Chemosphere xxx (2012) xxx–xxx

Contents lists available at SciVerse ScienceDirect

Chemosphere

journal homepage: www.elsevier.com/locate/chemosphere

Genetic susceptibility to dioxin-like chemicals' induction of cytochrome P4501A2 in the human adult linked to specific AhRR polymorphism

Wan-Ting Hung [a], George H. Lambert [b], Ping-Wei Huang [c], Donald G. Patterson Jr. [d], Yue Leon Guo [a,c,*]

[a] Department of Environmental and Occupational Medicine, College of Medicine, National Taiwan University and National Taiwan University Hospital, Taipei, Taiwan
[b] Department of Pediatrics, University of Medicine and Dentistry of New Jersey–Robert Wood Johnson Medical School, Piscataway, NJ, USA
[c] Institute of Occupational Medicine and Industrial Hygiene, College of Public Health, National Taiwan University, Taipei, Taiwan
[d] EnviroSolutions Consulting, Inc., Jasper, GA, USA

HIGHLIGHTS

- AhR, ARNT, and AhRR play important roles for dioxin-like chemicals signaling.
- CYP1A2 induction is an excellent biomarker of dioxin-like chemicals exposure.
- We study the blood samples from a highly dioxin-like chemicals exposed cohort.
- AhRR genetic polymorphisms are significant related to CYP1A2 induction.
- AhRR genetic polymorphisms predict susceptibility to dioxin-like chemicals.

ARTICLE INFO

Article history:
Received 29 January 2012
Received in revised form 16 October 2012
Accepted 17 October 2012
Available online xxxx

Keywords:
Aryl hydrocarbon receptor
Aryl hydrocarbon receptor repressor
CYP1A2 activity and induction
Single nucleotide polymorphisms
Dioxin-like chemicals
PCBs and PCDFs

ABSTRACT

Background: Dioxin-like chemicals are known to exert their effect by binding to aryl hydrocarbon receptor (AhR), forming complexes with aryl hydrocarbon nuclear translocator (ARNT), and binding to dioxin responsive elements (DREs) in promoter region to regulate the transcription of specific genes. In a previous study of the Yucheng cohort of humans who were exposed to high toxic levels of dioxin-like chemicals (PCDFs and PCBs), we reported marked induction of cytochrome P450 1A2 (CYP1A2) activity and this induction was an excellent biomarker of the exposure and adverse human health effects seen in the Yucheng cohort.
Objectives: The goal of this study was to determine the relationship between inducibility of CYP1A2 and genetic polymorphisms of AhR, ARNT, and AhRR in human.
Methods: The Yucheng victims who completed blood sample collecting in 1994–1995 for serum concentrations of PCB, PCDF, and PCDD congeners, and also completed the caffeine breath tests for CYP1A2 activity were identified. From the collected blood samples, six single nucleotide polymorphisms were selected for genotyping, including AhR (rs2066853), AhRR (rs2292596), ARNT (rs7517566), ARNT (rs3820541), ARNT (rs3768016), and ARNT (rs2228099).
Results: AhRR (rs2292596) polymorphism was significantly related to CYP1A2 inducibility ($p = 0.01$). A linear trend test was observed between people with AhRR (rs2292596) GG, GC, and CC genotype ($p = 0.0014$).
Conclusion: Overall, AhRR (rs2292596) genotypes predict the inducibility of CYP1A2 in people highly exposed to toxic dioxin-like chemicals. Future studies and analysis will determine to what degree these polymorphisms can predict a human's susceptibility to dioxin-related adverse human health effects.

