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The inhibition effect of 2,3,7,8-tetrachlorinated dibenzo-*p*-dioxin-induced aryl hydrocarbon receptor activation in human hepatoma cells with the treatment of cadmium chloride

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ABSTRACT

Polychlorinated dibenzo-*p*-dioxins (PCDDs) and dibenzofurans (PCDFs), considered as endocrine disruptors, tend to accumulate in fatty tissues. Dioxin-responsive element chemical activated luciferase gene expression assay (DRE-luciferase assay) has been recognized as a semi-quantitative method for screening dioxins for its fast and low-cost as compared with HRGC/HRMS. However, some problems with the bioassay, including specificity, detection variation resulted from different cleanup strategies, and uncertainty of false-negative or false-positive results, remain to be overcome. Cadmium is a prevalent environmental contaminant around the world. This study was aimed to examine the effects of cadmium on the 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD)-induced activation of aryl hydrocarbon receptor (AhR)-mediated gene expression in human hepatoma cells (Huh7-DRE-Luc cells and Huh7 cells). Ethoxyresorufin-*O*-deethylase (EROD) and DRE-luciferase assay were employed to determine the enzyme activity of cytochrome P450 1A1 (CYP1A1) and activation of AhR, respectively. The results showed that Cd^{2+} levels significantly inhibited the induction of TCDD-induced CYP1A1 and DRE luciferase activation in hepatoma cells. The 50% inhibited concentrations (IC_{50}) of $CdCl_2$ were 0.414 μM (95% confidence interval (C.I.): 0.230–0.602 μM) in Huh7-DRE-Luc cells and 23.2 μM (95% C.I.: 21.7–25.4 μM) in Huh7 cells. Accordingly, prevention of interference with non-dioxin-like compounds in a DRE-luciferase assay is of great importance in an extensive cleanup procedure.

1. Introduction

Polychlorinated dibenzo-*p*-dioxins (PCDDs) and dibenzofurans (PCDFs) are chlorinated aromatic compounds that are currently considered as endocrine disruptors [1]. Dioxins accumulate mainly in fatty tissues due to their high lipophilicity, long biological half-lives, and high resistance to biodegradation. General population exposure to PCDD/DFs can induce several adverse health effects, including reproductive [2], hormonal [3], and immunological [4] effects and cancer [5]. Measurements of these dioxin and dioxin-like compounds by cytochrome P450 1A1 (CYP1A1) enzyme activity in an ethoxyresorufin-*O*-deethylase (EROD) bioassay and chemical activated luciferase expression (CALUX) bioassay were extensively applied [6]. TCDD is the most toxic compounds in all 210 PCDD/Fs. PCDD/Fs toxicity are always expressed as TEQ. TEQ = $\sum \text{conc}_i \times \text{TEF}_i$, 2,3,7,8-TCDD has the highest TEF. Most studies used TCDD as cotreatment target.

The dioxin-responsive element chemical activated luciferase gene expression (DRE-CALUX) assay is recognized as a semi-quantitative method for screening dioxins. The DRE-CALUX bioassay is an in vitro system utilizing dioxin toxicity in gene expression to determine dioxins/PCBs toxic equivalency (TEQ) levels presented as CALUX-TEQ values [6]. The DRE-CALUX bioassay uses the expression of the firefly luciferase gene induced by dioxin-like compounds binding with the aryl hydrocarbon receptor (AhR) in a genetically recombinant rat/mouse hepatoma cell [7]. This

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dioxin bioassay has been widely used to detect CALUX-TEQ levels in various matrices, such as fish [8], breast milk [9], sediments [10], and water effluents [11]. A high correlation coefficient ($r = 0.91$) was found between CALUX-TEQ and dioxin-World Health Organization (WHO)-TEQ levels in retail fish [8]. Compared to a high-resolution gas chromatograph equipped with a high resolution mass detector (HRGC/HRMS), the DRE-CALUX bioassay has the advantages of shorter time requirement and lower cost. In addition, the DRE-CALUX bioassay may be a good screening method for dioxin surveillance.

The systems of DRE-CALUX are established in the recombinant mouse (Xenobiotic Detection System, Inc.) or rat hepatoma (BioDetection System b. v.) cells. In our previous study [12], we set up a stable system of a DRE-driven luciferase reporter gene in genetically modified human hepatoma cells (Huh7-DRE-Luc cells). It has been reported that a non-aryl hydrocarbon receptor (AhR) ligand, such as arsenic [12] or arecoline [13], interferes with TCDD-induced luciferase activation in Huh7-DRE-Luc cells. It has also been reported that sodium arsenite, cadmium chloride, and chromium trioxide could induce luciferase activities in Hepa 1c1c7 cells transiently transfected with a xenobiotic-responsive element-driven luciferase reporter gene [14]. Furthermore, the analytical results of the DRE-CALUX bioassay are probably correlated with extensive cleanup procedures due to the absence of spiked internal standards and the contaminants, particularly for unknown AhR agonists and antagonists, in solvents and samples [15]. The DRE-CALUX bioassay may require further improvement for application in various matrices and field surveillance.

