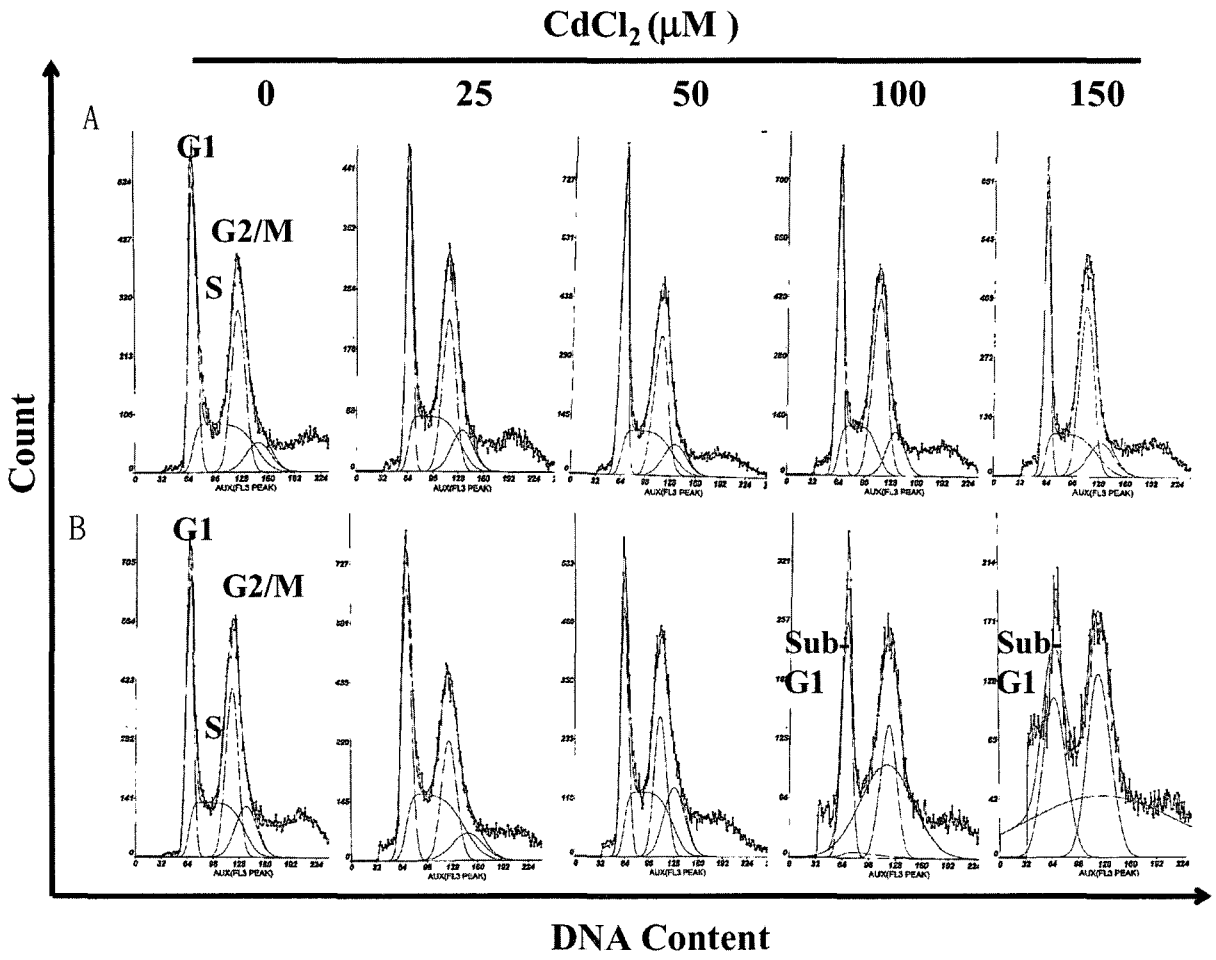


**Fig. 4.** Cell cycle was analyzed in untreated or cadmium (25 μM, 50 μM, 100 μM or 150 μM) treated MDA-MB-231 cells for 12 hr (A) and 24 hr (B).

