Cadmium-induced Oxidative Cellular Damage in Human Fetal Lung Fibroblasts (MRC-5 Cells)

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Epidemiological evidence suggests that cadmium (Cd) exposure causes pulmonary damage such as emphysema and lung cancer. However, relatively little is known about the mechanisms involved in Cd pulmonary toxicity. In the present study, the effects of Cd exposure on human fetal lung fibroblasts (MRC-5 cells) were evaluated by determination of lipid peroxidation, intracellular production of reactive oxygen species (ROS), and changes of mitochondrial membrane potential. A time- and dose-dependent increase of both lactate dehydrogenase leakage and malondialdehyde formation was observed in Cd-treated cells. A close correlation between these two events suggests that lipid peroxidation may be one of the main pathways causing its cytotoxicity. It was also noted that Cd-induced cell injury and lipid peroxidation were inhibited by catalase and superoxide dismutase, two antioxidant enzymes. By using the fluorescent probe 2′,7′-dichlorofluorescin diacetate, a significant increase of ROS production in Cd-treated MRC-5 cells was detected. The inhibition of dichlorofluorescein fluorescence by catalase, not superoxide dismutase, suggests that hydrogen peroxide is the main ROS involved. Moreover, the significant dose-dependent changes of mitochondrial membrane potential in Cd-treated MRC-5 cells, demonstrated by increased fluorescence of rhodamine 123 examined using a laser-scanning confocal microscope, also indicate the involvement of mitochondrial damage in Cd cytotoxicity. These findings provide *in vitro* evidence that Cd causes oxidative cellular damage in human fetal lung fibroblasts, which may be closely associated with the pulmonary toxicity of Cd. *Key words*: cadmium, cytotoxicity, lipid peroxidation, mitochondrial membrane potential, MRC-5 cells, ROS. *Environ Health Perspect* 105:712–716 (1997)

Cadmium (Cd), one of the common toxic heavy metals, is widely used in modern industry. Due to its extensive use and long biological half-life, the potential health effects of Cd to humans have attracted much attention over the years. Extensive studies have been carried out, and it is generally acknowledged that Cd exposure causes renal, skeletal, vascular, and respiratory disorders in humans (*1*). Based on evidence from both experimental and epidemiological investigations, the International Agency for Research on Cancer (IARC) has classified Cd as a Group 1 carcinogen in humans (*2*).

The lung is one of the main target organs for Cd toxicity, and several studies have shown that emphysema is one of the primary consequences resulting from Cd exposure (*3,4*), suggesting the possible involvement of lung fibroblasts in Cd pulmonary toxicity. However, studies on the toxic effects of Cd on lung fibroblasts are relatively rare compared to other target cells. Chambers et al. (*5*) demonstrated that Cd selectively inhibits procollagen production and proliferation of rat fetal fibroblasts, the cell type implicated in the pathogenesis of emphysema. Therefore, further studies on the adverse effects of Cd on lung fibroblasts may help to gain a better understanding of the mechanism involved in the pulmonary toxicity of Cd.

Although a number of studies have suggested that oxidative damage is involved in Cd-induced cytotoxicity, genotoxicity, and carcinogenicity, the exact mechanisms have not been fully elucidated. It has been shown that Cd is able to induce lipid peroxidation, one of the main manifestations of oxidative damage, under both *in vivo* and *in vitro* conditions (*6–10*). For instance, treatment of rats with a single dose of $CdCl_2$ increased the level of lipid peroxidation, iron content, and cellular production of reactive oxygen species (ROS), as well as DNA strand breakage in testicular Leydig cells, one of the target cells for Cd carcinogenesis (*8,9*). It has been proposed that Cd may initiate oxidative damage through the following two pathways: 1) depleting antioxidants such as glutathione and protein-bound sulfhydryl groups and 2) enhancing production of ROS (*11*).

On the other hand, there is growing evidence implicating mitochondria as important subcellular targets in xenobiotic-induced cell injury, particularly in oxidative cellular damage (*12,13*). The functional changes of mitochondria are usually manifested by the loss of mitochondrial membrane potential (MMP), which can be assessed using a fluorescent cationic dye, as the diffusion of the dye is proportional to the degree of MMP (*14*). At present, no reports are available showing the MMP changes in Cd-treated cells or tissues.

The primary objective of the present study was to evaluate Cd-induced oxidative cellular damage in a human fetal lung fibroblast cell line (MRC-5 cells) by studying Cd-induced lipid peroxidation, ROS production, and MMP changes. In addition, the effects of antioxidant enzymes such as catalase and superoxide dismutase on Cd-induced lipid peroxidation and ROS production in MRC-5 cells were also investigated. Results from the present study provide a better understanding on the mechanisms of Cd-induced pulmonary toxicity.