The dioxin-induced cytochrome P450 1A1 (CYP1A1) enzyme activity in an EROD bioassay and DRE-CALUX has been widely applied in various samples [16]. The induction fold, limit of detection, and limit of quantification were found to be similar for the DRE-CALUX and EROD bioassays by testing several dioxins and dioxin-like compounds in two rat hepatoma H4II cell lines [16]. Several studies have reported that metals (i.e. cadmium) can modify CYPs functions [17]. There was a significant dose-dependent induction of CYP1A1 in the hepatoma (HepG2) cell lines caused by Cd^{2+} in concentrations from 1.25 to 15 μM [18]. Elbekai and El-Kadi [17] co-administered Cd^{2+} with AhR ligand to inhibit the induction of CYP1A1 activation and to increase its mRNA levels and protein expression in Hepa 1c1c7 cells.

In previous studies, Chen et al. [19] found high amounts of Cd in the sediment by calculating its enrichment factors (EFs) ranged from 3.0 to 36.1. Liao et al. [20] investigated the metal distribution in the Nanjing area and found that the EF of Cd was 1.51. These results show that Cd is a prevalent environmental contaminant. It is also commonly found in high levels in ground water [21] and in the male population with a smoking habit in Taiwan [22]. The findings of our previous study showed that sodium arsenite (As^{3+}) inhibited the induction of CYP1A1 by 2,3,7,8-TCDD in both human hepatoma cells (Huh7) and recombinant Huh7-DRE-Luc cells [12]. The study suggested that extensive cleanup prevents any possible interference and guarantees the accuracy of the Huh7-DRE-Luc bioassay [12]. In this study, we examined the inhibitory effect of cadmium cations on the activation of Huh7-DRE-Luc and CYP1A1 enzyme activity in human hepatoma cell (Huh7) lines to further understand how cadmium influences the response of the Huh7 cell line while applying it in Cd–PCDD/Fs co-contaminated environmental samples.

2. Methods and materials

2.1. Materials and reagents

Cadmium chloride and 2,3,7,8-TCDD were purchased from Fluka Chemie GmbH (Buchs, Switzerland). Ethoxyresorufin, resorufin, salicylamide, and dimethyl sulfoxide (DMSO) were purchased from Sigma–Aldrich (St. Louis, MO, USA). 3-(4,5-Dimethyl-2-thiazolyl)-2,5-diphenyl-2H tetrazolium bromide (MTT) was obtained from Calbiochem (San Diego, CA, USA). The 4xDRE-TATA-Luc plasmid was constructed by cloning four copies of DRE of human CYP1A1 (DREs: −499, −895, −983, and −1056) [23] prior to a TATA box and a firefly luciferase gene. The human hepatoma cell line Huh7 and the pSUPER-EGFP-neo^r vector carrying EGFP with neo^r genes were respectively provided by Dr. Chiung-Tong Chen and Dr. Chi-Ying F. Huang (National Health Research Institutes, Taiwan). Quantification of protein concentration was determined using the Bradford method (BioRad, Hercules, CA, USA) [24].

2.2. Measurements of luciferase activation in recombinant Huh7-DRE-Luc cells

Stable transfection followed a previously described [7] procedure with slight modifications. Stable and available Huh7-DRE-Luc cells were established as described in detail in our previous report [12]. In brief, Huh7 cells seeded in 60 mm dishes were transfected with a reporter vector (4xDRE-TATA-Luc) and a selection vector (pSUPER-EGFP-neo), respectively. After 2 days of growth in a nonselective medium, the transfected cells were re-plated into a selection medium containing 800 and 200 μg/ml G418 for 1 week and 1 month, respectively. The Huh7-DRE-Luc cell clone was selected for the best inducible activations of DRE-luciferase activity by 2,3,7,8-TCDD from 45 individual clones (35 Huh7 and 10 HepG2 clones). The clones were continuously incubated for 14 months with stable DRE-luciferase activity.

The analytical method of the DRE luciferase bioassay was described in detail previously [12]. For analysis of the cadmium effect on TCDD-induced DRE luciferase activation, the cells were treated with 10 nM 2,3,7,8-TCDD for 24 h in various cadmium chloride concentrations (1, 5, and 25 μM). After treatments, cell lysates were collected in 0.2 ml of reporter lysis buffer (0.1 KH_2PO_4, pH 7.9, 0.5% Triton X-100, and 1 mM DTT) and then frozen-thawed at least three times to assure complete cell lysis. Luciferase activities from Huh7-DRE-Luc cells were measured using the Luciferase Assay System (Promega, Madison, WI, USA) with relative light units of (RLU)/μg protein.

