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Arsenic inhibits induction of cytochrome P450 1A1 by 2,3,7,8-tetrachlorodibenzo-*p*-dioxin in human hepatoma cells

How-Ran Chao [a], Tsui-Chun Tsou [b,*], Lih-Ann Li [b], Feng-Yuan Tsai [b], Ya-Fen Wang [c], Cheng-Hsien Tsai [d], Eddy Essen Chang [b], Zhi-Feng Miao [b], Chia-Hsin Wu [e], Wen-Jhy Lee [f,g]

[a] *Department of Environmental Science and Engineering, National Pingtung University of Science and Technology, Pingtung 912, Taiwan*
[b] *Division of Environmental Health and Occupational Medicine, National Health Research Institutes, Miaoli 350, Taiwan*
[c] *Department of Bioenvironmental Engineering, Chung Yuan Christian University, Chung-Li 320, Taiwan*
[d] *Department of Chemical Engineering, National Kaohsiung University of Applied Sciences, Kaohsiung 807, Taiwan*
[e] *Department of Chemical Engineering, Chung Yuan Christian University, Chungli 320, Taiwan*
[f] *Department of Environmental Engineering, National Cheng Kung University, Tainan 701, Taiwan*
[g] *Sustainable Environment Research Center, National Cheng Kung University, Tainan 701, Taiwan*

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Abstract

The aim of this study was to examine the arsenic effect on activation of aryl hydrocarbon receptor (AhR)-mediated gene expression by 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (2,3,7,8-TCDD) in human hepatoma cells. The human hepatoma Huh7 cells were treated with sodium arsenite ($NaAsO_2$) from 0.5 to 20 μM for 24 h. Our data revealed that $NaAsO_2 \leq 10$ μM caused no significant cytotoxic effect on Huh7 cells ($p > 0.05$). We also established a dioxin-responsive element (DRE)-mediated Chemical Activated LUciferase eXpression (CALUX) cell line, Huh7-DRE-Luc, by stable transfection of Huh7 with a DRE-driven firefly luciferase reporter plasmid (4xDRE-TATA-Luc). Treatments of Huh7-DRE-Luc and Huh7 with $NaAsO_2$ attenuated the 2,3,7,8-TCDD-induced DRE-CALUX and cytochrome P450 1A1 (CYP1A1) activations, respectively, in a dose-dependent manner. We found that the calculated CALUX-toxic equivalent (TEQ) levels induced by cotreatment of $NaAsO_2 \geq 3.0$ μM and 10 nM 2,3,7,8-TCDD were significantly lower than that induced by 2,3,7,8-TCDD alone ($p < 0.05$). In the present study, we demonstrated that arsenic not only inhibited the TCDD-induced CYP1A1 activation but also interfered with DRE-CALUX bioassay in human hepatoma cells. Our finding also suggests that extensive cleanup of sample for removal of any possible interfering factor is critical to guarantee the accuracy of dioxin-TEQ levels using DRE-CALUX bioassay.

Keywords: 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin; Arsenite; Chemical Activated LUciferase eXpression; Ethoxyresorufin-*O*-deethylase; Human hepatoma

1. Introduction

Halogenated aromatic hydrocarbons, such as polychlorinated dibenzo-*p*-dioxins (PCDDs), dibenzofurans (PCDFs), and biphenyls (PCBs), 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. PCDD/DF/Bs can persistently contaminate our environment leading to raising public concern on human exposure to these chemicals. Therefore, it is of great importance to effectively monitor the background levels of PCDD/DF/Bs in the environment, foodstuffs, and human bodies.

High-resolution gas chromatography/high-resolution mass spectrometry (HRGC/HRMS) is a certified method to determine dioxin concentrations in Taiwan. Moreover, the Chemical Activated LUciferase eXpression (CALUX) bioassay has been recently established in both Environmental Analysis Laboratories of Environmental Protection Administration (EPA) and Bureau of Food and Drugs Analysis of Department of Health (DOH) in Taiwan. The CALUX is an in vitro luciferase-reporter-gene assay for detecting the TEQ levels of dioxins and dioxin-

* Corresponding author at: Division of Environmental Health and Occupational Medicine, National Health Research Institutes, 35 Keyan Road, Zhunan Town, Miaoli County 350, Taiwan.
Tel.: +886 37 246 166x36511; fax: +886 37 587 406.
E-mail address: tctsou@nhri.org.tw (T.-C. Tsou).

