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Short communication

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

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Abstract

Dioxin-responsive element-mediated chemical activated luciferase expression (DRE-CALUX) is one of alternative bioassays for the determination of dioxin levels. We have previously established a DRE-CALUX cell line, Huh7-DRE-Luc, by using stable transfection of Huh-7 cells with a reporter plasmid (4xDRE-TATA-Luc) carrying a DRE-driven firefly luciferase gene. It was also shown that arecoline, a major areca nut alkaloid, inhibited the 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD)-induced cytochrome P450 1A1 (CYP1A1) activation in Huh-7 cells. The TCDD-activated aryl hydrocarbon receptor (AhR) induces the DRE-CALUX activation and CYP1A1 gene expression via binding to DRE in promoter regions of these dioxin-responsive genes. In the present study, the effect of arecoline on the TCDD-induced activation of DRE-CALUX and CYP1A1 enzyme in Huh7-DRE-Luc and Huh-7 cells, respectively, was examined. It was found that arecoline inhibited TCDD-induced CYP1A1 activation and however enhanced TCDD-induced DRE-CALUX activation. This finding indicates the differential effect of arecoline on the endogenous dioxin-responsive CYP1A1 and on a stably transfected DRE-driven reporter in human hepatoma cells. The present study suggests that induction of DRE-CALUX alone does not necessarily parallel with endogenous CYP1A1 gene expression, and that the reporter assay may detect interactions that are not functional in endogenous gene.

Keywords: 2,3,7,8-Tetrachlorinated dibenzo-*p*-dioxin; Arecoline, Chemical activated luciferase expression; Cytochrome P450 1A1; Human hepatoma

1. Introduction

Polychlorinated dibenzo-*p*-dioxins (PCDDs) and dibenzofurans (PCDFs) are halogenated aromatic compounds that are recently considered as environmental endocrine disruptors. These organochlorine compounds tend to accumulate mainly in fatty tissues due to their high resistance to biodegradation, high lipophilicity, and long biological half-life. Currently, high-resolution gas chromatograph with high-resolution mass spectrometry (HRGC/HRMS) is the most certified and powerful assay for the determination of dioxin levels. However, HRGC/HRMS is time-consuming and costly which makes it a poor screening method for dioxin surveillance.

DRE-CALUX bioassay is an *in vitro* luciferase reporter assay for detecting the TCDD equivalency (TEQs) levels of dioxins/PCBs (polychlorinated biphenyls) [1]. DRE-CALUX

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assay is recognized as one of the best possible alternative methods for the detection of dioxins. Recently, the DRE-CALUX bioassay has been applied in detection of TEQ levels in both biota [2] and the environment [1]. Compared with HRGC/HRMS, DRE-CALUX is a rapid, low-cost, and high-throughput screening method, particularly for a large number of highly dioxin-contaminated samples from the environment and food.

However, there are still a lot of parameters that may potentially interfere with the DRE-CALUX bioassay and thus require further evaluations for its future application. Our previous studies have revealed that arsenic [3] as well as arecoline [4] inhibits induction of CYP1A1 by TCDD. In arsenic study, as expected, CYP1A1 activities by ethoxyresorufin-*O*-deethylase (EROD) assay were highly parallel with those by DRE-CALUX bioassay [3]. Combined with our previous arecoline study [4], here we demonstrate that arecoline inhibits the TCDD-induced CYP1A1 activations, however with enhanced TCDD-induced DRE-CALUX activations. This finding indicates the differential effect of arecoline on the endogenous dioxin-responsive CYP1A1 and on a stably transfected DRE-driven reporter in human hepatoma cells. This study employs arecoline as an example to demonstrate that induction of DRE-CALUX alone does not necessarily parallel with endogenous gene expression as well as that the reporter assay may detect interactions that are not functional in endogenous CYP1A1 gene.

2. Methods and materials

TCDD was obtained from Fluka Chemie GmbH (Buchs, Switzerland). Arecoline, ethoxyresorufin, resorufin, salicylamide, and dimethyl sulfoxide (DMSO) were obtained from Sigma–Aldrich (St. Louis, MO, USA). The human hepatoma cell line Huh-7 was kindly provided by Dr. Chiung-Tong Chen (National Health Research Institutes, Taiwan). Huh-7 cells were maintained in DMEM supplemented with fetal bovine serum (10%), penicillin (100 units/ml), streptomycin (100 units/ml), and sodium bicarbonate (3.7 mg/ml) in a humidified atmosphere of 5% CO_2 and 95% air at 37 °C.

