CADMIUM (II) CHLORIDE INHIBITS THE 2,3,7,8-TCDD-INDUCED ACTIVATION OF CYTOCHROME P450 1A1 IN A DIOXIN-RESPONSIVE CHEMICAL ACTIVATED LUCIFERASE EXPRESSION (DRE-CALUX) CELL LINES

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Abstract

The effect of cadmium chloride ($CdCl_2$) on activation of aryl hydrocarbon receptor (AhR)-mediated gene expression by 2,3,7,8-tetrachlorinated dibenzo-p-dioxin (2,3,7,8-TCDD) in human hepatoma cells was investigated in this study. The results showed that $CdCl_2$ markedly attenuated the TCDD-induced activations of DRE-CALUX (dioxin-responsive element-mediated Chemical Activated LUciferase eXpression) and EROD in a dose-dependent manner with no marked cytotoxicity. Cadmium not only inhibited the TCDD-induced CYP1A1 activation but also interfered with DRE-CALUX bioassay in human hepatoma cells.

Introduction

Polychlorinated dibenzo-p-dioxins (PCDDs), dibenzofurans (PCDFs), andbiphenyls (PCBs), belong to halogenated aromatic hydrocarbons (HAHs), are lipophilic and anthropogenic chemicals and toxicants[1]. These organochlorines compounds have been found widespread in ambient air[2], stack flue gas[3], sediment[4], fish[5], blood and placentas[6], and breast milk[7,8] in Taiwan.

Chemical Activated LUciferase eXpression (CALUX) has been widely used in recent years for its rapid screening and low-cost while applying in identification of highly dioxin-contaminated samples from environment and food. Previous studies revealed that treatment of human hepatoma (HepG2) cells with 0.5 μM of $NaAsO_2$ caused a marked decrease in CYP1A1 induction by 2,3,7,8-TCDD[9]. It's interested to understand more about other metals, such as Cd, would also lead to inhibition on CALUX. In this study, the cadmium chloride ($CdCl_2$) effect on activation of AhR-mediated gene expression by 2,3,7,8-TCDD in human hepatoma cells was investigated and evaluated.

Materials and Methods

A dioxin-responsive element (DRE)-mediated Chemical Activated LUciferase eXpression (CALUX), Huh7-DRE-Luc cells, by stable transfection of Huh7 with a DRE-driven firefly luciferase reporter plasmid (4xDRE-TATA-Luc), were established. Different concentrations of $CdCl_2$ (1, 5 and 25 μM) with 10nM TCDD were cotreatment in cells for 24hr. The luciferase activity in Huh7-DRE-Luc cells were measured by Luciferase Assay System (Promega, Masison, WI, USA). Cell viability was determined by MTT assay, following the procedure previously described [10].The EROD activity was determined as previously described [11] using 5μM of ethoxyresorufin in DMEM medium as the substrate of CYP1A1 enzyme, in the presence of 1.5mM of salicylamide to inhibit conjugating enzymes. After incubation for 30 min at 37℃, fluorescence was measured by using a Fluoroskan multi-well fluorescence plate reader (Labsystems), with excitation at 530 nm and emission at 590 nm. Resorufin standard curve were used to convert fluorescence to pmole of resorufin formed. The CYP1A1 activity was defined as resorufin formation (in pmol) per 100 min of reaction time (pmol resorufin/100 min).The differences between the untreated controls and the cadmium-treated data were determined by the student t test.

Results and Discussion

Induction of DRE-CALUX by 2,3,7,8-TCDD in Huh7-DRE-Luc cells

Using the Huh7-DRE-Luc cells for DRE-CALUX bioassay, increasing concentrations of 2,3,7,8-TCDD were treated with the cells for 24 h and a dose–response induction of DRE-CALUX activity by 2,3,7,8-TCDD was shown in Fig. 1. This DRE-CALUX bioassay used a semi-logarithmic dose–response curve as a standard curve with a sigmoid appearance ($R^2 > 0.95$, $p < 0.001$). The coefficient of variation (CV) from quadruplicate measurements was below 20%. The detection limit for 2,3,7,8-TCDD was 6.4 pM, as defined by three times standard deviation above the average RLU value of the zero standards (or without 2,3,7,8-TCDD treatment).