tions of zinc were cotreated with 100 μM of cadmium for 12 hr (Figs. 8, 9). In MCF-7 cells, 100 μM of zinc treatment inhibited the cadmium-induced S phase arrest and recovered to similar distribution of cell population in untreated MCF-7 cells (Figs. 5, 8A, 9A). Moreover, cotreatment of zinc was effectively reduced the G2/M phase population and abolished the cell arrest in MDA-MB-231 cells (Figs. 8B, 9B).

### Zinc recovers cadmium-induced changes in expression of cell cycle regulators

Zinc in increasing concentration was cotreated with 100 μM of cadmium for 12 hr. In MCF-7 cells, the reduced expression of Cdk2 in 100 μM cadmium treatment alone was recovered to control level by zinc cotreatment. Cyclin A expression was also enhanced in high concentration of zinc even though it did not reach to the control level (Fig. 10). In MDA-MB-231 cells, expressions of cyclin B1 and Cdc2 were partially recovered by cotreatment with cadmium and zinc (Fig. 11).

### Cadmium activates MAPK pathway

Considering that MAP kinases have crucial role in cell cycle and cell survival, we have analyzed active p-ERK expression (Fig. 12). Both cell lines exhibited concentration-dependent increase in p-ERK expression above 50 μM cadmium treatment, while in relatively low concentration, 25 μM, p-ERK was decreased compared with control. Furthermore, the basal expression of p-ERK in MDA-MB-231 cells was much higher than in MCF-7 cells. These shows concentration and cell line specific expression of p-ERK.

## DISCUSSIONS

It has been reported that cadmium generates ROS and induces dissociation of cytochrome C in mitochondria and leads to apoptotic cell death via signal cas-