The promoter activity of DRE was determined with the DRE-CALUX bioassay by using Huh7-DRE-Luc cells as previously described in detail in Ref. [3]. Luciferase activity was determined in the programmed microplate luminometer MicroLumatPlus LB96V (EG&G Berthold, Germany). The luciferase activity was expressed as relative light units (RLU)/μg protein. The CYP1A1 enzyme activity was determined with EROD assay by using Huh-7 cells as previously described in detail in Ref. [3]. The CYP1A1 activity was defined as resorufin formation (in pmole) per 100 min of reaction time (pmole resorufin/100 min).

Each experiment was performed in at least four times. The statistical analysis was presented as mean ± standard error (S.E.) from each independent experiment. Mann–Whitney *U*-tests were used to examine the differences between the untreated controls and the chemical-treated data. Analyses were carried out by using SPSS.

3. Results

We have previously demonstrated that arecoline (50, 100, 200, and 300 μM) caused decreases in survival rate (by 1, 15, 23, and 22%, respectively) in Huh-7; however, there was no statistical significance except in treatments with arecoline up to 300 μM [4]. Moreover, the study also revealed that TCDD (10 nM) exhibited no significant cytotoxicity in Huh-7 cells in combined treatments with or without arecoline. Since Huh7-DRE-Luc cells were established from the parental Huh-7 cells [3], an arecoline concentration ranging from 50 to 300 μM was employed in treatments of Huh7-DRE-Luc cells for the DRE promoter activity assay (DRE-CALUX bioassay) and in treatments of Huh-7 cells for the CYP1A1 enzyme activity assay (EROD assay).

To address the effect of arecoline on the DRE-CALUX activity, Huh7-DRE-Luc cells were treated with arecoline in the presence or absence of 10 nM TCDD for 24 h. As shown in Fig. 1, 10 nM TCDD alone caused a marked induction of DRE-CALUX activity as expected. Treatments with 50, 100, 200, and 300 μM of arecoline alone caused increases in DRE-CALUX activity by 2.35-, 4.46-, 7.95-, and 8.31-fold, respectively, as compared with the untreated control. Moreover, treatments with 50, 100, 200, and 300 μM of arecoline further potentiated the TCDD-induced DRE-CALUX activation by 1.14-, 1.55-, 2.10-, and 2.60-fold, respectively.

To address the effect of arecoline on the dioxin-responsive CYP1A1 enzyme activity, Huh-7 cells were treated with arecoline in the presence or absence of 10 nM TCDD for 24 h and then CYP1A1 enzyme activity was analyzed by the EROD assay. As shown in Table 1, 10 nM TCDD alone caused a 4.35-fold induction of CYP1A1 enzyme activity (from 17.01 to 73.96 pmole resorufin/100 min). Treatments with arecoline alone, up to 300 μM, had no significant effect on the basal CYP1A1 enzyme activity. On the other hand, treatments with 50, 100, 200, and 300 μM of arecoline inhibited the TCDD-induced

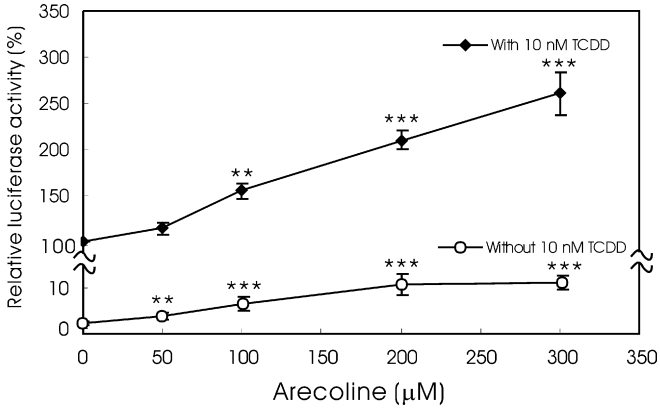

Fig. 1. Effect of arecoline on DRE-CALUX activity. Huh7-DRE-Luc cells were treated with different concentrations of arecoline (50, 100, 200, and 300 μM) in the presence or absence of TCDD (10 nM) for 24 h. The measurements of luciferase activity are presented as mean ± S.D. ($n = 4$). Our data are expressed as relative luciferase activity (%) as compared with that of 10 nM TCDD-treated control (100%). $^{**}p < 0.01$ and $^{***}p < 0.001$, compared with the respective untreated control (with or without 10 nM TCDD).

