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Original Article

The cytotoxic effects of cadmium chloride on the human lung carcinoma (Calu-6) cell line

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Abstract

Cadmium has been known to be harmful to human health, mainly via contaminated drinking water, food supplies, tobacco and industrial pollutants. The aim of this study was to determine the ability of cadmium chloride ($CdCl_2$) to cause cell death in the human lung carcinoma cell line (Calu-6). The cells were grown in RPMI-1640 medium supplemented with 10% FCS, penicillin/streptomycin (100 U/ml, 100 μg/ml) at 37 °C in 5% CO_2/95% air. The cells were plated in 96 multi-well plates. After 24 h, the medium was replaced with fresh medium containing different concentrations of $CdCl_2$ and incubated for 48 and 72 h. MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide) cell viability test was used to study the cytotoxic effects of Cd. Exposure of monolayers to different metal concentrations (1-1000 μM) in different times showed a significant decrease ($P<0.05$) in cell viability when compared with that of controls. In a dose-dependent manner, a significant cytotoxicity was observed at 200 μM for $CdCl_2$ during 48 h and 1 μM during 72 h exposure. However the concentrations greater than 200 μM had no significant effect to accelerate the power of cytotoxicity. Thus, $CdCl_2$ has this ability to induce cytotoxicity in the human lung carcinoma cell line in the lower micromolar concentrations. In conclusion, while high concentrations of Cd are harmful to human, lower concentrations induce a significant cytotoxicity in the cancer cells. This finding may introduce a new view on the mode of action and possible application of trace elements in the cancer treatment.

Keywords: Cytotoxicity; Human lung carcinoma; Cadmium

INTRODUCTION

Cadmium (Cd) is a soft, bluish-white bivalent heavy metal. It is widely used in industry mainly in nickel-Cd battery manufacturing, welding and electroplating, manipulation of paints and plastic stabilizers, and ore smelting. Because of its rising accumulation in the environment, Cd is a prevalent environmental contaminant. Elemental Cd and Cd compounds have been known to be harmful to human health. In the general population, the main contaminated sources are drinking water, food supplies, industrial pollutants and tobacco or cigarette smoking (1-3). Epidemiological studies have shown that some correlation exists between this heavy metal occupational exposure and human cancers such as lung, renal and prostate cancer. Therefore, Cd has been classified as a carcinogen by International Agency for Research in Cancer (IARC) (4,5). The half life of Cd in the human body is more than 20 years. So it has enough time to accumulate and cause tissue damage in several organs including lung, liver, kidney, cardiovascular, and nervous system, due to occupational intoxication (1). Cd has also been demonstrated to be a cytotoxic agent that induces cell death either by necrotic or apoptotic mechanisms and affect cells through very complex functions such as: reactive oxygen species (ROS) generation and oxidative damage, disruption of mitochondrial function, lysosomal damage, interfering with calcium and zinc and inhibition of DNA repair. While high concentrations of Cd are harmful to human, limited administration of

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lower concentrations may induce cytotoxicity in cancer cells (6). The main cause of lung diseases such as emphysema, chronic bronchitis and lung cancer is smoking. Tobacco smoking is the most prevalent source of Cd exposure in human so that blood and urine Cd levels of smokers are significantly higher than non-smokers (7).

The aim of this study was to determine the ability of $CdCl_2$ to cause cell death in the human lung carcinoma cell line (Calu-6).

MATERIALS AND METHODS

Chemicals

$CdCl_2$ and other chemicals were of analytical grade. All culture media, reagents and growth supplements were purchased from Gibco. Flasks, petri dishes and multi-well plates were obtained from Nunc via local venders.

Cell lines and cell culture conditions

Human lung adenocarcinoma cell line (Calu-6) was obtained from Pasture Institute of Iran and grown in RPMI-1640 media supplemented with 10% FCS, 100 U/ml penicillin and 100 µg/ml streptomycin at 37 °C in 5% CO_2/95% air. The cells were cultured in 25 cm^2 cell culture flasks and examined regularly for contamination and growth assessment.