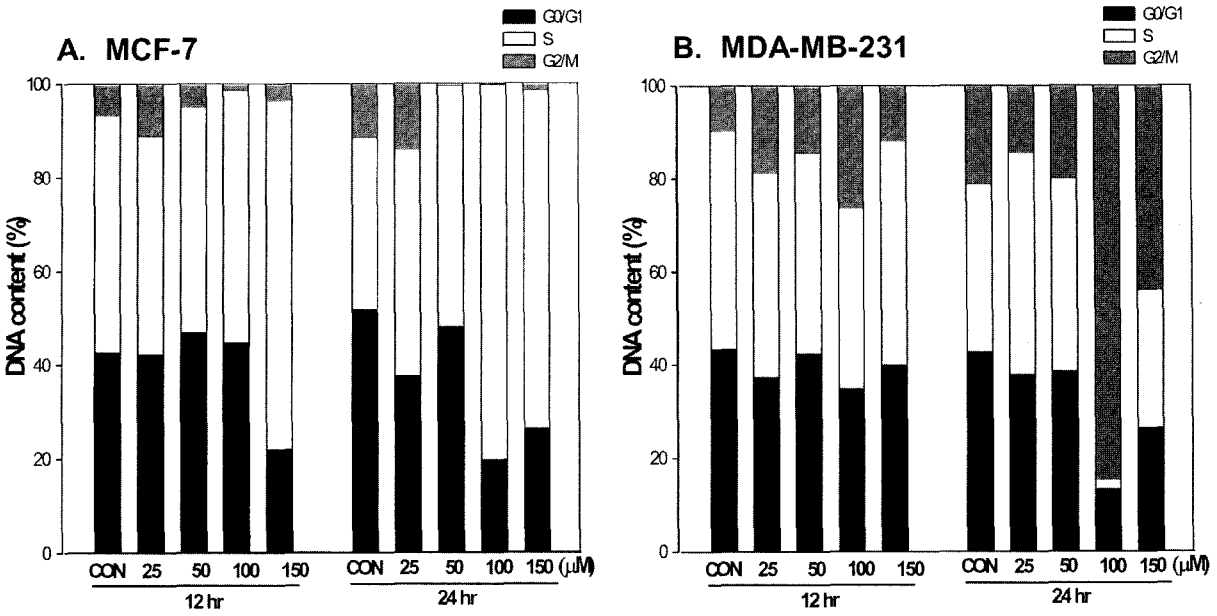


**Fig. 5.** Effects of cadmium on cell cycle distribution in MCF-7 cells (A) and MDA-MB-231 cells (B). Data represents the mean of more than duplicate measurements.

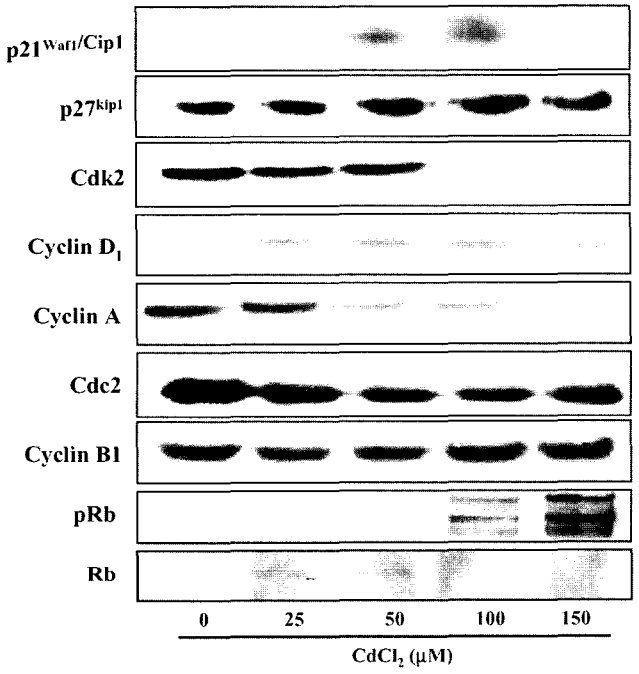


**Fig. 6.** Concentration-dependent effect of cadmium on the expression of $p21^{Waf/Cip1}$, $p27^{kip1}$, Cdk2, cyclin $D_1$, cyclin A, Cdc2, cyclin $B_1$, pRB and Rb in MCF-7 cells.

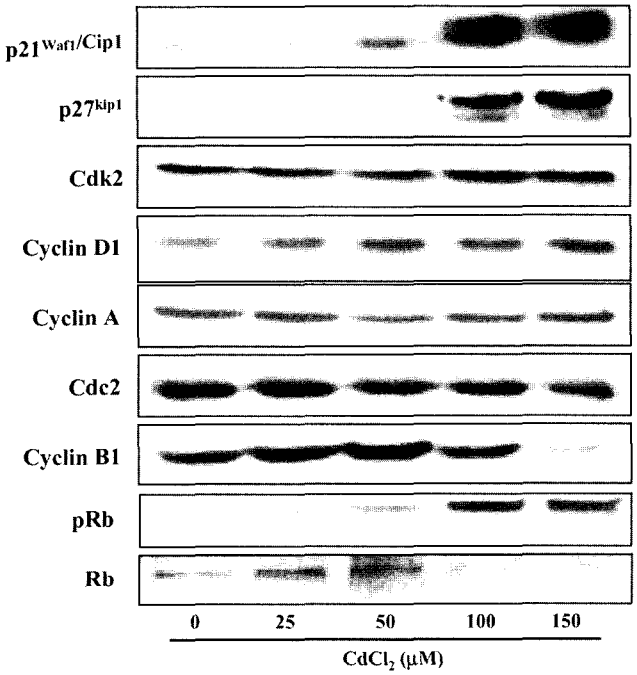


**Fig. 7.** Concentration-dependent effect of cadmium on the expression of $p21^{Waf/Cip1}$, $p27^{kip1}$, Cdk2, cyclin D1, cyclin A, Cdc2, cyclin $B_1$, pRB and Rb in MDA-MB-231 cells.