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like compounds based on their high affinity with aryl hydrocarbon receptor (AhR). Compared with HRGC/HRMS, CALUX bioassay is a rapid and low-cost screening method particularly for fast-screen identification of highly dioxin-contaminated samples from environment and food. Recently, the CALUX bioassay has been used in detection of the levels of dioxin-TEQ in both biota [9,10] and environment [11,12] in some developed countries. The CALUX bioassay has been certified a new method for detecting dioxins in Japan in September 2005. Taiwanese EPA and DOH will soon announce to adopt the CALUX bioassay as a standard method for fast screening the dioxin-TEQ levels in food and environment.

Previous studies revealed that arsenic could inhibit induction of CYP1A enzymes in rat hepatocytes [13] and thus might alter the potential carcinogenic effect of dioxins on human hepatocytes [14]. 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 [15]. Since dioxins and arsenic are common contaminants in both environment and food, incomplete sample clean-up for CALUX bioassay may result in over- or under-estimations of dioxin-TEQ levels. However, there is still limited information regarding to the inhibitory effect of arsenic on CALUX bioassay, especially for human hepatoma cells.

Currently, there are two commercially available recombinant DRE-CALUX cell lines, mouse hepatoma cells (Hepa 1c1c7) (Xenobiotic Detection Systems, Inc.) and rat hepatoma cells (H4IIE) (BioDetection System b.v.). Both cell lines were stably transfected with DRE-driven firefly luciferase-reporter plasmid (pGudLuc6.1) and pSVneo [9,11]. In the present study, we examined the inhibitory effect of $NaAsO_2$ on activation of DRE-CALUX by 2,3,7,8-TCDD in the human hepatoma cell line Huh7. The calculated CALUX-TEQ levels were determined based on the luciferase activity in response to co-treatments with 2,3,7,8-TCDD and $NaAsO_2$.

2. Materials and methods

2.1. Caution

Appropriate personal protective methods were used throughout all experiments to avoid of exposure to the hazardous halogenated and polycyclic aromatic hydrocarbons.

2.2. Materials and reagents

2,3,7,8-TCDD and $NaAsO_2$ were obtained from Fluka Chemie GmbH (Buchs, Switzerland). Ethoxyresorufin, resorufin, Salicylamide, and dimethyl sulfoxide (DMSO) were obtained 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 4×DRE-TATA-Luc plasmid was constructed by cloning four copies of DRE of human CYP1A1 (DREs: −499, −895, −983 and −1056) [16] in front of a TATA box and a firefly luciferase gene. The pSUPER-EGFP-neo vector carrying EGFP and neo^r genes was kindly provided by Dr. Chi-Ying F. Huang (National Health Research Institutes, Taiwan). The human hepatoma cell line Huh7 was kindly provided by Dr. Chiung-Tong Chen (National Health Research Institutes, Taiwan). Quantification of protein concentration was determined by the Bradford method (BioRad, Hercules, CA, USA) [17].

2.3. Establishment of recombinant Huh7-DRE-Luc cells for DRE-CALUX bioassay

Stable transfection was performed as previously described [18] with modifications. Huh7 cells seeded in 60 mm dishes were transfected with a reporter vector, 4×DRE-TATA-Luc, and a selection vector, pSUPER-EGFP-neo, according to the Lipofectamine reagent protocol (Life Technologies, Grand Island, NY, USA). Following 2 days of growth in nonselective medium, the transfected cells were split 1–6 and were re-plated into selection medium containing 800 μg/ml G418 for one week, then in medium containing 200 μg/ml G418 for 1 month. After the G418 selection, 35 Huh7 cell clones with G418-resistant phenotype and EGFP expression were selected for their inducible activations of luciferase activity by 2,3,7,8-TCDD. The Huh7-DRE-Luc cell clone was finally established for its sharp dose–response performance in response to 2,3,7,8-TCDD. Huh7-DRE-Luc cells have been tested for 8 months with stable induction of luciferase activity by 2,3,7,8-TCDD.