Table 1
Inhibitory effect of arecoline on TCDD-induced CYP1A1 activation in Huh-7 cells

Arecoline concentration (μM)	CYP1A1 activity (pmole resorufin/100 min)				
	0	50	100	200	300
Arecoline + 10nM TCDD ($n=7$)					
Mean ± S.E.	73.96 ± 10.00	64.60 ± 4.91	62.31 ± 5.51	49.17 ± 3.19	35.52 ± 3.97
95% C.I.[a]	64.71–83.21	49.56–79.64	45.43–79.19	39.41–58.92	23.37–7.67
p-Value	–	0.209	0.209	0.002**	0.001**
Arecolinealone ($n=8$)					
Mean ± S.E.	17.01 ± 1.32	15.30 ± 1.09	15.05 ± 1.32	12.24 ± 2.27	9.10 ± 3.11
95% C.I.	13.35–20.67	12.28–18.32	11.38–18.71	8.75–15.75	3.07–15.12
p-Value	–	0.645	0.442	0.061	0.050

[a] 95 C.I. means 95% of confidence interval.
** $p<0.01$.

CYP1A1 enzyme activation by 16.4, 20.5, 43.5% ($p<0.01$), and 67.5% ($p<0.01$), respectively.

4. Discussion

The DRE-CALUX bioassay will be soon adopted as one of the standard methods for fast screening of dioxin-contaminated samples because of 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 that may potentially interfere with the DRE-CALUX bioassay and thus require further evaluation. By using Huh7-DRE-Luc and its parental Huh-7 cells, our previous study has revealed that arsenic attenuates the TCDD-induced DRE-CALUX and CYP1A1 activations in a similar pattern [3]. We have also demonstrated that arecoline inhibits the TCDD-induced CYP1A1 activation in Huh-7 cells [4]. In this study, it was found that arecoline inhibits TCDD-induced CYP1A1 activation and unexpectedly potentiates TCDD-induced DRE-CALUX activation. This example indicates that some chemicals may produce a differential effect on endogenous dioxin-responsive CYP1A1 and on a stably transfected DRE-driven reporter, and this possibility has to be considered in order to utilize the fast/inexpensive technique properly.

The possible mechanism proposed for the present observation is that arecoline and/or its intracellular metabolites do bind to and activate the AhR, which enhances DRE-CALUX activation in Huh7-DRE-Luc cells. The promoter region for DRE-CALUX bioassay is very concise containing only four copies of DRE and a TATA box in front of a firefly luciferase gene [3], whereas the endogenous CYP1A1 promoter is much more complex that regulation of CYP1A1 gene involves a cross-talk between enhancer and promoter sequences with changes in the chromatin structure [5]. Therefore, the requirements for the CYP1A1 transcription in Huh-7 cells may include receptor conformation, interaction with response element, recruitment of coactivator and adaptor proteins, alterations in chromatin architecture, and recruitment of RNA polymerase and basal transcriptional machinery. Thus, the mechanisms required for the CYP1A1 gene transcription may not be necessary for the induction of an artificial DRE-driven luciferase reporter assay. Moreover, differences in post-transcriptional (such as mRNA stability) and/or post-translational (such protein stability) regulation between CYP1A1 and luciferase may also contribute to the resulting differences between the endogenous dioxin-responsive CYP1A1 activity and the DRE-driven luciferase activity. Similar to our findings, a previous study has revealed that the induction of estrogen receptor (ER)-mediated luciferase by selected PAHs does not accurately predict endogenous ER-dependent gene transcription [6]. However, the underlying mechanisms remain to be clarified.

In conclusion, effect of arecoline on the TCDD-induced activations of CYP1A1 enzyme (in Huh-7 cells) and DRE-CALUX (in Huh7-DRE-Luc cells) was examined in the present study. It was found that arecoline inhibited TCDD-induced CYP1A1 activation and, unexpectedly, potentiated TCDD-induced DRE-CALUX activation. Our present study reveals the differential effect of arecoline on the endogenous dioxin-responsive CYP1A1 and on a stably transfected DRE-driven reporter, suggesting that induction of DRE-CALUX alone does not necessarily parallel with endogenous CYP1A1 gene expression, and that the reporter assay may detect interactions that are not functional in endogenous gene.

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