cade of caspase activation, especially caspase 9 (Bagchi and Stohs, 1995; Kim *et al.*, 2000; Yuan *et al.*, 2000). ROS produced during the xenobiotic metabolism, cause cellular damage including DNA mutation and modulate expression of protooncogenes and tumor suppressor genes (Zimmermann *et al.*, 2001). Moreover, since ROS modifies transcription factors and protein kinase cascades, it is possible that cells proceed abnormal proliferation or differentiation, and result in apoptotic cell death and cell cycle arrest, occasionally, cancer (Chao

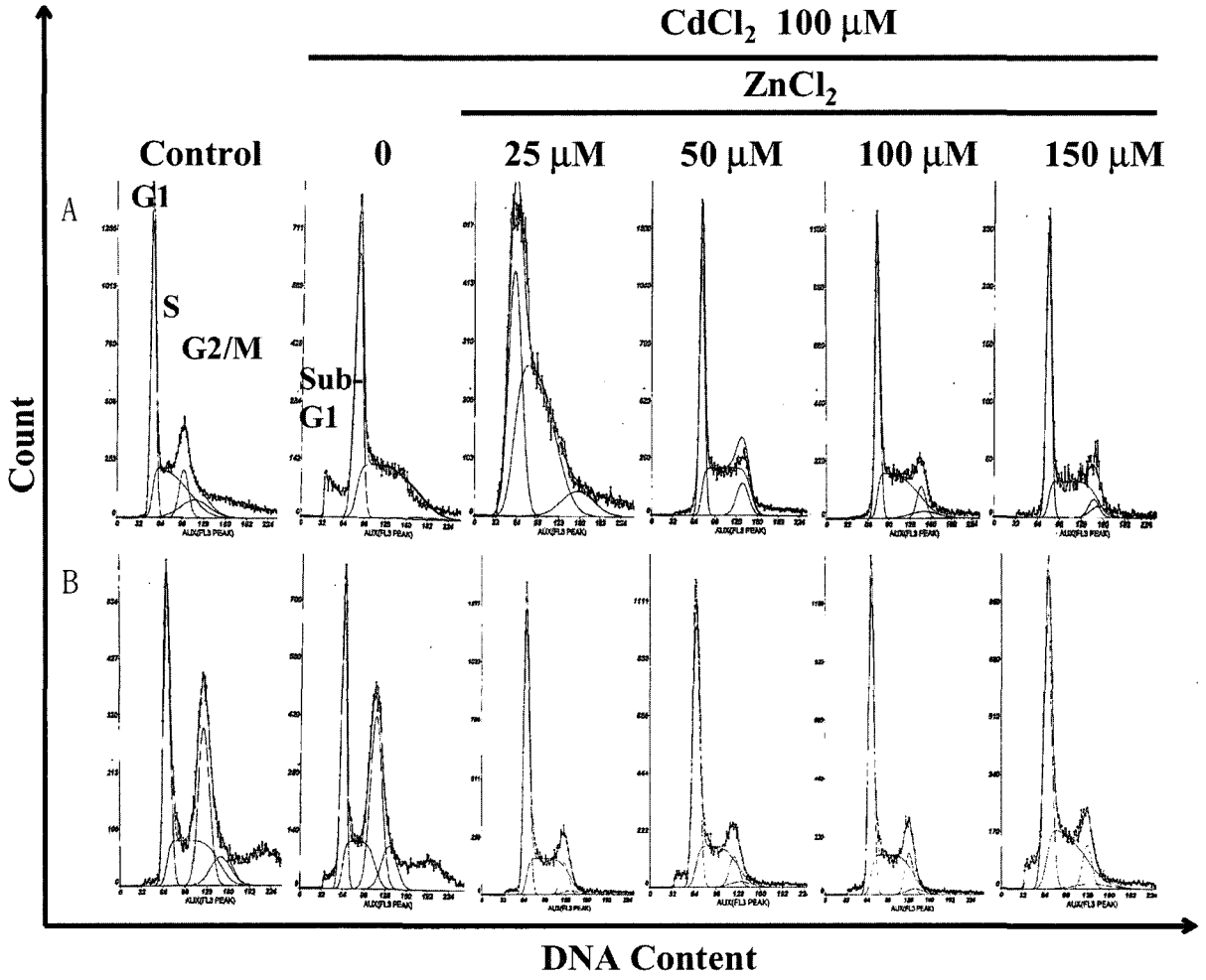


**Fig. 8.** Cell cycle analysis in MCF-7 (A) and MDA-MB-231 cells (B) cotreated with cadmium and a range of zinc. Cells were incubated for 12 hr with mixture of cadmium (100 μM) and zinc (25 μM, 50 μM, 100 μM or 150 μM).

and Yang, 2001).

In this study, codmium treatment caused cell cycle arrest in both cell lines; MCF-7 cells in S phase and MDA-MB-231 cells in G2/M phase. Since there was no remarkable change in G1 phase, we didn't included G1 specific cell cycle inhibitors in this study. Cadmium leads to cell cycle arrest by increasing cell cycle inhibitors as well as decreasing active cyclin/Cdk complexes, even though cadmium stimulated the Rb activation. These suggest that DNA synthesis keep on normally functioning but reduced active kinases are responsible for