TCDD RESPONSIVENESS OF A NONMALIGNANT HUMAN LUNG PERIPHERAL EPITHELIAL CELL LINE

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Introduction

Associations of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) with human lung disease have been reported in the past decade due to the availability to study larger cohorts as well as sufficient latency period for detection. [1,2] In rodents, TCDD exposure induces lung tumors in Osborne-Mendel rats and B6C3F1 mice [3] and recent studies from this laboratory have shown that in female Sprague-Dawley rats, TCDD induces metaplasia and hyperplasia in the alveolar/bronchiolar epithelium. [4] Since the expression of TCDD-inducible metabolic enzymes such as CYP1A1 in human [5] and rat [4] peripheral lung epithelial cells have been demonstrated, the relationship between TCDD exposure and lung cancer warrants further investigations.

Atypical adenomatous hyperplasia is a type of lung lesion believed to be the precursor of pulmonary adenocarcinoma that shares characteristics of type ii pneumocytes and Clara cells. [6] The A549 cell line, an established and well-characterized adenocarcinoma (type ii pneumocytes) expresses CYP1A1, CYP1B1 [7] and other cytochrome P450 enzymes [8]. In the A549 cell line, TCDD induced expression of CYP1A1 and CYP1B1 is inhibited by TGFβ1 [9,10]. Other studies have shown that their expression can be regulated differently by AhR inhibitors causing inhibition of TCDD-induced CYP1A1 but not CYP1B1, [8] such as genestein and staurosporine

The A549 cell line has proven valuable in evaluating the response of TCDD toxicity in human lung cells, but may not be appropriate for assessing TCDD effects in "normal" nonmalignant epithelial cells. The HPL1A cell line is a recently established normal human peripheral lung epithelial cell line that retains type ii pneumoctye specific multivesicular bodies and multilamellar body-like structures [11]. Cell proliferation and differentiation responses in the HPL1A cell line are altered by growth factors (EGF, HGF and TGFβ1) that may be involved in the pathogenesis of lung cancer.

The aim of this study was to assess comparative responses of gene expression in a nonmalignant type ii pneumocyte cell line (HPL1A) and a malignant type ii pneumocyte cell line (A549). Expression patterns of CYP1A1, and CYP1B1, AhR and AhR repressor (AhRR) were examined by real time RT PCR.

Methods and Materials

Cell Culture

All cell culture reagents unless otherwise stated were purchased from Life Technologies (Rockville MA). A549 cells (ATCC No. CCL-185) were purchased from American Type Culture Collection (Manassas, VI) and plated in Falcon tissue culture 75 cm^2 flasks at 4.2 X 10^4 cells/ml in F12 nutrient mixture (HAM) with glutamine and supplemented with 10% defined FBS (HyClone, Logan, UT), 100 units/ml penicillin G sodium, 100 μg/ml streptomycin sulfate, 25 μg/ml amphotericin B, and 15 mM HEPES. HPL1A cells were plated at 5.4 X 10^4 cells/ml in media as described previously. [11] A stock TCDD solution was prepared in FBS as reported previously [12]

and diluted with FBS for treatments. Twenty four hours after incubation, half of the culture media was removed and spiked to achieve final concentrations of 0, 0.1, 1, and 10 nM TCDD.

RNA extraction and cDNA synthesis
Media was removed 24 hours after incubation. Cells were scraped and lysed using TRI Reagent (Sigma, St. Louis, MO). Total RNA was isolated according to the manufacturer recommendations, quantitated, diluted to 40 ng/µl with DEPC-treated water, and stored at –70 °C. Total RNA (100 ng) was then reverse transcribed with random hexamer primers (2.5 mM) and MMLV reverse transcriptase (1.25 U/µl) in 10 µl reactions using a three step cycle: 25°C, 10 mins; 48° C, 30 mins; and 95° C, 5 mins. Reverse transcription products were purchased from PE Applied Biosystems (Foster City, CA).

Real Time Fluorescence detection PCR Analysis
Real time fluorescence detection was carried out using the ABI Prism 7700 Sequence Detection System, MicroAmp 96 well reaction plate, PCR buffer 1X (containing Sybergreen, a nonspecific dsDNA binding dye), $MgCl_2$ (5 mM), dATP, dCTP, dGTP (2.5mM each), dUTP (5mM), Taq Polymerase (0.025 U/µl) (PE Applied Biosystems, Foster City, CA), forward and reverse primers (Research Genetics, Huntsville, Al.) at 0.2 µM, and cDNA (10µl) in a final PCR reaction volume of 50 µl. PE 7700 amplification parameters were: denaturation at 94°C 10 mins, followed by 40 cycles of; 95°C, 15 sec.; 60° C, 60 sec. Data is reported in C_T values, where C_T is based on when fluorescence signal emitted is significantly above background levels and corresponds to when exponential PCR amplification begins (which is dependent on the initial template copy number). Actin was used as an endogenous control. Fold induction was calculated using the formula $2^{-\Delta\Delta C_T}$, where ΔC_T = target gene C_T - actin C_T, and $\Delta\Delta C_T$ is based on the mean ΔC_T of respective control (non-TCDD treated).

Some amplification products performed in the ABI prism 7700 were checked by electrophoresis on 1 % ethidium bromide stained agarose gels. The estimated size of the amplified products matched the calculated size for transcript.