Materials and Methods

Cells and chemicals. The human fetal lung fibroblast cell line (MRC-5 cells) was from American Type Culture Collection (ATCC; Rockville, MD). Cadmium chloride ($CdCl_2$) and thiobarbituric acid (TBA) were purchased from Merck (Darmstadt, Germany); minimum essential medium (MEM) was from Gibco (Buffalo, NY); and fetal bovine serum (FBS) was from Cytosystems (Castle Hill, Australia). Rhodamine 123 (Rh-123), sodium dodecyl sulphate (SDS), penicillin, streptomycin, catalase (CAT), and superoxide dismutase (SOD) were from Sigma Chemical Co. (St. Louis, MO). 2′,7′,-Dichlorofluorescin diacetate (DCFH-DA) was purchased from Molecular Probes, Eugene, OR.

Cell culture and treatments. MRC-5 cells were cultured in complete MEM (10% FBS, 100 units/ml penicillin, 100 mg/ml streptomycin, pH 7.4) at 37°C in 95% O_2 and 5% CO_2. Cells in logarithmic growth phases (approximately 90% confluence) were used for various experiments.

In the dose–response study, various

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concentrations of $CdCl_2$ dissolved in FBS-free MEM were incubated with MRC-5 cells for 16 hr. In the time-course study, cells were treated with 35 µM $CdCl_2$ up to 20 hr. The inhibitory effects of CAT (1000 U/ml) and SOD (500 U/ml) on Cd-induced cell damage were tested after they were cultured together with 35 µM $CdCl_2$ for 16 hr.

Determination of lactate dehydrogenase leakage. Lactate dehydrogenase (LDH) activity was determined using an Abbott VP Biochemical Analyzer with the test kit (Abbott Laboratories, Chicago, IL), as described by Shen et al. (*15*). At the end of the experiment, an aliquot of medium (0.2 ml) was taken out for measuring extracellular LDH activity. The total LDH activity was determined after cells were disrupted thoroughly using sonication. The percentage of LDH leakage was then calculated to reflect the cytotoxicity of $CdCl_2$.

Measurement of lipid peroxidation. Malondialdehyde (MDA), an end product of lipid peroxidation, was measured to estimate the extent of lipid peroxidation in MRC-5 cells. MDA concentration in cell homogenate was determined using a TBA method as described by Uchiyama and Mihara (*16*), with modifications. Briefly, at the end of the experiment, cells were collected using a cell scraper and washed with PBS. Cell homogenate (0.5 ml in PBS with 1% SDS) was mixed with 3 ml 1% phosphoric acid and 1 ml 0.67% TBA and heated in boiling water for 60 min. After cooling, 1.5 ml *n*-butanol was added. After centrifugation, the absorbance of the butanol phase was read at 535 nm and 520 nm. The difference between 535 nm and 520 nm was used to calculate the MDA concentration, which was expressed as nanomoles per milligram protein.

Detection of ROS formation and effects of antioxidant enzymes. Cd-induced ROS formation in MRC-5 cells was detected by using a fluorescent probe, 2′,7′-diclorofluorescin diacetate (DCFH-DA), as described by Shen et al. (*17*). DCFH-DA diffuses through the cell membrane readily and is enzymatically hydrolyzed by intracellular esterases to nonfluorescent diclorofluorescin (DCFH), which is then rapidly oxidized to highly fluorescent diclorofluorescein (DCF) in the presence of ROS. The DCF fluorescence intensity is believed to be parallel to the amount of ROS formed intracellularly (*18*). The stock DCFH-DA (2 mM) was prepared in absolute ethanol and kept at -70°C in the dark. Cells collected from culture flasks using a cell scraper were washed twice with PBS prior to the analysis. Each fluorescence cuvette contained 0.6×10^5 cells in 3 ml PBS. $CdCl_2$ was added to the cells simultaneously with DCFH-DA (final concentration 2 µM) and incubated at 37°C up to 4 hr. The fluorescence intensity was monitored using a Perkin-Elmer spectrofluorometer LS-5B (Perkin Elmer, Beaconsfield, U.K.) with excitation wavelength at 485 nm and emission wavelength at 530 nm.

The inhibitory effects of CAT (1,000 U/ml) and SOD (500 U/ml) on ROS production were evaluated by the following approach: both enzymes were first pre-incubated with MRC-5 cells in culture flasks for 6 hr; cells were then collected and washed with PBS for the fluorescence test as described above.