the failure in progression S to G/2 or G2 to mitotic phase. Specially, S phase arrest in MCF-7 cells was correlated with reduction in Cdk2 and cyclin A, which have critical role in progression S to G2/M phase. In MDA-MB-231 cell arrested in G2/M phase, cyclin B1 and Cdc2 protein, which participate in G2/M phase transition, were down-regulated but cyclin A, cyclin D1 and Cdk2 were not affected. From these observation, cadmium-evoked cellular signals resulted in cell cycle arrest in cell line specific manner. In cotreated cells with cadmium (100 μM) and zinc (100 μM), both cells kept in similar cell distribution with control cells as well as did not change in expression of Cdk2 and cyclin A in MCF-7 cell and Cdc2 and cyclin B1 in MDA-MB-231 cells. This suggest antioxidative metal, zinc, protect cell from cadmium-induced oxidative damages, even if the exact mechanism in protection could not be identified in this study.

Further studies using other antioxidant analyzed the recovery of viability. Vit. E (α-tocopherol), ascorbic acid (Vit. C) and resveratrol, which are known to protect human from oxidative stress by scavenging ROS and inhibition of lipid peroxidation in cellular membrane (Warren *et al.*, 2000; Hsu *et al.*, 1998). However, none of antioxidant except zinc showed observable protective effect in both cell lines (data were not shown).