2.4. CALUX bioassay

Huh7-DRE-Luc cells were seeded on 12-well plates at 90% confluence in DMEM medium. Stock solution of 2,3,7,8-TCDD was prepared in DMSO. After incubation for 24 h, the cells were subjected to different concentrations of 2,3,7,8-TCDD treatments (10^{-2}, 10^{-1}, 1, 10, 10^2, 10^3, and 10^4 pM) for 24 h. For analysis of arsenic effect on the TCDD-induced CALUX activation, the cells were treated with 10 nM 2,3,7,8-TCDD for 24 h in the presence of different concentrations of $NaAsO_2$ (0.5, 1, 3, 5, 10 and 20 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 were freeze–thaw three times to ensure complete cell lysis. Luciferase activity was measured by using Luciferase Assay System (Promega, Madison, WI, USA) according to the standard protocol provided. Luciferase activity was determined in the programmed microplate luminometer MicroLumatPlus LB96V (EG&G Berthold, Germany) with excitation and emission set at 400 and 460 nm wavelengths, respectively. The luciferase activity was expressed as relative light units (RLU)/μg protein.

2.5. Cell viability assay

Huh7 cells were seeded in a 96-well plate at a density of 4×10^4/well and cultured in DMEM medium for 24 h. Then, the cells were left untreated or treated with $NaAsO_2$ (0.5, 1.0, 3.0, 5.0, 10.0 and 20 μM) for another 24 h. Cell viability was determined by MTT assay, following the procedure previously described [19].

2.6. Ethoxyresorufin-O-deethylase (EROD) assay

Huh-7 cells, seeded at 90% confluence on 24-well plates with complete medium, were left untreated or treated with 10 nM 2,3,7,8-TCDD in the presence of different concentrations of $NaAsO_2$ (0.5, 1, 3, 5, 10, 20 μM) for 24 h. At the end of the incubation, the medium was removed and the wells were washed twice with fresh medium. The EROD activity was determined as previously described [20] using 5 μM of ethoxyresorufin in DMEM medium as the substrate of CYP1A1 enzyme, in the presence of 1.5 mM of salicylamide to inhibit conjugating enzymes. After incubation for 30 min at 37 °C, 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).

2.7. Statistics

Each experiment was performed in triplicate or quadruplicate. The statistical analysis was performed on the mean ± standard deviation (S.D.) from each independent experiment. The Mann–Whitney *U*-test was used to determine the differences between the untreated controls and the arsenic-treated data. Analyses were carried out using the Statistical Package for Social Science (SPSS) version 12.0 (SPSS Inc., Chicago, IL, USA).

3. Results

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

Using our Huh7-DRE-Luc cells for DRE-CALUX bioassay, we treated the cells with increasing concentrations of 2,3,7,8-TCDD for 24 h and demonstrated a dose–response induction of DRE-CALUX activity by 2,3,7,8-TCDD as shown in Fig. 1. This

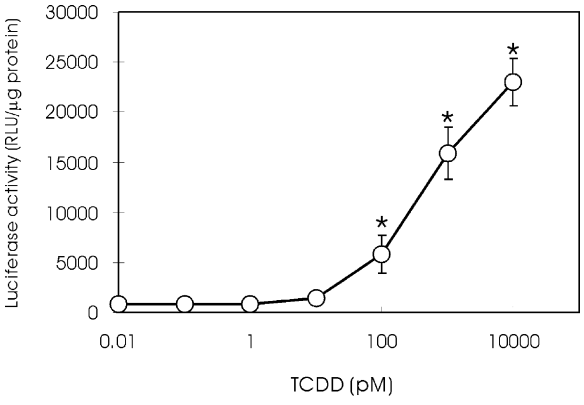

Fig. 1. Dose–response induction of luciferase activity by 2,3,7,8-TCDD. Huh7 cells at ≈90% confluence were incubated with 2,3,7,8-TCDD for 24 h. The measurements of luciferase activity are shown as means ± S.D., $n = 4$. $^{*}p < 0.05$, compared with the treatment with 0.01 pM TCDD.

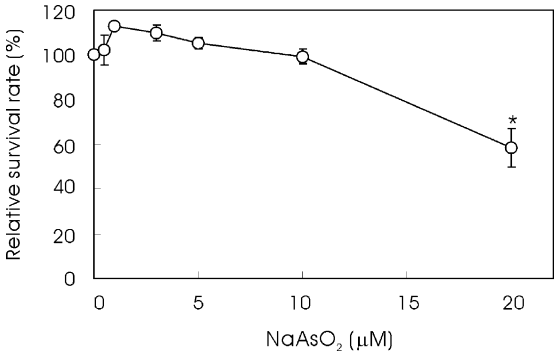

Fig. 2. Cytotoxic effect of arsenic on Huh7 cells. The cells were left untreated or treated with different concentrations of $NaAsO_2$ (0.5, 1, 3, 5, 10, and 20 M) for 24 h. After treatments, survival rates were determined by using MTT assay. Data are presented as means ± S.D., $n = 3$, and are expressed as percentage of survival rate as compared with that of untreated control. $^{*}p < 0.05$, compared with the untreated control.