Determination of mitochondrial membrane potential. MMP in intact MRC-5 cells was determined using Rh-123, a fluorescent dye. Mitochondria are stained by Rh-123 because of the high negative electrical potential across the mitochondrial membrane, and the diffusion of Rh-123 is directly proportional to the degree of MMP (*14*). MRC-5 cells were cultured in MEM in coverglass chambers. Before analysis, the cultured cells were washed once with HEPES-containing Hanks' balanced salt buffer (HBSS; 1.26 mM $CaCl_2$, 5.36 mM KCl, 0.44 mM KH_2PO_4, 0.49 mM $MgCl_2 \cdot 6H_2O$, 0.41 mM $MgSO_4 \cdot 7H_2O$, 0.137 M NaCl, 0.34 mM $Na_2HPO_4 \cdot 7H_2O$, 20 mM HEPES). Cells were then incubated with different concentrations of $CdCl_2$ (0, 8.75, 17.5, and 35 µM) for 1 hr, followed by incubation with 6 µg/ml Rh-123 for 30 min. After the removal of $CdCl_2$ and Rh-123 with HBSS, cells were evaluated immediately using a laser-scanning inverted confocal microscope (Carl Zeiss LSM 410, Jena, Germany). Rh-123 was excited using 488 nm laser line with a laser power of 10%. The emission signal was observed with a combination of a 510 nm dichroic mirror and a 515-516 nm cut-off filter. A heat platform was fitted to the microscope and set at 37°C throughout the analysis. The quantification of the Rh-123 fluorescence intensity in different groups was performed.

Statistical analysis. Data are presented as mean ± standard deviation (SD) and analyzed using one-way analysis of variance (ANOVA) with Scheffe's test or Student's *t*-test. A *p*-value of <0.05 was considered statistically significant.

Results

Cd-induced cytotoxicity in MRC-5 cells. The dose-dependent increase of LDH leakage in Cd-treated MRC-5 cells is shown in Figure 1A. It was found, with incubation for 16 hr, that the lowest concentration of $CdCl_2$ able to cause a significant change of LDH leakage was 8.75 µM. Based on this dose–response relationship, the median lethal concentration (LC_{50}) of $CdCl_2$ was calculated to be 40 µM. In the time-course study, MRC-5 cells were cultured with $CdCl_2$ (35 µM) up to 20 hr; the results are presented in Figure 1B. In the Cd-treated group, a significant increase of LDH activity in the medium was observed at 12 hr; with 20 hr of incubation, the percentage of LDH leakage in Cd-treated cells reached about 75%. On the other hand, LDH leakage in the control cells remained at a constantly low level within the entire incubation.

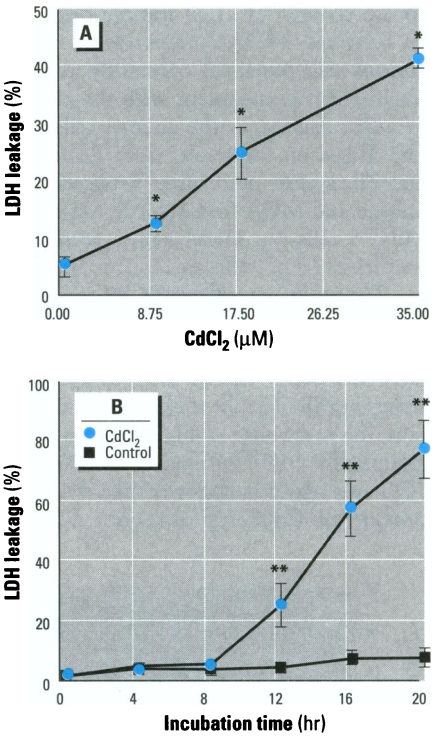


Figure 1. Cd-induced lactate dehydrogenase (LDH) leakage in cultured MRC-5 cells as shown by (A) dose response and (B) time-course. Data are expressed as mean ± standard deviation (n = 5–6). In the dose–response study, cells were incubated with $CdCl_2$ for 16 hr. In the time-course study, the exposure concentration of $CdCl_2$ was 35 µM.
*p<0.05 compared to zero $CdCl_2$ concentration (ANOVA with Scheffe's test).
**p<0.01 compared to their respective control groups (Student's t-test).

Cd-induced lipid peroxidation in MRC-5 cells. A clear dose-dependent increase of MDA concentration in Cd-treated MRC-5 cells is shown in Figure 2A. Similar to the dose response of LDH leakage, a significant increase in MDA concentration was observed with the lowest Cd dose (8.75 µM). Figure 2B shows the time-course changes in MDA concentration in Cd-treated MRC-5 cells. A significant increase in MDA concentration occurred at 12 hr after treatment, reaching the highest level at 20 hr, which is parallel to the time-

course changes of LDH leakage (Fig. 1B). In contrast, MDA concentration in the control cells remained constantly at a low level, which is consistent with the changes of LDH leakage in the control cells (Fig. 2B). Based on the results from Figures 1B and 2B, a close correlation between LDH leakage and MDA formation in Cd-treated MRC-5 cells was found with a correlation coefficient (r) of 0.96 (see insert in Fig. 2B).

The inhibitory effects of CAT and SOD on LDH leakage and MDA formation. Figure 3A shows the inhibitory effects of CAT and SOD on Cd-induced cytotoxic effects in MRC-5 cells. After a 16 hr incubation, the percentage of LDH leakage in the groups with 1,000 U/ml CAT or 500 U/ml SOD was significantly lower than the group treated with $CdCl_2$ (35 µM) only. A similar inhibitory effect was also observed in Cd-induced lipid peroxidation. CAT or SOD significantly reduced the MDA concentration in Cd-treated MRC-5 cells (Fig. 3B).