2.3. Cell viability assay

For the cytotoxicity tests, Huh7 cells were seeded in a 96-well plate at a density of 4×10^4/well and cultured in DMEM (Dulbecco's modified Eagle's medium) for 24 h. The Huh7 cells were then treated with Cd^{2+} (1.0, 5.0, and 25 μM) for another 24 h or left untreated. Cell viability was determined using the MTT assay, following a previously described procedure [25].

2.4. CYP1A1 activity determined using the EROD assay

The EROD bioassay with human hepatoma Huh7 cells was performed as described in Ref. [26]. Huh7 cells were seeded into 24-well cell culture plates up to a density of 90% confluence in the medium. Cells were treated for 24 h with 10 nM 2,3,7,8-TCDD and Cd^{2+} (1.0, 5.0, and 25 μM) or with just 10 nM 2,3,7,8-TCDD. After treatment, the medium was removed and the well was washed twice with fresh medium at the end of the incubation. EROD activity was determined as described in Ref. [27] using 5 μM of ethoxyresorufin in DMEM medium as the substrate of the CYP1A1 enzyme with 1.5 mM of salicylamide to inhibit enzyme conjugation. After an incubation of 30 min at 37 °C, fluorescence was measured using a Fluoroskan multi-well fluorescence plate reader (Labsystems). Resorufin standard curves were used to convert fluorescence to p moles of resorufin formed. The CYP1A1 activity was defined as resorufin formation (in p moles) per 100 min of reaction time (p

Table 1
Inhibitory effect of cadmium on TCDD-induced luciferase activation in Huh7-DRE-Luc cells (RLU/μg protein).

Cadmium chloride concentration	0 μM	1 μM	5 μM	25 μM	*p*-Value[a]	*p*-Value[b]
10 nM TCDD + Cd^{2+} (n = 9)						
Mean	2820	759	70.7	53.5	<0.001	<0.001
95% C.I.[c]	1550–4080	551–967	42.8–98.6	41.6–65.3	–	–
p-Value[d]	–	<0.001	<0.001	<0.001	–	–
p-Value[e]	<0.001	<0.001	0.500	0.690	–	–
Base level (n = 9)[f]						
Mean ± SE			50.7			
95% C.I.			43.1–58.4		–	–

[a] ANOVA tests.
[b] Jonkheere–Terpstra tests.
[c] 95 C.I. means 95% of confidence interval.
[d] Mann–Whiney *U*-tests comparing the untreated control to the cotreatment of Cd^{2+} and TCDD.
[e] The significant differences in luciferase activities between the cotreatment and the base level were examined using Moses extreme reaction tests.
[f] There were no significant differences in luciferase activities between the base level and Cd^{2+} alone (n = 3).

moles resorufin/100 min). Additionally, quantification of protein concentration was determined using the Bradford method (BioRad, Hercules, CA, USA) [24].

2.5. Statistics

Each experiment was performed at least three times. The statistical analysis was expressed using the mean ± standard error (SE) from each independent experiment. To avoid statistical bias, analysis of variance (ANOVA) tests and nonparametric tests with Jonkheere–Terpstra and Mann–Whiney *U*-tests were used to examine the differences between the untreated controls and the chemically treated samples. We also used Moses extreme reaction tests to compare the responses of using Cd^{2+} alone with those of using the cotreatment of Cd^{2+} and 10 nM TCDD. IC_{50} is the concentration of Cd^{2+} that causes 50% inhibition of TCDD-specific activation in comparison to control by the Probit model. Analyses were carried out using the Statistical Package for Social Sciences (SPSS) version 15.0 (SPSS Inc., Chicago, IL, USA).

3. Results

3.1. Effects of cadmium chloride on DRE luciferase induction by 2,3,7,8-TCDD

Following our previous study [12], we treated Huh7-DRE-Luc cells with increasing 2,3,7,8-TCDD concentrations for 24 h, revealing a dose–response induction of DRE-luciferase activities by TCDD. A semi-logarithmic dose–response curve was exhibited by the Huh7-DRE-Luc cells after treatments of 2,3,7,8-TCDD from 0.01 to 10,000 pM (data not shown). 10 nM TCDD was chosen as simulating the situation of seriously PCDD/Fs polluted area because the metal pollution was always coexisted in serious PCDD/Fs polluted area in Taiwan. Huh7-DRE-Luc cells were treated with 10 nM 2,3,7,8-TCDD in the presence of different cadmium chloride concentrations in order to evaluate the Cd^{2+} effects on luciferase induction by 2,3,7,8-TCDD. The Cd^{2+} treatment significantly attenuated the TCDD-induced luciferase activation in a dose-dependent manner (Fig. 1). IC_{50} of Cd^{2+} was predicted to be 0.414 μM (95% confidence interval (C.I.): 0.230–0.602 μM). The inhibited effects of cadmium on TCDD-induced activation in Huh7-DRE-Luc cells are shown in Table 1. $Cd^{2+} \geq 1$ μM significantly inhibited TCDD-induced luciferase enzyme activities ($p < 0.001$). Compared to base levels, significant differences were found between luciferase activation (RLU/μg protein) in 10 nM TCDD alone ($p < 0.001$) and that after the cotreatment of 10 nM TCDD and 1 μM Cd^{2+} ($p < 0.001$). The luciferase activities in the presence of 5 ($p = 0.500$) and 25 μM Cd^{2+} ($p = 0.690$) with 10 nM TCDD did not have a higher magnitude than those in the Cd^{2+} alone.