Park *et al.* (2002) reported that cytotoxic concentration

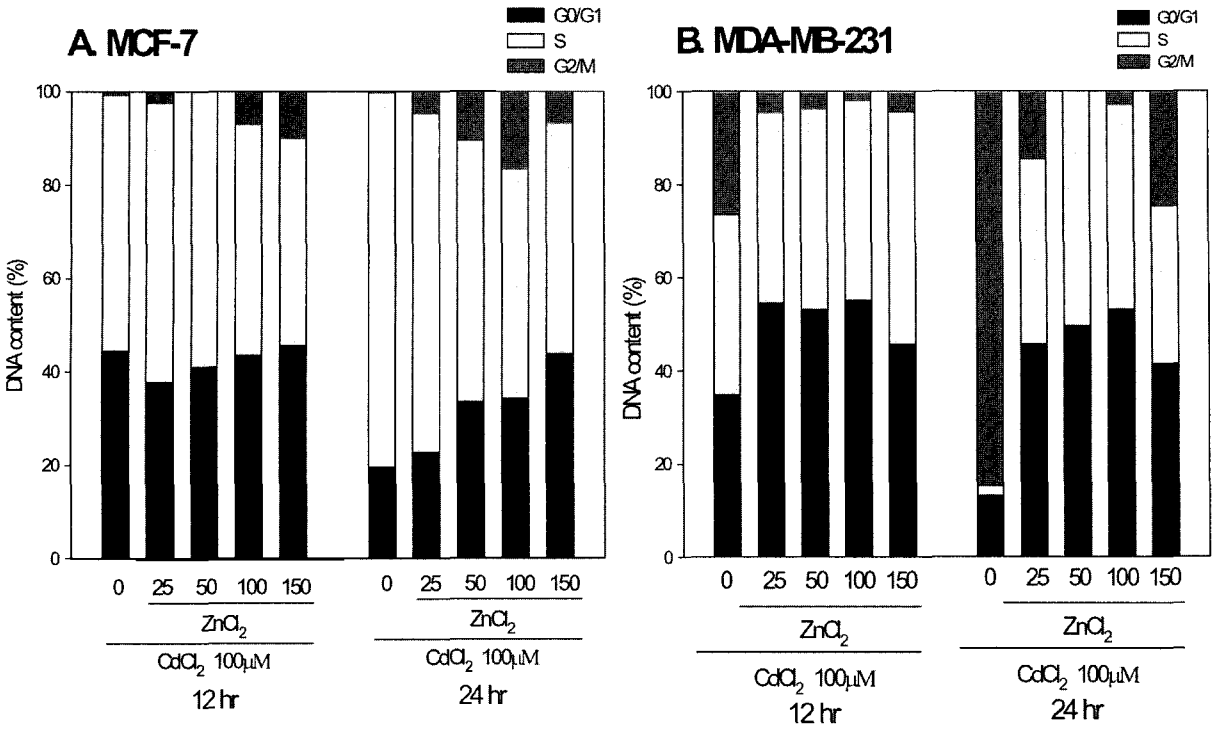


**Fig. 9.** Effect of zinc supplementation on cadmium induced cell arrest in MCF-7 (A) and MDA-MB-231 cells (B). Cells were incubated for 12 hr or 24 hr with cadmium or with mixture of cadmium (100 μM) and zinc (25 μM, 50 μM, 100 μM or 150 μM). Data represents the mean of more than duplicate measurements.

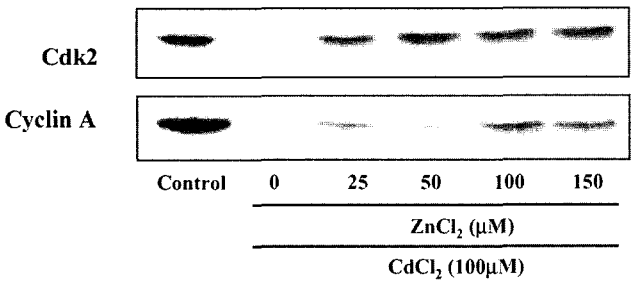


**Fig. 10.** Effect of mixture of cadmium (100 μM) and zinc (25 μM, 50 μM, 100 μM or 150 μM) supplementation on the expression of cdc2 and cyclin A in MCF-7 cells. Cells were treated with cadmium alone or mixture of cadmium and zinc for 12 hr.