Cd-induced ROS formation and the inhibitory effects of CAT and SOD. In this part of the study, intracellular ROS formation was estimated by the changes in DCF fluorescence intensity. Figure 4A shows the dose-dependent increase of DCF fluorescence in MRC-5 cells when incubated with $CdCl_2$ for 4 hr. The time-course changes in ROS production in both the control and Cd-treated MRC-5 cells are presented in Figure 4B. It was found that with 30 min of incubation, the fluorescence intensity in Cd-treated cells was significantly higher than that of the control cells. A nearly 100% increase was noted in the Cd-treated cells compared to the control cells with a 4-hr incubation.

The inhibitory effects of CAT and SOD on ROS formation in Cd-treated MRC-5 cells are shown in Figure 5. CAT (1,000 U/ml) completely inhibited the increase of Cd-induced ROS formation, and the fluorescence intensities in the groups of CAT pretreatment alone and CAT-Cd co-treatment were even significantly lower than that in the control cells. In contrast, SOD failed to show any inhibitory effects on Cd-induced ROS formation in MRC-5 cells.

Cd-induced MMP changes in MRC-5 cells. Figure 6 shows the dose-dependent changes of Rh-123 fluorescence intensity quantified using a confocal microscope. In addition, the fluorescence images of the control cells and cells treated with the highest $CdCl_2$ concentration (35 µM) are presented in Figure 7. In the control cells, the Rh-123 fluorescence is located in areas around the nucleus that correspond to the distribution of mitochondria (Fig. 7A). After a 1-hr exposure to $CdCl_2$, Rh-123 fluorescence intensity increased and appeared diffusely in the cytoplasm, with poorly defined and irregular cell morphology (Fig. 7B).

Discussion

Evidence has suggested that occupational exposure to Cd causes serious lung damage including emphysema (*3,4*). However, at present, the mechanisms responsible for

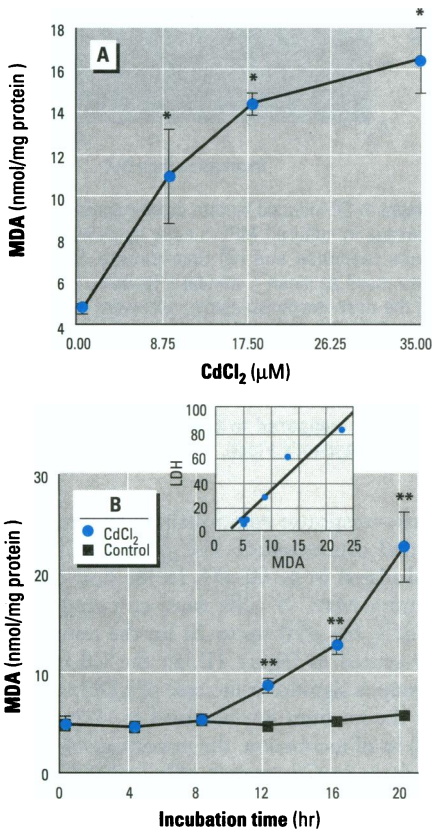


Figure 2. Cd-induced malondialdehyde (MDA) formation in cultured MRC-5 cells as shown by (A) dose response and (B) time-course. Data are expressed as mean ± standard deviation (n = 5–6). In the dose–response study, cells were incubated with $CdCl_2$ for 16 hr. In the time-course study, the exposure concentration of $CdCl_2$ was 35 µM. The insert in B indicates the correlation between the percentage of lactate dehydrogenase (LDH) leakage and MDA concentration (nmol/mg protein) in Cd-treated MRC-5 cells, based on the data from Figs. 1B and 2B. $y = 13.737 + 4.3705x$; $r = 0.96$.
*$p<0.05$ compared to zero $CdCl_2$ concentration (ANOVA with Scheffe's test).
**$p<0.01$ compared to their respective control groups (Student's t-test).