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). The detection limit for 2,3,7,8-TCDD (6.4 pM) in Huh7-DRE-Luc cells was 10-fold higher than those in rat hepatoma cells (H4IIE) from Biodetection Systems b.v. (≈0.4 pM) [21] and in mouse hepatoma cells (Hep1c1c7) from Xenobiotic Detection System Inc (≈0.4 pM) [9].

3.2. Cytotoxic effect of arsenic on Huh7 cells

Cytotoxic effect of arsenic has been extensively studied in various types of cells. Thus, it is essential to define the non-toxic range of $NaAsO_2$ for Huh7 cells, the parental cells of Huh7-DRE-Luc cells, for evaluating the arsenic effects on DRE-CALUX activation by 2,3,7,8-TCDD. To analyze the cytotoxic effect of $NaAsO_2$, Huh7 cells were treated with different concentrations of $NaAsO_2$ for 24 h and then survival rate was determined with MTT assay. As shown in Fig. 2, treatments with $NaAsO_2 \leq 10$ μM caused no significant effect on cytotoxicity of Huh7 cells ($p > 0.05$), whereas treatment with 20 μM $NaAsO_2$ caused significant cytotoxic effect with a significant decrease in survival rate by 42% ($p < 0.001$).

To evaluate the cytotoxicity of $NaAsO_2$ in the presence of 2,3,7,8-TCDD, Huh7 cells were also treated with $NaAsO_2$ (5 and 10 μM) with or without 10 nM 2,3,7,8-TCDD for 24 h. Our data indicates that, in the presence of 2,3,7,8-TCDD, the 5 and 10 μM $NaAsO_2$ treatments exhibits no marked cytotoxicity and 10% decrease in survival rate, respectively (data not shown).

3.3. Inhibition of arsenic on DRE-CALUX induction by 2,3,7,8-TCDD

To address the arsenic effect on the DRE-CALUX induction by 2,3,7,8-TCDD, Huh7-DRE-Luc cells were treated with 10 nM 2,3,7,8-TCDD in the presence of different concentrations

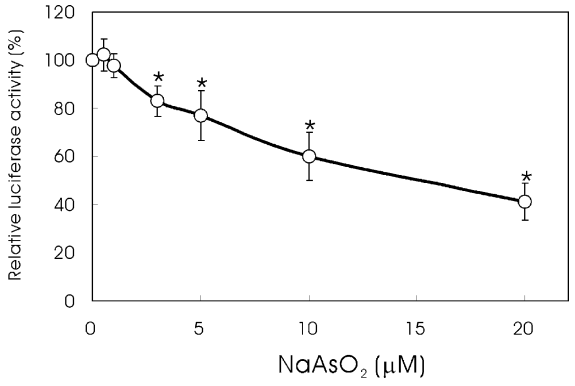

Fig. 3. Inhibitory effect of arsenic on the TCDD-induced luciferase activation in Huh7-DRE-Luc cells. The cells were treated with 10 nM TCDD in the presence of different concentrations of $NaAsO_2$ (0.5, 1, 3, 5, 10, and 20 μM) for 24 h. After treatments, luciferase activities were determined with CALUX bioassay as described in detail under Section 2. Data are presented as means ± S.D., $n = 4$, and are expressed as relative luciferase activity as compared with that of 10 nM TCDD-treated control. $^{*}p < 0.05$, compared with the treatment with 10 nM TCDD only.