3.2. Effects of Cd^{2+} on CYP1A1 induction by 2,3,7,8-TCDD

The EROD assay was used to analyze the effect of Cd^{2+} on CYP1A1 enzyme activation induced by 10 nM 2,3,7,8-TCDD in Huh7 cells. The inhibition of Cd^{2+} for the TCDD-induced EROD activation was only found at the highest concentration (25 μM, $p = 0.037$) in a dose-dependent manner (Fig. 2). This result is consistent with that using the DRE luciferase bioassay. IC_{50} of Cd^{2+} was also evaluated. 23.2 μM (95% C.I.: 21.7–25.4 μM) of Cd^{2+} could significantly inhibit 50% of EROD activation in the presence of 10 nM TCDD in Huh7 cells. As shown in Table 2, the CYP1A1 enzyme activation in the coexistence of 10 nM TCDD and cadmium was significantly higher than that in treatment with cadmium alone ($p < 0.001$).

3.3. Cytotoxic effects on the cells

To address the cytotoxic effects of TCDD and Cd^{2+} on Huh7-DRE-Luc cells, it is essential to define the non-toxic range of TCDD and

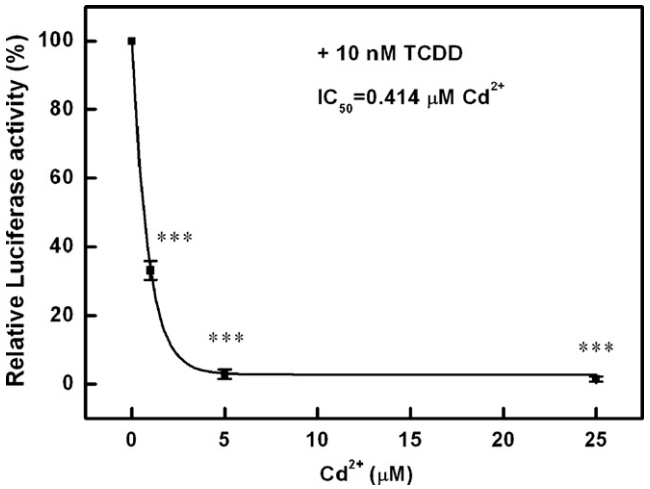

Fig. 1. Inhibitory effect of cadmium on TCDD-induced luciferase activation in Huh7-DRE-Luc cells (IC_{50} = 0.414 of μM Cd^{2+}). The cells were treated with 10 nM TCDD in the presence of different concentrations of Cd^{2+} (1, 5, and 25 μM) for 24 h. Data are presented as mean ± SD, n = 4, and are expressed as relative luciferase activity (%) compared to that of the 10 nM TCDD-treated control. ***$p < 0.001$, compared with the relative untreated control.

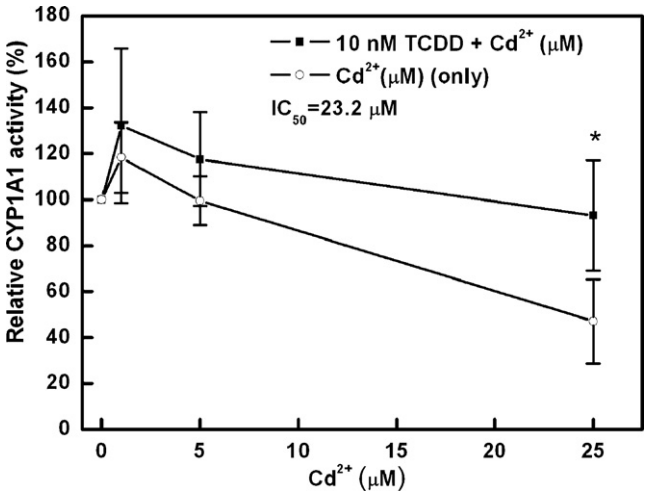

Fig. 2. Inhibitory effect of cadmium on TCDD-induced CYP1A1 activation in Huh7 cells (IC_{50} = 23.2 μM Cd^{2+}). The cells were treated with 10 nM TCDD in the presence of different concentrations of Cd^{2+} (1, 5, and 25 μM) for 24 h and without it. CYP1A1 activities are presented as mean ± SD, n = 3, and are expressed as relative CYP1A1 activity (%). *p < 0.05, compared with the relative untreated control (with or without 10 nM TCDD).