Results and Discussion

Although, AhR transcript levels were abundant, no changes in mRNA were observed by 24 hours after treatment, at any doses tested. This corresponds with the literature where there is no major TCDD regulation of the AhR mRNA in certain systems but may occur at the level of its protein.[13,14]

The AhRR is a TCDD-responsive gene that modulates the AhR/ARNT signal transduction pathway and is expressed primarily in the heart and lung of male C57BL/6J mice. It is reported to regulate the AhR by a competitive mechanism involving both the AhR/ARNT complex and XRE binding activity.[15] Results show that AhRR is inducible in a dose-dependent manner with maximal induction of 3-4X over its respective control in both the A549 and HPL1A cells. TCDD-induced expression of CYP1B1 in both cell lines was comparable to those reported by Hukkannen et al., (2000). A dose dependent increase was observed, but only at the higher two TCDD doses were relative fold induction for CYP1B1, AhRR, and CYP1A1 significant to control. CYP1A1 was the most TCDD-inducible in both cell lines with slightly greater fold induction in the HPL1A (30X compared to 13X).

In summary, our results demonstrate that the HPL1A cell line is TCDD responsive and may be suitable for analyzing the effects of TCDD in normal human peripheral lung epithelial cells. By establishing the characteristics of TCDD-dose response in this cell line, future studies could be aimed at species comparative responses since normal (nonmalignant) rat type ii pneumocyte cell lines are also available.

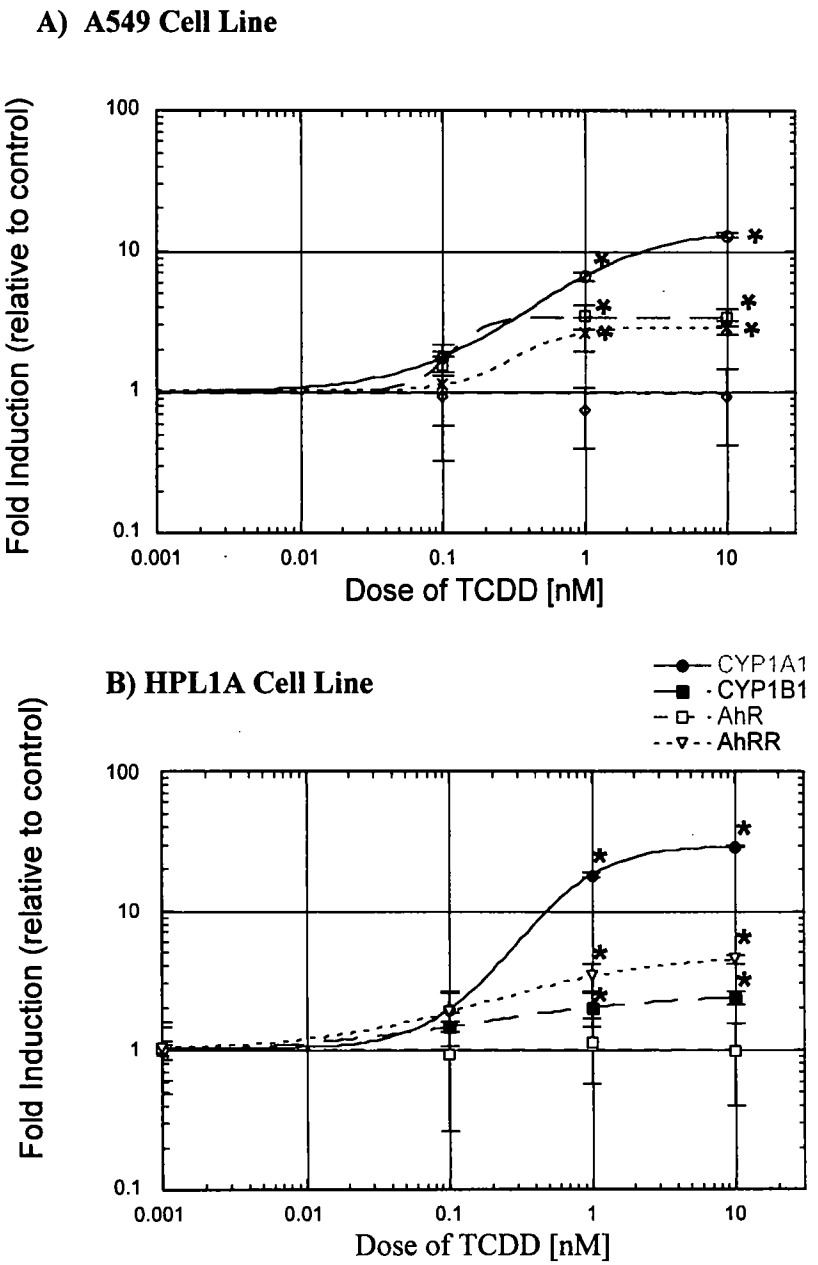

Figure 1. Relative quantitation of CYP1A1, and CYP1B1, AhR and AhRR using Real Time RT PCR. Experiments were repeated 3X and samples were analyzed in duplicate. Asterisks indicate statistically different from control using the Tukey-Kramer test, significance level 5%.

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