1. Introduction

Polychlorinated dibenzo-p-dioxins (PCDDs), dibenzofurans (PCDFs), and biphenyls (PCBs) are persistent toxic chemicals widely found in low concentrations in the environment and in humans throughout world (Jensen, 1987; Srogi, 2008). Due to these chemicals' multiple adverse health effects (Carpenter, 2006) and long half-lives (Kimbrough, 1985) they are a serious world-wide public health concern. The most well-studied dioxin-like chemicals, 2,3,7,8-tetrachloro-dibenzo-p-dioxins (TCDD) is known to bind with aryl hydrocarbon receptor (AhR) in the cell plasma, translocate into the nucleus, form complexes with aryl hydrocarbon nuclear

* Corresponding author. Address: No. 1, Sec. 1, Ren-ai Rd., Zhongzheng Dist., Taipei City 100, Taiwan. Tel.: +886 2 33668216; fax: +886 2 23278515.
E-mail address: leonguo@ntu.edu.tw (Y.L. Guo).

0045-6535/$ - see front matter © 2012 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.chemosphere.2012.10.026

translocator (ARNT), and bind to specific DNA sequence, i.e. dioxin responsive elements (DREs), in the promoter region to enhance or repress the transcription of specific gene (Rowlands and Gustafsson, 1997; Abel and Haarmann-Stemmann, 2010). On the other hand, aryl hydrocarbon receptor repressor (AhRR), which could be induced by the AhR–ARNT complex, represses AhR transactivation in vitro and in animal models, although understanding of the mechanism is yet limited (Evans et al., 2005, 2008). Through AhR binding, TCDD and other dioxin-like-chemicals highly induce a number of phase I and phase II enzymes that are related to drug and xenobiotic metabolism, including the cytochrome P450 (CYP) superfamily members, especially CYP1A1, 1A2, and 1B1.

The CYP isoenzymes play important roles in biotransformation of exogenous and endogenous compound. For example, CYP1A2 metabolizes approximately 8–10% of clinical medicines that are metabolized by CYPs and takes part in the bioactivation of a series of procarcinogens and several compounds from natural and herbal sources (Zhou et al., 2010). Although interindividual variation of CYP1A2 protein expression and activity existed (Schweikl et al., 1993), induction of CYP1A2 was shown to be an excellent biomarker for exposure of dioxin-like chemicals and, most importantly, adverse human health effects in the Yucheng cohort (Lambert et al., 2006).

Single nucleotide polymorphisms (SNPs) of the genetic background in human population have been known to affect health outcomes caused by environmental exposure, and are considered important susceptibility factors. Among the genes associated with toxicities of dioxin-like chemicals, AhR, AhRR, and ARNT polymorphisms are conceivably related to their toxic mechanisms. The inducibility of CYP1A2 has been found related to the SNPs in AhR. A study in populations of healthy smoking young women indicated that CYP1A2 activity, assessed by the urinary caffeine metabolite ratio, is significantly higher in the group with at least one AhR Lys554 allele than in the homozygous Arg554/Arg554 group (Harper et al., 2002). With regard to the polymorphisms of ARNT, a G > C silent mutation in codon 189 (rs2228099) has been investigated, but no relationship was found between poor CYP1A1 inducibility (Anttila et al., 2000) and associated clinical findings such as bladder cancer risk (Figueroa et al., 2008), male infertility (Merisalu et al., 2007), or endometriosis (Tsuchiya et al., 2005). Another SNP, rs7517566, locates at in the 5′ near region of ARNT gene. This SNP was found associated with bladder cancer risk (Figueroa et al., 2008) but not type 2 diabetes in either African American or European American (Das et al., 2008). To date, most studies on SNPs in AhRR have focused on the Ala185Pro polymorphism (rs2292596). This SNP was first reported by Watanabe et al. (2001). It is a missense mutation located at codon 185 in exon 6 and is just behind the Per–Arnt–Sim (PAS) region (codon 112–182), which stabilizes the dimerization of AhRR and ARNT. Subsequent studies have revealed some links between this SNP and human reproductive disorders, such as micropenis, male infertility, and endometriosis (Watanabe et al., 2004; Soneda et al., 2005; Tsuchiya et al., 2005). However, the relationship between CYP1A2 inducibility and gene polymorphisms of AhR, AhRR and ARNT has not been well documented up to this point.