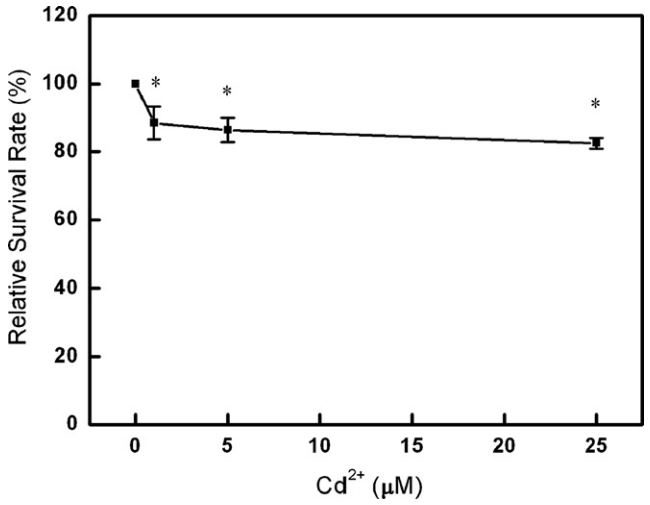

Fig. 3. The cytotoxic effect of cadmium on Huh7 cells. The cells were left untreated or were treated with different concentrations of Cd^{2+} (1, 5, and 25 μM) for 24 h. After treatments, survival rates were determined using the MTT assay. Data are presented as mean ± SD, n = 3, and are expressed as percentage of survival rate compared to that of the untreated control. *p < 0.05, compared with the relative untreated control (with or without 10 nM TCDD).

Cd^{2+} for Huh7 cells, which are the parental cells of Huh7-DRE-Luc cells, for the estimate of Cd^{2+} effects on luciferase activation by 2,3,7,8-TCDD. Huh7 cells were treated with various Cd^{2+} concentrations for 24 h. The survival rate of the cells was determined using the MTT assay to analyze the cytotoxic effect of Cd^{2+}. Concentrations of $Cd^{2+} \geq 1$ μM caused cytotoxic effects with decrease in the survival rate of 89–84% ($p < 0.001$), as shown in Fig. 3. The relative survival rates of treatments with 5 and 25 μM Cd^{2+} were not significantly lower than that with 1 μM of Cd^{2+} ($p > 0.05$). Although slightly cytotoxic effects were found in Huh7 cells treated with ≥1 μM Cd^{2+}, they did not significantly affect the performance of Huh7 and Huh7-DRE-Luc cells using the EROD and DRE luciferase bioassays, respectively. We also evaluated the cytotoxicity of Cd^{2+} in the presence of TCDD to consider the combined effects in Huh7 cells. Huh7 cells were also treated with Cd^{2+} with 10 nM TCDD for 24 h. Our data indicate that the presence of TCDD and Cd^{2+} treatments exhibits no marked cytotoxicity in the survival rate (data not shown). Additionally, Huh7-DRE-Luc cells for cytotoxicity test were also performed. The result indicated that there was no significant difference for cytotoxicity between metal alone and cotreatment test (data not shown).

4. Discussion

Cadmium (II) chloride significantly inhibited TCDD-induced luciferase activation in a dose-dependent manner, as shown in Fig. 1. Few studies have indicated that heavy metals could interfere with TCDD-induced DRE-luciferase activities in recombinant hepatoma cells. According to our previous study [12], concentrations of sodium arsenite ($NaAsO_2$) higher than 3 μM significantly inhibited TCDD-induced luciferase activation in Huh7-DRE-Luc cells. Treatment with Cd^{2+} inhibited both TCDD-induced luciferase (Huh7-DRE-Luc cells) (Fig. 1) and EROD (Huh7 cells) (Fig. 2) activation in a dose-dependent manner without significantly affecting cytotoxicity in Huh7 cells (Fig. 3).

We found that Cd^{2+} alone did not have significant toxic effects on CYP1A1 and DRE luciferase activation in either cell after the relative survival rates of cytotoxic effects were adjusted ($p > 0.05$). The results of some studies were consistent with ours, but those of others were not. Hepa 1c1c7 treated with 5 μM Cd^{2+} did not show an inducible effect on EROD activities [28]. HepG2 treated with 5 μM cadmium chloride was not significantly different compared with the untreated control [27]. In in vitro studies with Cd^{2+} treatment ranging from 0 to 26.7 μM [29] and 0 to 15 μM [18], a significant

Table 2
Inhibitory effect of cadmium on TCDD-induced CYP1A1 activation in Huh7 cells (p mole resorufin/100 min).