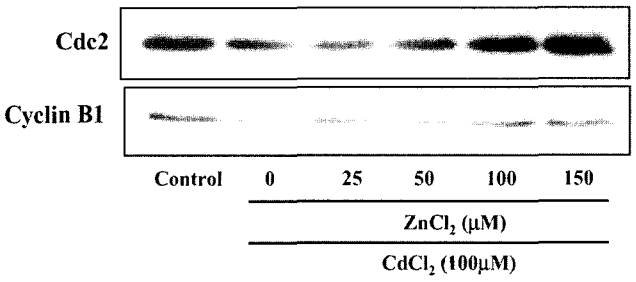


**Fig. 11.** Effect of mixture of cadmium (100 μM) and zinc (25 μM, 50 μM, 100 μM or 150 μM) supplementation on the expression of Cdc2 and cyclin B1 in MDA-MB-231 cells. Cells were treated with cadmium alone or mixture of cadmium and zinc for 12 hr.

of zinc inhibited cell growth by activation of $p21^{WAF1/Cip}$ via ERK dependent pathway. In present study, we have observed concentration-dependent increase in expression of p-ERK in both cell lines. Although it is generally known that JNK and p38 MAP kinase are mainly involved in apoptotic cell death, recent reports showed that ERK MAP kinase pathway took part in apoptosis as well (Chao and Yang, 2001; Son *et al.*, 2001). It has been known that transient ERK activation leads to proliferation while persistent activation mediates growth arrest. In present study, high concentration of cadmium (50~150 μM) significantly increased p-ERK expression and is likely to result in apoptosis. On the contrary, low concentration of cadmium somewhat decreased the p-ERK, which might contribute to cell cycle arrest (Chuang *et al.*, 2001). Thus, It could be inferred that cadmium-induced stress signals were mediated by ERK MAPK pathways and ERK activation were partially responsible for the cell cycle arrest in our study.

Cadmium differentially arrested MCF-7 in S phase and MDA-MB-231 cells in G2/M phase. We couldn't conclude the existence or lack of estrogen receptor in

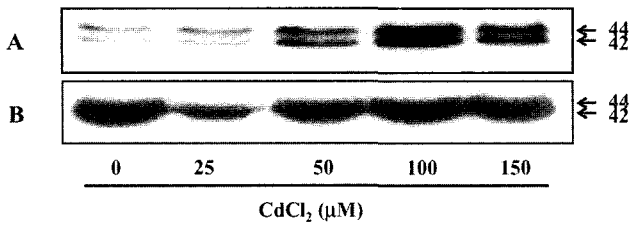


**Fig. 12.** Concentration-dependent effect of cadmium on the expression of p-ERK in MCF-7 cells (A) and MDA-MB-231 cells (B). Bands at 42 kDa and 44 kDa represent ERK1 and ERK 2 respectively.

the two cell lines caused the differences in cell cycle distribution, because these cell lines are basically driven from different origin and show distinguished cell characteristics. Thus further studies are needed to identify the attributed cell specific characters in cadmium-induced cell death and cell cycle arrest.

In conclusion, we observed that cadmium could induce apoptosis and cell cycle arrest in MCF-7 cells and MDA-MB-231 cells and that these effects could prevent by adding effective antioxidant. These findings, even though *in vitro* assay, could be usefull for miniming the adverse effect of cadmium exposure on human health.

## ACKNOWLEDGMENT

This study was supported by the 2003 research fund of the National Institute of Toxicological research/Korea Food & Drug Administration.

## REFERENCES

Chao, J.I. and Yang, J.L. (2001): Opposite roles of ERK and p38 Mitogen-Activated Protein Kinases in cadmium-induced genotoxicity and mitotic arrest. *Chem. Res. Toxicol.*, **14**, 1193-1202.

Chuang, S.M., Wang, I.C. and Yang, J.L. (2000): Roles of JNK, p38 and ERK mitogen-activated protein kinases in the growth inhibition and apoptosis induced by cadmium. *Carcinogenesis*, **21**, 1423-1432.

Garcia-Morales, P., Saceda, M., Kenney, N., Kim, N., Salomon, D.S., Gottardis, M.M., Solomon, H.B., Sholler, P.F., Jordan, V.C. and Martin, M.B. (1994): Effect of cadmium on estrogen receptor levels and estrogen-induced responses human breast cancer cells. *J. Biol. Chem.*, **269**, 16896-16901.