In 1978–1979, a rice oil poisoning event occurred in central Taiwan. A PCB mixture, presumably Japanese brand Kanechlor 500, was used as heat transfer medium in the production of rice oil in a Taiwan oil company. PCBs and the heat-degraded by-products, mainly PCDFs leaked into the rice oil. Approximately two thousand people who had consumed the oil were poisoned (Guo and Yu, 2003) and developed symptoms such as fatigue, chloracne, pigmentation of nails, skin, and gum, and hypersecretion of Meibomian glands. The syndrome was referred to as *Yucheng, oil syndrome* in Chinese. Fourteen years after exposure, the total blood PCB/PCDF concentrations of 56 directly exposed women were still much higher than control group, with 7-fold higher total PCBs and 40–130 times higher PCDF congeners than pooled local controls (Guo et al., 1997) and these Yucheng people are among the most highly exposed population to dioxin-like chemicals. We previously reported that CYP1A2 was induced among the Yucheng subjects, up to ten folds higher than could be induced by smoking (Lambert et al., 2006). Here we examine the hypothesis that inducibility of CYP1A2 in humans was modified by AhR, AhRR and ARNT polymorphisms.

2. Materials and methods

2.1. Subjects

The Yucheng rice oil poisoning incident registry recruited 2016 victims in 1979. Those who were older than 30 years old and still alive were matched with healthy people by sex, age, ethics, race, residence, and education status in 1993. The blood samples for measurements of serum concentrations of congeners and genotyping were taken during 1994–1995. The measurements of the serum concentrations of PCB, PCDF, and PCDD congeners were done by the U.S. Centers for Disease Control and Prevention in 2004 and 2008. Caffeine breath tests (CBTs) were conducted to determine CYP1A2 activity measurements in 1995. The details were described elsewhere (Lambert et al., 2006). Overall, data of 86 Yucheng patients and 69 control subjects were obtained successfully (Fig. 1, Table 1).

2.2. Measurement of internal exposure to PCBs/PCDFs/PCDDs

Serum concentrations of PCBs/PCDFs/PCDDs of Yucheng patients were previously determined (Lambert et al., 2006). Total TCDD toxic equivalent (TEQ) were determined by using WHO-TEF with following formula: Total TEQ = $[(\mathrm{PCDD}i \times \mathrm{TEF}i)n]$ +

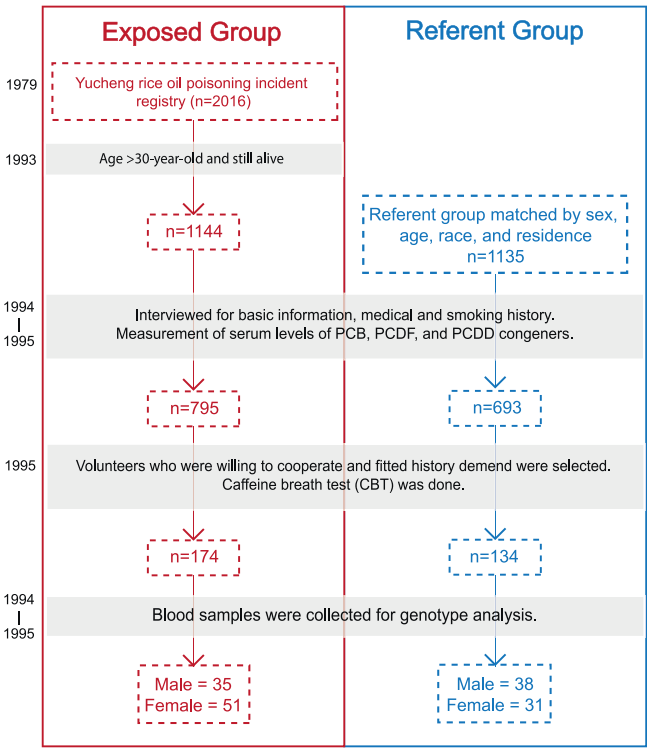

Fig. 1. Study design and major events.