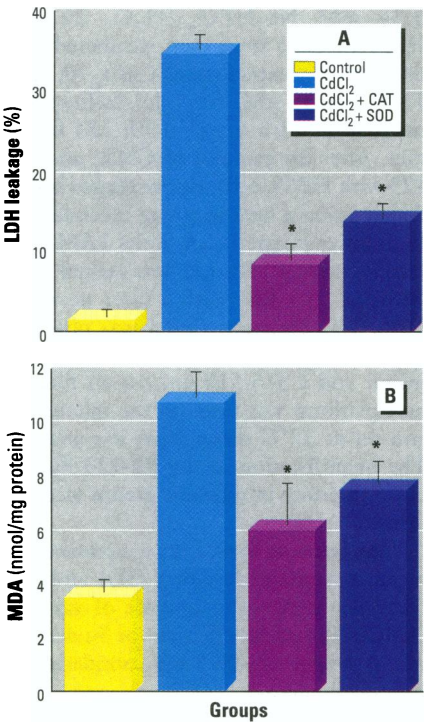


Figure 3. The inhibitory effects of catalase (CAT) or superoxide dismutase (SOD) on (A) lactate dehydrogenase (LDH) leakage and (B) malondialdehyde (MDA) formation in Cd-treated MRC-5 cells. Data are expressed as mean ± standard deviation (n = 5). Cells were treated with 35 µM $CdCl_2$ for 16 hr with or without catalase (1,000 U/ml) and SOD (500 U/ml).
*$p<0.05$ compared to the group treated with $CdCl_2$ only (ANOVA with Scheffe's test).

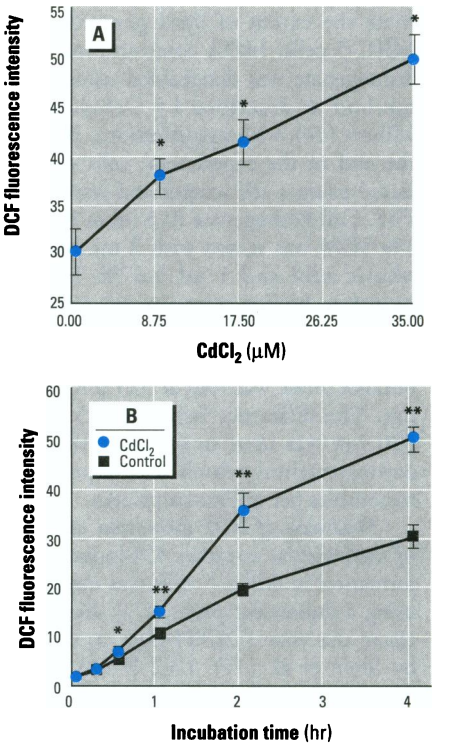


Figure 4. Elevated level of dichlorofluorescein (DCF) fluorescence in Cd-treated MRC-5 cells as shown by (A) dose response and (B) time-course. The reaction took place with 0.6×10^5 cells and 2 µM 2′,7′-dichlorofluorescin diacetate in 3 ml PBS. Data are expressed as mean ± SD (n = 5–6). In the dose–response study, cells were incubated with $CdCl_2$ for 4 hr. In the time-course study, the exposure concentration of $CdCl_2$ was 35 µM.
*$p<0.05$ compared to 0 $CdCl_2$ concentration (ANOVA with Scheffe's test).
**$p<0.01$ compared to their respective control groups (Student's t-test).

Cd-induced pulmonary toxicity have not been fully understood. In the present study, the cytotoxic effects of Cd on a human fetal lung fibroblast cell line (MRC-5 cells) were evaluated by studying Cd-induced lipid peroxidation, ROS production, and MMP changes. The results clearly indicate that Cd is able to cause oxidative cellular damage in lung fibroblasts, manifested by lipid peroxidation, elevated level of ROS formation, and mitochondrial membrane damage, which eventually leads to irreversible cell injury.

Lipid peroxidation is one of the main manifestations of oxidative damage and is closely associated with the toxicity of many heavy metals including Cd (*10,11*). Free radical scavengers and antioxidants, such as glutathione, vitamin E, vitamin C, butylated hydroxyanisole, butylated hydroxytoluene, and metallothionein, etc., are capable of protecting against Cd toxicity (*6,19–21*). However, most of these studies used kidney/renal cells or testis/testicular cells as experimental models. In the present study, Cd-induced cytotoxicity and lipid peroxidation were studied by measuring LDH leakage and MDA production in lung fibroblasts. Both time- and dose-dependent changes of these two parameters were noted in Cd-treated cells (Fig. 1, 2). Moreover, a close correlation between these two events was also found. Thus, lipid peroxidation may be one of the important events responsible for pulmonary toxicity of Cd. Manca et al. (*7,22*) investigated the susceptibility of various organs (liver, kidney, brain, lung, heart, and testis) of rats administered Cd and found that lung and brain had the greatest increases in lipid peroxidation. Similar findings, together with the changes of lung antioxidant systems in Cd-instillated rat lungs, were also found by Salovsky et al. (*23*).

In a series of studies of Cd-induced carcinogenesis, Koizumi et al. (*8,9*) found that cellular production of H_2O_2 one of the main components of ROS, was remarkably enhanced in Cd-treated rat testicular Leydig cells, the target cell population for Cd carcinogenesis. However, so far there is no experimental evidence showing the elevated level of intracellular ROS production in Cd-treated lung cells. In the present study, Cd-induced ROS formation was detected with a fluorescent probe, DCFH-DA. The time- and dose-dependent increases of DCF fluorescence intensity (Fig. 4) clearly indicate the significant increase of ROS production in Cd-treated MRC-5 cells. Moreover, it was found that a significant increase in ROS production started as early as 0.5 hr after Cd treatment and apparently preceded LDH leakage and MDA formation, suggesting the causative role of ROS production in initiation of lipid peroxidation and other forms of oxidative damage.