Cytotoxic effect of cadmium on Huh7 cells

Before treating cadmium with cells, it is essential to define the non-toxic range of $CdCl_2$ for Huh7 cells, the parental cells of Huh7-DRE-Luc cells, for evaluating the cadmium effects on DRE-CALUX activation by 2,3,7,8-TCDD. To analyze the cytotoxic effect of $CdCl_2$, Huh7 cells were treated with different concentrations of $CdCl_2$ for 24 h and then survival rate was determined with MTT assay. As shown in Fig. 2, treatments with $CdCl_2 \geq 1\mu M$ caused significant effect on cytotoxicity of Huh7 cells ($p < 0.05$) but the survival rate still higher than 80 %.

Inhibition of cadmium on DRE-CALUX induction by 2,3,7,8-TCDD

To address the cadmium effect on the DRE-CALUX induction by 2,3,7,8-TCDD, Huh7-DRE-Luc cells were treated with 10nM 2,3,7,8-TCDD in the presence of different concentrations of $CdCl_2$. As shown in Fig. 3, treatments with $CdCl_2$ attenuated the TCDD-induced DRE-CALUX activation in a dose dependent manner and $CdCl_2 \geq 1$ μM could cause significant decreases in DRE-CALUX activity ($p < 0.001$).

Inhibition of cadmium on CYP1A1 induction by 2,3,7,8-TCDD

By using Huh7 cells, the effect of $CdCl_2$ on CYP1A1 enzyme activation induced by 10nM 2,3,7,8-TCDD was also analyzed as determined by using EROD assay. The result indicated that $CdCl_2$ inhibited the TCDD-induced EROD activation in a dose-dependent manner (Fig. 4). It was correlated with that from DRE-CALUX bioassay. Meanwhile, treatments with $CdCl_2$ alone caused no marked effect on CYP1A1/EROD activity. Comparing with the result of Elbekai and El-Kadi's work (2004)[12], the pattern of EROD activity was similar.

Moreover, the data showed that $CdCl_2$ inhibited induction of cytochrome P450 1A1 by 2,3,7,8-TCDD in human hepatoma cells in a similar pattern with $NaAsO_2$ of Chao et al.(2006)[13], suggesting that these inhibitory effects of $CdCl_2$ and $NaAsO_2$ were possibly regulated via the same mechanism(s). The present study also suggests that extensive cleanup for removal of any possible interfering factor is critical to guarantee the accuracy of DRE-CALUX bioassay.

In conclusion, the results showed that $CdCl_2$ markedly attenuated the TCDD-induced activations of DRE-CALUX (dioxin-responsive element-mediated Chemical Activated LUciferase eXpression) and EROD in a dose-dependent manner with no marked cytotoxicity. The calculated CALUX-toxic equivalent (TEQ) levels induced by cotreatment of $CdCl_2$ and 10nM 2,3,7,8-TCDD were significantly lower than that induced by 2,3,7,8-TCDD alone ($p < 0.05$). Cadmium not only inhibited the TCDD-induced CYP1A1 activation but also interfered with DRE-CALUX bioassay in human hepatoma cells.

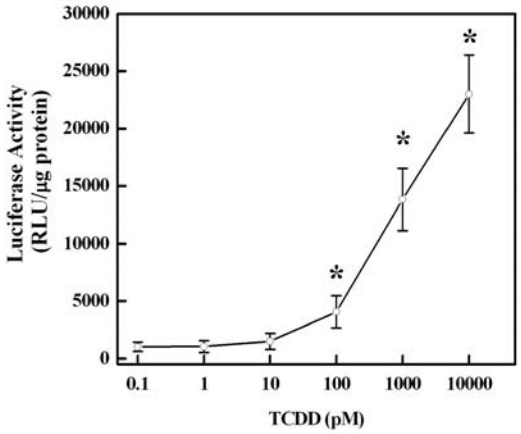

Fig. 1. Dose–response induction of luciferase activity by 2,3,7,8-TCDD. Huh7 cells at ≈ 90% confluence were incubated with 2,3,7,8-TCDD for 24 h. The measurements of luciferase activity are shown as means ± S.D., n=3. *p < 0.05, compared with the treatment with 0.01pM TCDD.