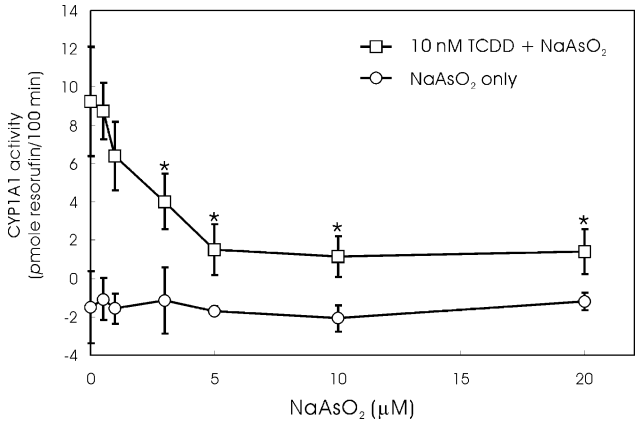

Fig. 4. Inhibitory effect of arsenic on the TCDD-induced CYP1A1 activation in Huh7 cells. The cells were treated with or without 10 nM TCDD in the presence of different concentrations of $NaAsO_2$ (0.5, 1, 3, 5, 10, and 20 μM) for 24 h. After treatments, CYP1A1 activities were determined with EROD assay as described in detail under Section 2. CYP1A1 activities are presented as means ± S.D., $n = 4$, and are expressed as resorufin formation (in pmol) per 100 min of reaction time (pmol resorufin/100 min). $^{*}p < 0.05$, compared with the treatment with 10 nM TCDD only.

of $NaAsO_2$. As shown in Fig. 3, treatments with $NaAsO_2$ attenuated the TCDD-induced DRE-CALUX activation in a dose-dependent manner. We then calculated the resulting CALUX-TEQ level of each treatment by fitting the model of equation ($\ln(Y) = 0.4435735568 \times \ln(X) + 18.51226416$, X and Y indicate CALUX-TEQ and luciferase activity, respectively). As shown in Table 1, the calculated CALUX-TEQ levels induced by 2,3,7,8-TCDD in the presence of 3, 5, 10 and 20 μM $NaAsO_2$ were 215, 186, 103, and 45.7 pg CALUX-TEQ/well, respectively. We found that these CALUX-TEQ levels were significantly lower than that induced by 2,3,7,8-TCDD alone (322 pg CALUX-TEQ/well, $p < 0.001$).

3.4. Inhibition of arsenic on CYP1A1 induction by 2,3,7,8-TCDD

By using Huh7 cells, we also analyze the effect of $NaAsO_2$ on CYP1A1 enzyme activation induced by 10 nM 2,3,7,8-TCDD as determined by using EROD assay. Our data indicated that $NaAsO_2$ inhibited the TCDD-induced EROD activation in a dose-dependent manner and $NaAsO_2 \geq 3$ μM could cause significant decreases in EROD activity ($p < 0.001$) (Fig. 4). This result was correlated with that from DRE-CALUX bioassay. Meanwhile, treatments with $NaAsO_2$ alone caused no marked effect on CYP1A1/EROD activity.

4. Discussion

Our established Huh7-DRE-Luc clone, despite its lower detection sensitivity for 2,3,7,8-TCDD, still provides a good in vitro model for evaluation of combined effects of environmental contaminants, such as arsenic, and 2,3,7,8-TCDD on regulation of AhR-mediated gene expression especially in human hepatocytes.

Our data indicted that $NaAsO_2 \leq 10$ μM significantly inhibited the TCDD-induced DRE-CALUX induction (Fig. 3) with no detectable cytotoxic effect on Huh7-DRE-Luc cells (Fig. 2), suggesting that the arsenic inhibition on DRE-CALUX activity was not due to cytotoxic effect of $NaAsO_2$. Our data from human hepatocytes agreed with a previous study [13], in which arsenic treatments between 2.5 and 10 μM could attenuate the 3-methylcholanthrene-induced CYP 1A1 enzyme activation in rat hepatocytes with no marked cytotoxic effect.

On the other hand, treatment with 20 μM $NaAsO_2$ significantly inhibited the TCDD-induced DRE-CALUX activation by

Table 1
Calculation of CALUX-TEQ levels based on induction of luciferase activity by $NaAsO_2$ and 2,3,7,8-TCDD

$NaAsO_2$ (μM)	0	0.5	1	3	5	10	20
Luciferase activities (RLU/μg protein)	11535	11792	11248	9648	9047	6968	4850
S.D.	1030	1118	990	1228	1676	973	895
Ratios	1.0	1.02	0.975	0.836	0.784	0.604	0.42
Calculated CALUX-TEQ levels (pg CALUX-TEQ/well)[a]	322	338	304	215	186	103	45.7
p-values[b]	–	0.789	0.843	0.045	0.012	<0.001	<0.001

[a] CALUX-TEQ levels were calculated according to the combined effect of $NaAsO_2$ and 2,3,7,8-TCDD (10 nM) on luciferase activity in Huh7-DRE-Luc cells. The calculated CALUX-TEQ levels are derived from the equation "$\ln(Y) = 0.4435735568 \times \ln(X) + 18.51226416$, $R^2 = 0.951$" and are presented as pg CALUX-TEQ/well. X and Y indicate CALUX-TEQ and luciferase activity, respectively.