Cadmium chloride concentration	0 μM	1 μM	5 μM	25 μM	p-Value[a]	p-Value[b]
10 nM TCDD + Cd^{2+} (n = 3)					0.007	0.033
Mean	56.0	66.5	55.9	26.8	–	–
95% C.I.[c]	51.6–60.3	49.9–83.0	44.1–67.6	11.1–42.5	–	–
p-Value[d]	–	0.400	0.700	0.043	–	–
Cd^{2+} alone (n = 3)					0.553	0.570
Mean ± SE	12.9	15.8	14.4	11.1	–	–
95% C.I.[c]	6.12–19.6	11.1–20.6	9.06–19.8	8.01–14.3	–	–
p-Value[d]	–	0.400	0.700	0.700	–	–
Nonparametric tests						
p-Value[e]	<0.001	<0.001	<0.001	<0.001		

[a] ANOVA tests.
[b] Jonkheere–Terpstra tests.
[c] 95 C.I. means 95% of confidence interval.
[d] Mann–Whiney U-tests comparing the untreated control to the cotreatment of Cd^{2+} and TCDD.
[e] The significant differences in EROD activities between the cotreatment and the treatment alone were examined using Moses extreme reaction tests.

increase in EROD activation was found at higher Cd^{2+} concentrations compared to that of the untreated control in human HepG2 cells.

As shown in Table 2, cadmium inhibited the induction of TCDD-mediated CYP1A1 activities with significantly antagonistic results. 5 μM Cd^{2+} attenuating the induction of EROD activation after treatment with 10 nM TCDD in Hepa 1c1c7 cells was also found in the study of Elbekai et al. [30]. They also indicated that the inhibition of TCDD-mediated EROD activation was positively associated with increased Cd^{2+} concentrations (1, 5, and 25 μM) in the same hepatoma cell line [17]. To our knowledge, few mammalian in vivo studies were designed to test EROD activation with the combined treatment of metals and TCDD.

From Tables 1 and 2, the statistical results are significantly different at Cd^{2+} concentrations of 5 and 25 μM between luciferase activities and CYP1A1 activation for the cotreatment with Cd^{2+} and TCDD and Cd^{2+} alone. IC_{50} of 0.414 μM of Cd^{2+} (95% C.I.: 0.230–0.602 μM) in the presence of 10 nM TCDD for Huh7-DRE-Luc cells was notably lower than that of 23.2 μM Cd^{2+} (95% C.I.: 21.7–25.4 μM) for Huh7 cells with a significance of $p < 0.001$. Our previous study also showed the differential effect of arecoline on a stably transfected DRE-driven reporter and on the endogenous AhR-ligand CYP1A1 in human hepatoma cells [13]. Elbekai et al. [30] demonstrated that As^{3+}, Cd^{2+}, and Cr^{6+} caused the activation of AhR and transcriptional induction of CYP1A1, and were involved in posttranscriptional modification while decreasing CYP1A1 activation at posttranslational levels. We further demonstrated that the differential effect of Cd^{2+} (non-AhR ligand) on DRE luciferase activation and on CYP1A1 activation had statistically different results. Huh7-DRE-Luc cells are genetically recombinant hepatoma cells that are specifically and directly activated to AhR function by AhR agonists, but might be easily accompanied by the downstream regulation of transcriptional, posttranscriptional, or posttranslational modification by the non-AhR ligand.

We attempted to mimic CYP1A1 activation by the activities of a DRE-driven reporter gene even though there were different biological outcomes. Based on the findings of our previous studies [12] and the present one, the biological outcomes in relation to AhR with the cotreatment of AhR agonist and non-AhR ligand by the EROD assay were more stable compared to those tested by the DRE luciferase bioassay. A previous study indicated that using the DRE-CALUX bioassay for detecting AhR agonists had many advantages over the EROD assay, including easy operation, more reliable outcomes, less time-consuming, and fewer inhibition effects [16]. Based on our results, EROD assay was more stable for pure compound cotreatment, however, if applying in matrix, the results of DRE luciferase bioassay have more reliable outcomes, this inference warrants further investigation in the future. We also recognize that our DRE luciferase bioassay or a commercial DRE-CALUX bioassay could be a valuable tool for rapid assessment and fast-screen investigations of AhR agonists in environmental contamination after an extensive cleanup procedure.

Food and tobacco smoke are well known major sources of cadmium with non-occupational exposure in the general population. Urinary cadmium levels in smokers are significantly higher than those in nonsmokers in the general population of Taiwan [22]. According to our previous study [26], arecoline, an alkaloid in the areca nut, inhibited the induction of TCDD-mediated CYP1A1 activation. Most Taiwanese males have a betel-quid chewing habit coupled with a smoking habit, and smokers who chewed betel-quid nearly tripled their oral cancer risks compared to nonsmokers [31]. It is still unknown whether arecoline and cadmium in a highly exposed population may increase or decrease AhR-agonist-induced adverse health effects (i.e. polycyclic aromatic hydrocarbons (PAHs) or dioxins). Further studies are needed to test whether the effects of TCDD/PAH-induced CYP1A1 activation caused by the combined treatment of cadmium and arecoline are agonistic or antagonistic.

