BLOOD AHR ACTIVATOR LEVELS AND AHR MESSENGER RNA EXPRESSION IN POPULATIONS WITH HIGH AND BACKGROUND EXPOSURE TO PCP WASTE

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Introduction

The toxic and biological effects of TCDD and dioxin-like compounds are mediated by aryl hydrocabon receptor (AhR)[1]. Polychlorinated dibenzo-p-dioxins (PCDDs), dibenzofurans (PCDFs), polychlorinated biphenyls (PCBs), polyaromatic hydrocarbons (PAHs), polychlorinated naphthalenes (PCNs), hexachlorobenzene, and derivatives of these compounds can also activate AhR[2]. AhR-dependent toxicity appears to be primarily driven by abnormal and persistent activation of AhR-dependent gene expression in target cells[3]. Natural and endogenous ligands of AhR -including flavonoids, carotenoids, tryptophan, and arachidonic acid metabolites- have relatively weak affinity compared to TCDD and are rapidly degraded[4]. Human exposure to PCDD/Fs and PCBs is associated with serious consequences to health including developmental[5,6], neurological[7], immunological[8], reproductive[9-12], and carcinogenic effects[13,14].

Pentachlorophenol (PCP) and chemical waste produced by a factory in southwestern Taiwan continued to contaminate the surrounding area even after the plant closed. Sera PCDD/PCDF levels of residents in the contaminated area have been found to be higher than those of non-contaminated area. These residents may have higher AhR ligand levels in their blood, which would probably raise the AhR activity abnormally and might lead to related health effects.

In this work we measured AhR activators with an alternative clean-up method conserving PAH, in order to maximize the estimation of the biological dose of AhR activators in blood samples of residents from the above mentioned contaminated area and other non-contaminated areas. We also correlated the Calux results with the AhR mRNA expression in the blood samples of these residents.

Materials and methods

A total of 504 subjects, 271 residents of the high exposure area (Annan District, Tainan) and 210 from a non-exposure area serving as reference group were recruited for the study.

Clean-up and CALUX were performed at Hiyoshi Corporation, Japan. Lipid fraction of 10 mL of whole blood with heparin was extracted with acetone and n-hexane, cleaned up using a celite-sodium sulphate column, dried with evaporator and transferred to silica-gel columns in hexane, without sulphuric acid silica gel layers, to retain PAH.

Thereafter H1L6.1 cell line, a mouse hepatoma (Hepa1c1c7)-derived cell line which has been stably transfected with the DRE-driven firefly luciferase reporter plasmid (pGudLuc6.1), was used for the CALUX system with 20 hours of incubation. CALUX results were expressed as relative luciferase activity (RLA), RLA per gram fat (RLA/g fat), and RLA per gram blood (RLA/g blood). Luciferase activity of 1 nM TCDD has RLA=1.

Buffy coat was obtained from blood anticoagulated with EDTA for AhR mRNA determination. After washing with red blood cells lysis buffer, white blood cells were kept in Trizol. Total RNA was isolated from white blood cells using with Acid Phenol-Guanidium thiocyanate- chloroform extraction method. Convertion to cDNA was performed with High Capacity cDNA Reverse Transcription Kit (Applied Biosystems). AhR and GAPDH

messenger RNA were measured using the ABI PRISM 7900HT Sequence Detection System with Taqman Hs00169233_m1 and Hs99999905_m1.

Statistical analyses were performed with SPSS 17.0.

Results

(1) Exposure area residents had relatively higher AhR activator levels than control area residents (average = 144.47 vs. 72.43 pg TEQ/g fat). After controlling for age, BMI, and smoking status, the differences were still statistically significant, either considering both genders together or separately.
(2) AhR expression (normalized with GAPDH) was also higher in the exposure area (1.03±0.05) compared to the control areas (0.83±0.03) (t=3.293, $p<0.001$).
(3) Significant correlation (Spearman's rho 0.207, $p<0.02$) between AhR ligand levels as determined with CALUX and AhR expression in terms of mRNA was found.

Conclusions

We have found higher levels of total AhR ligands and AhR mRNA expression in bloods of residents from a PCP contaminated area compared to other non-contaminated areas. Blood AhR ligand levels and AhR mRNA expression was associated, suggesting that the AhR ligands may be inducing AhR expression. Whether abnormal AhR expression would to AhR related health consequences would be our next subject of study.

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