[b] p-values were compared with that treated with TCDD alone (10 nM 2,3,7,8-TCDD without $NaAsO_2$), $n = 4$.

58% ($p < 0.001$) (Fig. 3) accompanied by a significant decrease in survival rate by 42% ($p < 0.001$) (Fig. 2), suggesting that the inhibitory effect of arsenic on DRE-CALUX activity was potentiated partly due to the arsenic-induced cytotoxic effect on Huh7-DRE-Luc cells. Moreover, regarding to the future application of DRE-CALUX bioassay in fast screening of a large number of samples with dioxin-like contaminants, our study also suggested that co-contaminants, such as arsenic, in samples due to incomplete cleanup may result in underestimation of CALUX-TEQ levels by using DRE-CALUX bioassay.

The toxicity of dioxins, coplanar PCBs, and 3-methylcholanthrene are mainly through the activation of AhR-mediated gene expression. To determine if the arsenic effect on DRE-CALUX activation performed in our Huh7-DRE-Luc system could represent the *true* AhR-mediated gene expression, we further analyzed CYP1A1 activity, as determined by EROD assay, in Huh7 cells subjected to the same treatments. Our data indicated that the inhibitory effects of arsenic on inductions of DRE-CALUX (Fig. 3) and CYP1A1 (Fig. 4) were quite similar. It has been recently demonstrated that arsenic treatments (between 0.25 and 5.0 μM) of human hepatoma Hep3B and HepG2 cells could attenuate the CYP1A1/EROD induction by 2,3,7,8-TCDD [14]. The study is well consistent with our present data.

Fig. 3 shows that 5 μM of $NaAsO_2$ inhibited TCDD-induced DRE-CALUX bioassay by 20% only, whereas Fig. 4 shows that $NaAsO_2$ inhibited EROD by 80%. The difference between these two assays could be resulted from posttranscriptional regulation and/or direct inhibitory effect of arsenite on CYP1A1. Moreover, a protein named AhR repressor (AhRR) has been revealed with the capacity to repress transcriptional activity by AhR [22]. The study showed that the liganded AhR complex could mediate gene expression of AhRR, which then inhibits AhR function by competing with AhR via dimerizing with Arnt and binding to the XRE sequence. Therefore, it is reasonable to suspect that the AhR-mediated AhRR expression in Huh7-DRE-Luc cells (for DRE-CALUX bioassay) is relatively lower than that in Huh7 cells (for EROD assay) and lead to inhibition of AhR function and CYP1A1 expression.

Co-contamination of high concentrations of arsenite with dioxin may attenuate the dioxin effect on DRE-CALUX reading such as making the positive results negative (Table 1). On the basis of our data by using Huh7-DRE-Luc bioassay, we suggest that arsenite concentrations higher than 3 μM (225 μg/l) can significantly underestimate the dioxin-CALUX-TEQ levels.

It is noteworthy that some studies have revealed that arsenite alone can induce CYP1A1 mRNA expression [23] and can activate the AhR possibly via a ligand-independent manner [24]. However, the former study also showed that arsenite inhibited the induction of CYP1A1 activity by 2,3,7,8-TCDD, accompanied by an upregulation of CYP1A1 mRNA expression. Thus, these data show that arsenite inhibits the 2,3,7,8-TCDD-induced CYP1A1 activation possibly at transcriptional and/or posttranscriptional levels. The precise regulatory mechanism remains unclear and needs to be further elucidated. In addition, we also conducted the similar experiments, i.e., DRE-CALUX, EROD, and MTT assays, by using $CdCl_2$. We demonstrated that treatments with $CdCl_2$ (1, 5, and 25 μM) significantly attenuated the TCDD-induced activations of DRE-CALUX and EROD in a dose-dependent manner with no marked cytotoxicity (data not shown). Therefore, our data showed that both $CdCl_2$ and $NaAsO_2$ inhibited induction of cytochrome P450 1A1 by 2,3,7,8-TCDD in human hepatoma cells in a similar pattern, suggesting that these inhibitory effects of $CdCl_2$ and $NaAsO_2$ were possibly regulated via the same mechanism(s).