5. Conclusions

Cadmium chloride significantly inhibits the induction of TCDD-mediated CYP1A1 activation in Huh7 cells and dioxin-responsive element-driven luciferase activities in Huh7-DRE-Luc cells. This study also demonstrates the differential effects of CYP1A1 activation in Huh7 cells and luciferase activation in Huh7-DRE-Luc cells with the cotreatment of Cd^{2+} and TCDD using IC_{50} values and statistical differences. Prevention of interference with non-dioxin-like compounds in a dioxin-responsive element-driven luciferase bioassay is of great importance in an extensive cleanup procedure.

Acknowledgments

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References

[1] L.S. Birnbaum, Developmental effects of dioxins and related endocrine disrupting chemicals, Toxicol. Lett. 82–83 (1995) 743–750.
[2] H.R. Chao, S.L. Wang, L.Y. Lin, W.J. Lee, O. Papke, Placental transfer of polychlorinated dibenzo-p-dioxins, dibenzofurans, and biphenyls in Taiwanese mothers in relation to menstrual cycle characteristics, Food Chem. Toxicol. 45 (2007) 259–265.
[3] S.L. Wang, Y.C. Chang, H.R. Chao, C.M. Li, L.A. Li, L.Y. Lin, O. Papke, Body burdens of polychlorinated dibenzo-p-dioxins, dibenzofurans, and biphenyls and their relations to estrogen metabolism in pregnant women, Environ. Health Perspect. 114 (2006) 740–745.
[4] S.L. Wang, P.H. Su, S.B. Jong, Y.L. Guo, W.L. Chou, O. Papke, In utero exposure to dioxins and polychlorinated biphenyls and its relations to thyroid function and growth hormone in newborns, Environ. Health Perspect. 113 (2005) 1645–1650.
[5] A. Schecter, J.R. Olson, Cancer risk assessment using blood dioxin levels and daily dietary TEQ intake in general populations of industrial and non-industrial countries, Chemosphere 34 (1997) 1569–1577.
[6] P.A. Behnisch, K. Hosoe, K. Shiozaki, H. Ozaki, K. Nakamura, S. Sakai, Low-temperature thermal decomposition of dioxin-like compounds in fly ash: combination of chemical analysis with in vitro bioassays (EROD and DR-CALUX), Environ. Sci. Technol. 36 (2002) 5211–5217.
[7] P.M. Garrison, K. Tullis, J.M.M.J.G. Aarts, A. Brouwer, J.P. Giesy, M.S. Denison, Species-specific recombinant cell lines as bioassay systems for the detection of 2,3,7,8-tetrachlorodibenzo-p-dioxin-like chemicals, Fundam. Appl. Toxicol. 30 (1996) 194–203.
[8] T. Tsutsumi, Y. Amakura, M. Nakamura, D.J. Brown, G.C. Clark, K. Sasaki, M. Toyoda, T. Maitani, Validation of the CALUX bioassay for the screening of PCDD/Fs and dioxin-like PCBs in retail fish, Analyst 128 (2003) 486–492.
[9] E.A. Nelson, L.L. Hui, T.W. Wong, A.J. Hedley, Demographic and lifestyle factors associated with dioxin-like activity (CALUX-TEQ) in human breast milk in Hong Kong, Environ. Sci. Technol. 40 (2006) 1432–1438.
[10] M.R. Hurst, J. Balaam, Y.L. Chan-Man, J.E. Thain, K.V. Thomas, Determination of dioxin and dioxin-like compounds in sediments from UK estuaries using a bioanalytical approach: chemical-activated luciferase expression (CALUX) assay, Mar. Pollut. Bull. 49 (2004) 648–658.
[11] M.R. Hurst, Y.L. Chan-Man, J. Balaam, J.E. Thain, K.V. Thomas, The stable aryl hydrocarbon receptor agonist potency of United Kingdom Continental Shelf (UKCS) offshore produced water effluents, Mar. Pollut. Bull. 50 (2005) 1694–1698.
[12] H.-R. Chao, T.-C. Tsou, L.-A. Li, F.-Y. Tsai, Y.-F. Wang, C.-H. Tsai, E.E. Chang, Z.-F. Miao, C.-H. Wu, W.-J. Lee, Arsenic inhibits induction of cytochrome P450 1A1 by 2,3,7,8-tetrachlorodibenzo-p-dioxin in human hepatoma cells, J. Hazard. Mater. 137 (2006) 716–722.
[13] H.-R. Chao, Y.-F. Wang, H.-T. Chen, Y.-C. Ko, E.E. Chang, Y.-J. Huang, F.-Y. Tsai, C.-H. Tsai, C.-H. Wu, T.-C. Tsou, Differential effect of arecoline on the endogenous dioxin-responsive cytochrome P450 1A1 and on a stably transfected dioxin-responsive element-driven reporter in human hepatoma cells, J. Hazard. Mater. 149 (2007) 234–237.
[14] R.H. Elbekai, A.O.S. El-Kadi, Transcriptional activation and posttranscriptional modification of Cyp1a1 by arsenite, cadmium, and chromium, Toxicol. Lett. 172 (2007) 106–119.
[15] L. Hoogenboom, W. Traag, T. Bovee, L. Goeyens, S. Carbonnelle, J. van Loco, H. Beernaert, G. Jacobs, G. Schoeters, L. Goeyens, W. Baeyens, The CALUX bioassay: current status of its application to screening food and feed, TrAC Trends Anal. Chem. 25 (2006) 410–420.