Hsu, P.C., Liu, M.Y., Hsu, C.C., Chen, L.Y. and Guo, Y.L. (1998): Effects of vitamin E and/or C on reactive oxygen species-related lead toxicity in the rat sperm. *Toxicology*, **128**, 169-179.

IARC (1993): Beryllium, cadmium, mercury and exposures in the glass manufacturing industry. In *IARC Monographs on the Evaluation of Carcinogenic Risks to Human*, 58, 41117, International Agency for Research on Cancer, Lyon.

Kim, M.S., Kim, B.J., Woo, H.N., Kim, K.W., Kim, K.B., Kim, I.K. and Jung, Y.K. (2000): Cadmium induces caspase-mediated cell death: suppression by Bcl-2. *Toxicology*, **145**, 27-37.

Koizumi, S. and Yamada, H. (2003): DNA microarray analysis of altered gene expression in cadmium-exposed human cells. *J. Occup. Health*, **45**, 331-334.

Park, K.S., Ahn, Y., Kim, J.A., Yun, M.S., Seong, B.L. and Choi, K.Y. (2002): Extracellular zinc stimulates ERK-dependent activation of $p21^{Cip/WAF1}$ and inhibits proliferation of colorectal cancer cells. *Br. J. Pharmacol.*, **137**, 597-607.

Pietenpol, J.A. and Stewart, Z.A. (2002): Cell cycle checkpoint signaling: cell cycle arrest versus apoptosis. *Toxicology*, **181-182**, 475-481.

Sarkar, S., Yadav, P. and Bhatnagar, D. (1998): Lipid peroxidative damage on cadmium exposure and alterations in antioxidant system in rat erythrocytes: a study with relation to time. *Biometals*, **11**, 153-157.

Son, M.H., Kang, K.W., Lee, C.H. and Kim, S.G. (2001): Potentiation of cadmium-induced cytotoxicity by sulfur amino acid deprivation through activation of exracellular signal-regulated kinase 1/2 (ERK1/2) in conjunction with p38 kinase or c-jun N terminal kinase (JNK). Complete inhibition of the potentiated toxicity by U0126 and ERK1/2 and p38 kinase inhibitor. *Biochem. Pharmacol.*, **62**, 1379-1390.

Stohs, S.J. and Bagchi, D. (1995): Oxidative mechanisms in the toxicity of metal ions. *Free Radic. Biol. Med.*, **18**, 321-336.

Szuster-Ciesielska, A., Stachura, A., Slotwinska, M., Kaminska, T., Sniezko, R., Paduch, R., Abramczyk, D., Filar, J. and Kandefer-Szerszen, M. (2000): The inhibitory effect of zinc on cadmium-induced cell apoptosis and reactive oxygen species (ROS) production in cell cultures. *Toxicology*, **145**, 159-171.

Waalkes, M.P. and Diwan, B.A. (1999): Cadmium-induced inhibition of the growth and metastasis of human lung carcinoma xenografts: role of apoptosis. *Carcinogenesis*, **20**, 65-70.

Waalkes, M.P. (2000): Cadmium carcinogenesis in review. *J. Inorg. Biochem.*, **79**, 241-4.

Warren, S., Patel, S. and Kapron, C.M. (2000): The effect of vitamin E exposure on cadmium toxicity in mouse embryo cells *in vitro*. *Toxicology*, **142**, 119-126.

Yuan, C., Kadiiska, M., Achanzar, W.E., Mason, R.P. and Waalkes, M.P. (2000): Possible role of caspase-3 inhibition in cadmium-induced blockage of apoptosis. *Toxicol. Appl. Pharmacol.*, **164**, 321-329.

Zimmermann, K.C., Bonzon, C. and Green, D.R. (2001): The machinery of programmed cell death. *Pharmacol. Ther.*, **92**, 57-70.