Significant changes of activities of CAT and SOD, two important antioxidant enzymes, have been noted in various tissues or cells with Cd exposure, indicating the involvement of these two enzymes in Cd toxicity (*10,23,24*). In the present study, the protective effects of CAT against Cd-induced LDH leakage, lipid peroxidation, and ROS formation (Fig. 3, 5) suggest that H_2O_2 is one of the main ROS generated in Cd-treated MRC-5 cells and is responsible for the oxidative damage observed. On the other hand, SOD inhibited Cd-induced LDH leakage and lipid peroxidation (Fig. 3), but failed to reduce DCF fluorescence formation in Cd-treated cells (Fig. 5). Similar results were also found in some other studies (*17,25,26*). Therefore, it seems that superoxide radicals play an important role in Cd-induced oxidative damage, although they are not directly involved in DCFH oxidation. As exogenous CAT and SOD are unable to pass the cell membrane freely, it has been suggested that CAT or SOD is able to enter cells through endocytosis, which is a relatively slow process (*27,28*). In the present study, CAT and SOD were either incubated together with Cd throughout the study (for the LDH and MDA tests) or preincubated for 6 hr before Cd treatment (for the DCF fluorescence test). It is thus believed that a significant amount of CAT or SOD entered MRC-5 cells to exert their protective effects.

Rh-123 is the most commonly used fluorescent probe for detecting MMP changes. It is believed that when Rh-123 accumulates electrophoretically in the mitochondrial matrix, its fluorescence is quenched due to stacking, aggregation, changes in polarity or ionization, and so forth (*13*). Therefore, the dose-dependent increase of Rh-123 fluorescence in Cd-treated MRC-5 cells (Fig. 6)

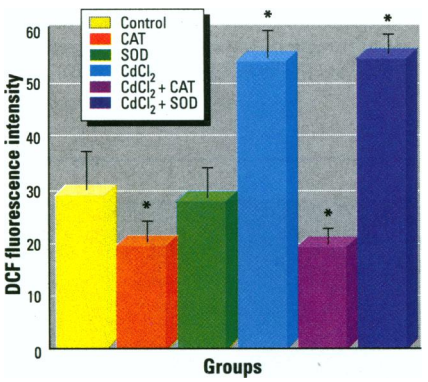


Figure 5. Effects of catalase (CAT) and superoxide dismutase (SOD) on reactive oxygen species production in Cd-treated MRC-5 cells. CAT (1000 U/ml) and SOD (500 U/ml) were preincubated for 6 hr prior to the analysis. Data are expressed as mean ± SD (n = 5–6).
*p<0.05 compared to the control group (ANOVA with Scheffe's test).

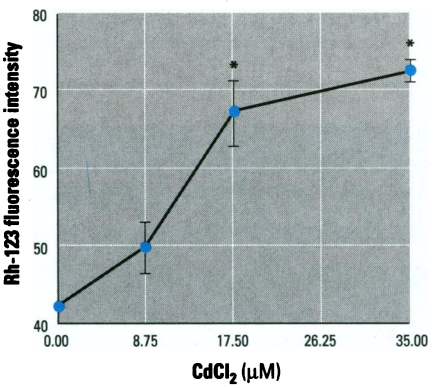


Figure 6. The dose-dependent increase of Rh-123 fluorescence in Cd-treated MRC-5 cells. Data are expressed as mean ± standard deviation (n = 3).
*p<0.05 compared to the control group (zero $CdCl_2$ concentration) (ANOVA with Scheffe's test).