[16] P.A. Behnisch, K. Hosoe, A. Brouwer, S. Sakai, Screening of dioxin-like toxicity equivalents for various matrices with wildtype and recombinant rat hepatoma H4IIE cells, Toxicol. Sci. 69 (2002) 125–130.
[17] R.H. Elbekai, A.O.S. El-Kadi, Modulation of aryl hydrocarbon receptor-regulated gene expression by arsenite, cadmium, and chromium, Toxicology 202 (2004) 249–269.
[18] D.B. Tully, B.J. Collins, J.D. Overstreet, C.S. Smith, G.E. Dinse, M.M. Mumtaz, R.E. Chapin, Effects of Arsenic, cadmium, chromium, and lead on gene expression regulated by a battery of 13 different promoters in recombinant HepG2 cells, Toxicol. Appl. Pharmacol. 168 (2000) 79–90.
[19] C.-W. Chen, C.-M. Kao, C.-F. Chen, C.-D. Dong, Distribution and accumulation of heavy metals in the sediments of Kaohsiung Harbor, Taiwan, Chemosphere 66 (2007) 1431–1440.
[20] Q.-l. Liao, L.J. Evans, X. Gu, D.-f. Fan, Y. Jin, H. Wang, A regional geochemical survey of soils in Jiangsu Province, China: preliminary assessment of soil fertility and soil contamination, Geoderma 142 (2007) 18–28.
[21] L.-j. Tsai, K.-c. Yu, S.-T. Ho, Cadmium distribution in sediment profiles of the six main rivers in southern Taiwan, J. Hazard. Mater. 148 (2007) 630–639.
[22] Y.C. Chen, Y.S. Pu, R.S. Lin, C.Y. Yang, M.K. Lai, S.H. Liu, F.C. Sung, Blood and urine cadmium levels in relation to demographic and life style in middle aged and elderly men, Bull. Environ. Contam. Toxicol. 66 (2001) 287–294.
[23] S. Kress, J. Reichert, M. Schwarz, Functional analysis of the human cytochrome P4501A1 (CYP1A1) gene enhancer, Eur. J. Biochem. 258 (1998) 803–812.
[24] M.M. Bradford, A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding, Anal. Biochem. 72 (1976) 248–254.
[25] T.C. Tsou, S.C. Yeh, F.Y. Tsai, L.W. Chang, The protective role of intracellular GSH status in the arsenite-induced vascular endothelial dysfunction, Chem. Res. Toxicol. 17 (2004) 208–217.
[26] E.E. Chang, Z.-F. Miao, W.-J. Lee, H.-R. Chao, L.-A. Li, Y.-F. Wang, Y.-C. Ko, F.-Y. Tsai, S.C. Yeh, T.-C. Tsou, Arecoline inhibits the 2,3,7,8-tetrachlorodibenzo-p-dioxin-induced cytochrome P450 1A1 activation in human hepatoma cells, J. Hazard. Mater. 146 (2007) 356–361.
[27] S.W. Kennedy, S.P. Jones, Simultaneous measurement of cytochrome P4501A catalytic activity and total protein concentration with a fluorescence plate reader, Anal. Biochem. 222 (1994) 217–223.
[28] R.H. Elbekai, A.O.S. El-Kadi, The role of oxidative stress in the modulation of aryl hydrocarbon receptor-regulated genes by As^{3+}, Cd^{2+}, and Cr^{6+}, Free Radic. Biol. Med. 39 (2005) 1499–1511.
[29] P.F. Dehn, C.M. White, D.E. Conners, G. Shipkey, T.A. Cumbo, Characterization of the human hepatocellular carcinoma (hepg2) cell line as an in vitro model for cadmium toxicity studies, In Vitro Cell Dev. Biol. Anim. 40 (2004) 172–182.
[30] R.H. Elbekai, J. Duke, A.O.S. El-Kadi, Ascorbic acid differentially modulates the induction of heme oxygenase-1, NAD(P)H:quinone oxidoreductase 1 and glutathione S-transferase Ya by As^{3+}, Cd^{2+} and Cr^{6+}, Cancer Lett. 246 (2007) 54–62.