Taiwanese EPA and DOH will soon adopted DRE-CALUX bioassay as one of standard methods to fast screening of dioxin-contaminated samples for its specificity, sensitivity, efficiency, easy sample cleanup, low-cost, and wide application to a variety of matrices. However, there are still a lot of parameters potentially interfering with DRE-CALUX bioassay require further evaluation. In the present study, we not only demonstrated an in-vitro evidence that arsenic could inhibit the TCDD-induced DRE-CALUX activation in our Huh7-DRE-Luc system but also presented an example that environmental contaminants, such as arsenic, could interfere with DRE-CALUX bioassay. Since arsenic is ubiquitous geographically, there are two endemic areas of long-term arsenic exposure from drinking water in Taiwan [25]. Samples with high arsenic contamination in the environment, seafood, and human specimens from these endemic areas without extensive cleanup will increase the difficulty of analyzing dioxin and PCB TEQ levels by DRE-CALUX bioassay. Thus, development of multi-stage cleanup procedures to prevent the interference for DRE-CALUX bioassay is necessary for specific monitoring of dioxin and PCB TEQ levels in various matrices in the future study.

The DRE-CALUX bioassay is a fast and inexpensive method especially for high-throughput screening of a large amount of sample with low dioxin-TEQ levels. However, on the basis of our study, the bioassay can be highly variable and inaccurate most probably due to incomplete sample cleanup for DRE-CALUX bioassay. Our data indicated that arsenite could affect the DRE-CALUX reading via inhibition of AhR function and CYP1A1 expression as well as via its cytotoxicity on Huh7-DRE-Luc cells. Therefore, in addition to those metals such as arsenite, cadmium, and chromium, it is noteworthy that some other co-contaminants with lipophilic property are usually very toxic to cells in culture. Most importantly, these lipophilic contaminants are extremely difficult to be separated from dioxin-like chemicals during extraction/purification process. Thus, the above precautions have to be taken properly in order to utilize the fast/inexpensive technique correctly.

Our study suggests that extensive clean-up of sample following the standard method can attenuate arsenic interference with DRE-CALUX bioassay. However, the concentration of 10 nM TCDD is an extremely high dose in the environment. In the future study, the low dose of TCDD with high dose of arsenic (i.e. 3 μM) tested in Huh7-DRE-Luc cells is needed. Therefore, DRE-CALUX bioassay coupled with HRGC/HRMS is recommended in analyzing dioxin levels in Arseniasis-endemic area. The public health in Arseniasis-endemic area in Taiwan is of great concern since elevated arsenic concentrations have been detected in human blood, urine, and hair [26]. Today, we are able to accurately measure TEQ levels of body burden by using

chemical and biological assays. However, in Arseniasis-endemic area, it is very possible that the TEQ levels we measured are not directly correlated with the dioxin-induced health effects due to the combined effects of dioxin(s) and arsenic. On the basis of our study, we suggest that the health effects induced by co-exposure of dioxin(s) and arsenic should be taken into serious consideration for toxicological and epidemiological studies in Arseniasis-endemic areas subjected to dioxin contamination.

5. Conclusion

In this study, we demonstrated that treatments of Huh7-DRE-Lux cells with $NaAsO_2 \geq 3\ \mu M$ significantly inhibited the TCDD-induced DRE-CALUX activation. Treatments of Huh7 cells with $NaAsO_2 \geq 3\ \mu M$ significantly inhibited the TCDD-induced CYP1A1 activation, as determined by EROD assay. Meanwhile, $NaAsO_2 \geq 20\ \mu M$ caused marked decreases in both DRE-CALUX and CYP1A1 activation induced by 2,3,7,8-TCDD partly due to its cytotoxic effect. Here, we not only demonstrated an in-vitro evidence that arsenic could inhibit the TCDD-induced CYP1A1 activation in human hepatoma cells but also presented an example that environmental contaminants, such as arsenic, could interfere with DRE-CALUX bioassay. Our present study also suggests that extensive cleanup for removal of any possible interfering factor is critical to guarantee the accuracy of DRE-CALUX bioassay. Otherwise, assessment of dioxin and PCB TEQ levels with DRE-CALUX bioassay in Arseniasis-endemic areas may lead to under-estimation of health impact induced by dioxins or dioxin-like chemicals.

Acknowledgments

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