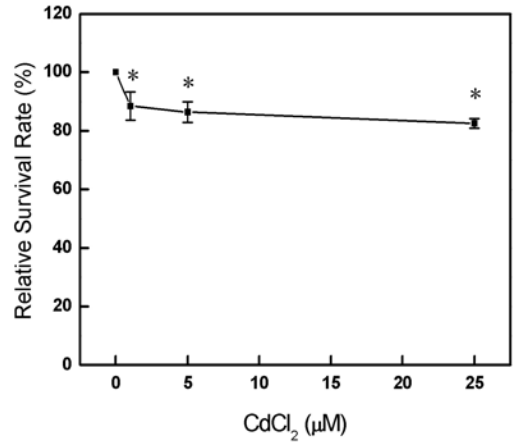

Figure 2.Cytotoxic effect of cadmium on Huh7 cells. The cells were left untreated or treated with different concentrations of $CdCl_2$ (1, 5, 25 μM) for 24 h. After treatments, survival rates were determined by using MTT assay. Data are presented as mean ± SD, n=3, and are expressed as percentage of survival rate as compared with that of untreated control.

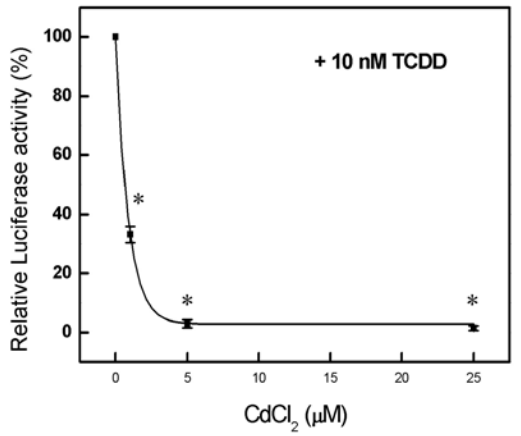

Figure 3. Inhibitory effect of cadmium on the TCDD-induced luciferase activation in Huh7-DRE-Luc cells. The cells were treated with 10 nM TCDD in the presence of different concentrations of $CdCl_2$ (1, 5, 25 μM) for 24 h. Data are presented as mean ± SD, n=3, and are expressed as relative luciferase activity as compared with that of 10 nM TCDD-treated control.

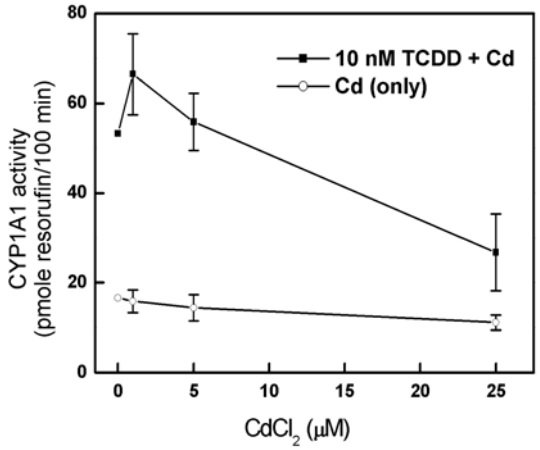

Figure 4. Inhibitory effect of cadmium on the TCDD-induced luciferase activation in Huh7 cells. The cells were treated with 10 nM TCDD in the presence of different concentrations of $CdCl_2$ (1, 5, 25 μM) for 24 h. CYP1A1 activities are presented as mean ± SD, n=3, and are expressed as resorufin formation (in pmole) per 100 min of reaction time (pmole resorufin/100 min).

Acknowledgements
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