Drug treatment

For experimental purposes, cells were cultured in 96 multi-well plates. The optimum cell concentration (30,000 cells/ml) were plated and allowed to attach to the wells. After 24 h, the medium was replaced with fresh medium containing different concentrations of $CdCl_2$ (1-1000 µM) and incubated for 48 and 72 h.

Cell viability assay

The number of living cells affected by different concentration of $CdCl_2$ was determined using MTT assay (8). Briefly, Calu-6 human lung adenocarcinoma cell line was cultured in the presence or absence of $CdCl_2$. At the end of incubation MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide) solution was added to each well (0.5 mg/ml) and incubation continued further for additional 4 h, then replaced with DMSO. The optical density of samples was read on spectrophotometer at 540 nm. The absorbance directly proportions to the number of living cells. The results are expressed as percent of control cells.

Statistical analysis

One-way analysis of variance (ANOVA) was performed followed by post-hoc Dunnett's test, using SPSS software, v. 11.0. Each experiment was carried out in triplicate and repeated three to four times independently. Differences were considered statistically significant when a $P<0.05$ was found. All data in the figures and text are presented as means ± S.D. of n observations (with n>9).

RESULTS

MTT cell viability test was used to study the cytotoxic effects of $CdCl_2$. Exposure of monolayers to different metal concentrations (1-1000 µM) in different times showed a significant decrease ($P<0.05$) in cell viability when compared with that of controls. In a dose and time dependent manner, a significant cytotoxicity was observed at 1 µM of $CdCl_2$. For example, at 1 µM concentration of $CdCl_2$, the percent of cell growth was reduced to 80% (i.e., about 20% of the cells died) of the control at 48 h (Fig. 1). $CdCl_2$ at 200 µM concentration was found to be very toxic for the cells and reduced cell viability to 50% (Fig.1). However the concentrations greater than 200 µM had no significant cytotoxic effects compared to lower effective doses. Increasing incubation time resulted in increased toxicity. As shown in Fig. 2, significant cytotoxicity of $CdCl_2$ was shifted to the lower micromolar concentration at 72 h, for example at 1 µM of $CdCl_2$ cell viability was reduced to 69% which reached to the maximum at 200 µM (nearly 43%). Like 48 h, higher concentrations of $CdCl_2$ did not potentiate the observed cytotoxicity.

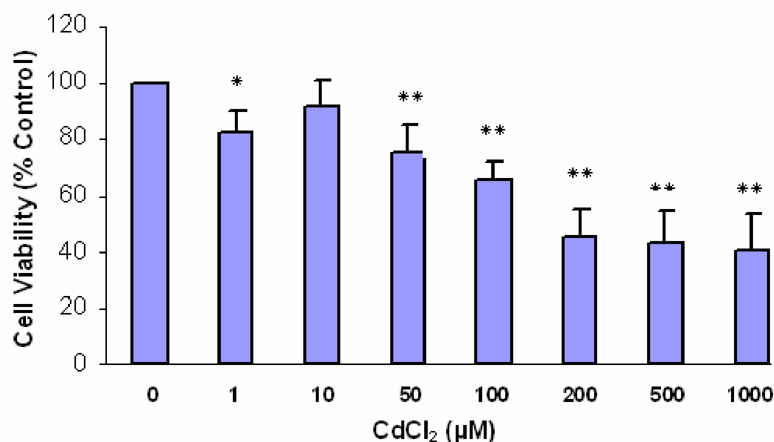

Fig. 1. Effects of cadmium chloride on survival of human lung adenocarcinoma (Calu-6) cell line at 48 h. Cells were treated with $CdCl_2$ at concentrations from 1-1000 μM and incubated for 48 h, then cell viability was quantified by MTT assay. Results (Mean ± SD) were calculated as percent of corresponding control values. Statistical analysis was performed by ANOVA followed by Dunnett's test. Each point represents 3 repeats of triplicate. Stars show that each value is significantly different when compared with control (*P< 0.05 and **P< 0.01)