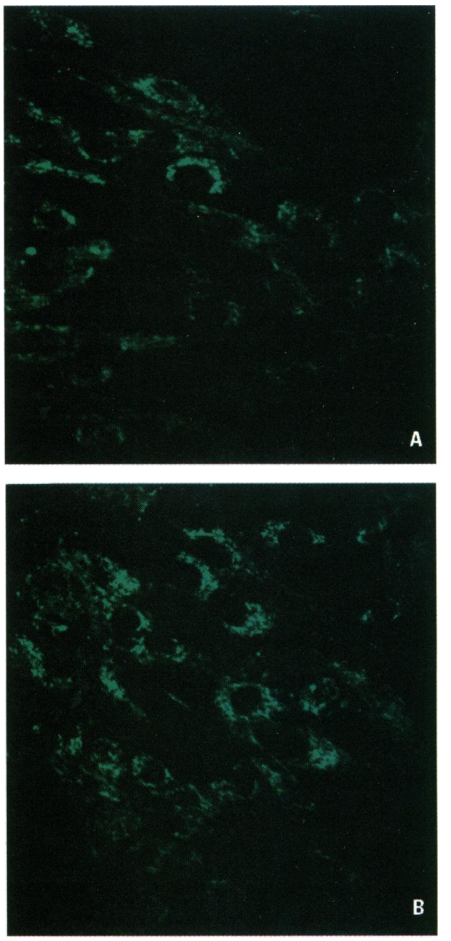
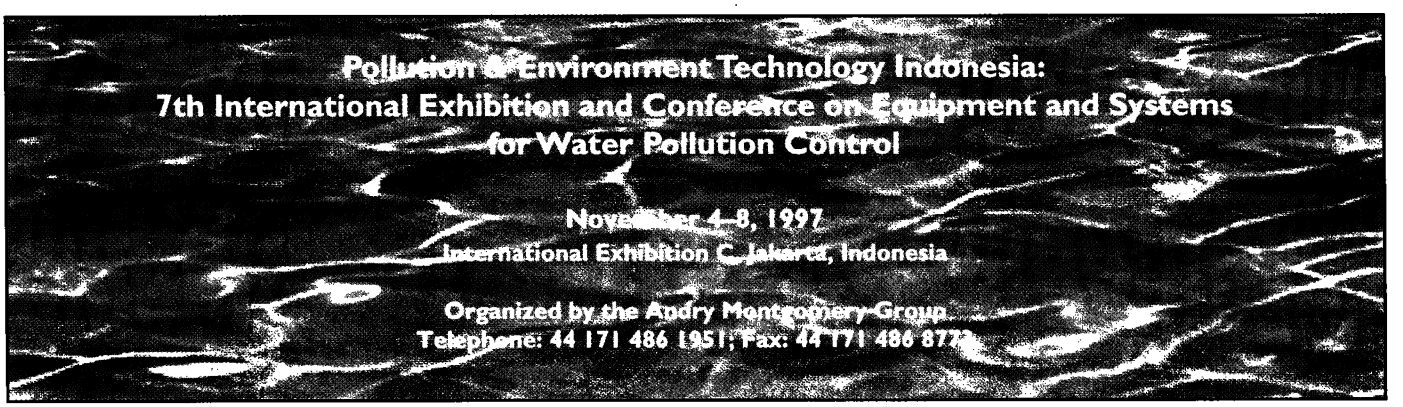


Figure 7. Images of MRC-5 cells showing mitochondrial membrane potential changes by the increase of Rh-123 fluorescence level in (A) control cells and (B) cells treated with 35 μM $CdCl_2$ for 1 hr.

indicates the decrease of MMP, resulting in the release of the probe from mitochondrial matrix into cytoplasm. The close relationship between MMP changes, lipid peroxidation, and cell injury has been found in liver and renal cells (*12,13,29*). In the present study, significant MMP changes in lung fibroblasts were observed with 1 hr Cd treatment. Thus, it seems that MMP changes occur well before MDA formation and LDH leakage. There are two possible consequences of mitochondrial damage: ATP depletion and further enhancement of intracellular ROS production, both of which could contribute to Cd-induced lipid peroxidation and irreversible cell injury. Nevertheless, the exact role of mitochondrial damage in Cd toxicity has yet to be investigated further.

In summary, the present study demonstrates the ability of Cd in inducing oxidative cellular damage in cultured human fetal lung fibroblasts (MRC-5 cells). The results show that Cd exposure enhances intracellular ROS production, causes changes of MMP, and increases lipid peroxidation, which eventually leads to cell damage and death. It is thus believed that Cd-induced oxidative cellular damage plays an important role in Cd-induced pulmonary toxicity.

References

1. Nordberg GF. Application of the critical effect and critical concentration concept to human risk assessment for cadmium. In: Cadmium in the Human Environment: Toxicity and Carcinogenicity (Nordberg GF, Herber RFM, Alessio L, eds). Lyon:IARC Scientific Publications No 118, 1992;3–14.
2. IARC. IARC Monographs on the Evaluation of Carcinogenic Risks to Humans: Beryllium, Cadmium, Mercury and Exposures in the Glass Manufacturing Industry. Vol 58. Lyon: Intenational Agency for Research on Cancer, 1993.
3. Elinder CGT, Kjellstrom C, Hogstedt K, Andersson H, Spang G. Cancer motality of cadmium workers. Br J Ind Med 42:651–655 (1985).
4. Davison AG, Newman Taylor AJ, Darbyshire J, Chettle DR, Guthrie CJG, O'Malley D, Mason HJ, Fayers PM, Venables KM, Pickering CAC. Cadmium fume inhalation and emphysema. Lancet 1:663–667 (1988).
5. Chambers RC, McAnulty RJ, Shock A, Campa JS, Newman AJ, Laurent GJ. Cadmium selectively inhibits fibroblast procollagen production and proliferation. Am J Physiol 267:L300–L308 (1994).
6. Fariss MW. Cadmium toxicity: unique cytoprotective properties of alpha tocopheryl succinate in hepatocytes. Toxicology 69:63–77 (1991).
7. Manca D, Ricard AC, Trottier B, Chevalier G. Studies on lipid peroxidation in rat tissues following administration of low and moderate doses of cadmium chloride. Toxicology 67:303–323 (1991).
8. Koizumi T, Li ZG. Role of oxidative stress in single-dose, cadmium-induced testicular cancer. J Toxicol Environ Health 37:25–36 (1992).
9. Koizumi T, Li ZG, Tatsumoto H. DNA damaging activity of cadmium in Leydig cells, a target cell population for cadmium. Toxicol Lett 63:211–220 (1992).
10. Sarkar S, Yadav P, Trivedi R, Bansal AK, Bhatnagar D. Cadmium-induced lipid peroxidation and the status of the antioxidant system in rat tissues. J Trace Elem Med Biol 9:144–149 (1995).
11. Stohs SJ, Bagchi D. Oxidative mechanisms in the toxicity of metal ions. Free Radical Biol Med 18:321–336 (1995).
12. Carini R, Parola M, Dianzani MU, Albano E. Mitochondrial damage and its role in causing hepatocyte injury during stimulation of lipid peroxidation by iron nitriloacetate. Arch Biochem Biophys 297:110–118 (1992).
13. Palmeira CM, Moreno AJM, Madeira VMC, Wallace KB. Continuous monitoring of mitochondrial membrane potential in hepatocyte cell suspensions. J Pharmacol Toxicol Methods 35:35–43 (1996).
14. Chen LB. Mitochondrial membrane potential in living cells. Annu Rev Cell Biol 4:155–181 (1988).
15. Shen HM, Ong CN, Shi CY. Involvement of reactive oxygen species in aflatoxin B_1-induced cell injury in cultured rat hepatocytes. Toxicology 99:115–123 (1995).
16. Uchiyama M, Mihara M. Determination of malonaldehyde precursor in tissues by thiobarbituric acid test. Anal Biochem 86:271–278 (1978).
17. Shen HM, Shi CY, Shen Y, Ong CN. Detection of elevated reactive oxygen species level in cultured rat hepatocytes treated with aflatoxin B_1. Free Radical Biol Med 21:139–146 (1996).
18. LeBel CP, Ischiopoulos H, Bondy SC. Evaluation of the probe 2′,7′-dichlorofluorescin as an indicator of reactive oxygen species formation and oxidative stress. Chem Res Toxicol 5:227–231 (1992).
19. Fox MRS, Fry BE Jr. Cadmium toxicity decreased by dietary L-ascorbic acid supplements. Science 169:989–991 (1970).
20. Chubastu LS, Gennari M, Meneghini R. Glutathione is the antioxidant responsible for resistance to oxidative stress in V79 Chinese hamster fibroblasts rendered resistant to cadmium. Chem Biol Interact 82:99–110 (1992).
21. Peters JM, Duncan JR, Wiley LM, Keen CL. Influence of antioxidants on cadmium toxicity of mouse preimplantation embryos *in vitro*. Toxicology 99:11–18 (1995).
22. Manca D, Ricard AC, Tra HV, Chevalier G. Relation between lipid peroxidation and inflammation in the pulmonary toxicity of cadmium. Arch Toxicol 68:364–369 (1994).
23. Salovsky P, Shopova V, Dancheva V, Marev R. Changes in antioxidant lung protection after single intratracheal cadmium acetate instillation in rats. Hum Exp Toxicol 11:217–222 (1992).
24. Sharma G, Nath R, Gill KD. Effect of ethanol on cadmium-induced lipid peroxidation and antioxidant enzymes in rat liver. Biochem Pharmacol 42(suppl):9–16 (1991).
25. Carter QO, Narayanan PK, Robinson JP. Intracellular hydrogen peroxide and superoxide anion detection in endothelial cells. J Leukocyte Biol 55:253–258 (1994).
26. Zhu H, Bannerberg GL, Moldeus P, Shertzer HG. Oxidation pathways for the intracellular probe 2′,7′-dichlorofluorescein assay. Anal Biochem 68:111–116 (1994).
27. Beckman JS, Minor RL Jr, White CW, Repine GM, Rosen JE, Freeman BA. Superoxide dismutase and CAT conjugated to polyethylene glycol increases endothelial enzyme activity and oxidant resistance. J Biol Chem 263:6884–6892 (1988).
28. Ito Y, Hiraishi H, Razandi M, Terano A, Harada T, Ivey KJ. Role of cellular superoxide dismutase against reactive oxygen metabolite-induced cell damage in cultured rat hepatocytes. Hepatology 16:247–254 (1992).
29. Wu EY, Smith MT, Bellomo G, DiMonte D. Relationships between the mitochondrial transmembrane potential, ATP concentration, and cytotoxicity in isolated rat hepatocytes. Arch Biochem Biophys 282:358–362 (1990).