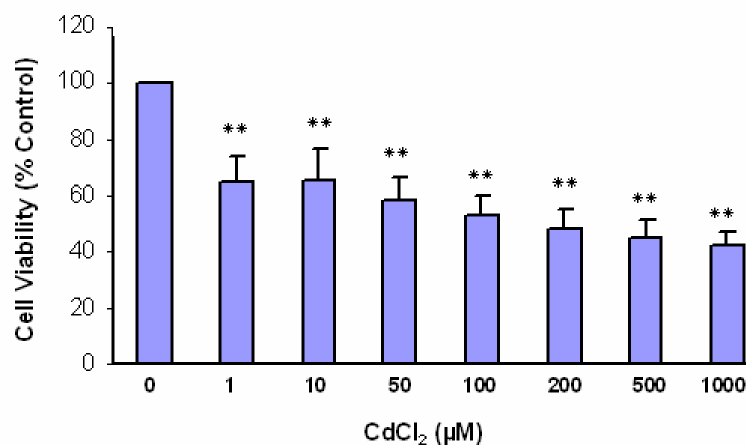

Fig. 2. Effects of cadmium chloride on survival of human lung adenocarcinoma (Calu-6) cell line at 72 h. Cells were treated with $CdCl_2$ at concentrations from 1-1000 μM and incubated for 72 h, then cell viability was quantified by MTT assay. Results (Mean ± SD) were calculated as percent of corresponding control values. Statistical analysis was performed by ANOVA followed by Dunnett's test. Each point represents 3 repeats of triplicate. Stars show that each value is significantly different when compared with control (*P< 0.05 and **P< 0.01).

DISCUSSION

There is a clear association between occupational Cd exposure and human cancers (4-6). Via the contamination of the outermost layer of soil (topsoil), Cd accumulates in plants and through consumption of food and tobacco enters the body. However it is also important to consider its occupational industrial sources (9). Interestingly, a comprehensive study in the vicinity of Cd related industrial sites showed an increased lung cancer risk in relation to elevated urinary Cd (9).The main cause of lung cancer (as the most

common cause of cancer related death) is smoking and exposure to 60 known carcinogens (including Cd), in tobacco smoke causes seriously DNA damages of lung tissue (10-11). These findings demonstrate the need for more research that addresses the role of Cd in human health (12).

Data obtained from the present study clearly indicate that $CdCl_2$ is highly cytotoxic to human lung adenocarcinoma cells in a dose and time dependent manner. A significant cytotoxicity was observed at 200 μM of $CdCl_2$ during 48 h in which the cell viability reduced to 50%. Increasing the incubation time from 48 h to 72 h resulted in increased toxicity. However, the higher concentrations of $CdCl_2$ did not lead to more inhibition of cell viability. This indicates that Cd even in low concentrations is very cytotoxic in the human lung carcinoma cell line. The results from our studies are in accordance with previous *in vitro* and *in vivo* studies concerning the exposure of different cell lines to $CdCl_2$ (13-16). Study of the cytotoxic effect of $CdCl_2$ in the human breast cancer cell line, MDA-MB468, showed similar results (data not shown).

Cd induces apoptosis in various tissues and cells ranging from normal to cancerous cells through very complex functions (5,13). It has been suggested that Cd disrupts mitochondrial function (14) and induces lysosomal damage (15) as the first target which leads to other cellular events including ROS production and oxidative damage, interfering with essential metals (7) lysosomal damage, lipid peroxidation, (15,16), DNA strand breaks, gene expression, chromosomal aberrations, inhibition of DNA repair processes and induction of apoptosis (10). It has been observed that some antioxidants are capable of reversing some deleterious effect of the metal on apoptosis.

CONCLUSION

To the best of our knowledge this is the first preliminary study to demonstrate the cytotoxic effects of Cd in the human lung adenocarcinoma cells. In conclusion, while high concentrations of Cd are harmful to human, lower concentrations induce a significant cytotoxicity in the cancer cells. However the cytotoxic mechanism of Cd in the Calu-6 cells remains to be elucidated. This finding suggests an approach to the mode of action and possible application of trace elements in the cancer treatment.

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