Environmental Pollution 208 (2016) 506–511

Contents lists available at ScienceDirect

Environmental Pollution

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

Measurement of human CYP1A2 induction by inhalation exposure to benzo(a)pyrene based on *in vivo* isotope breath method

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ARTICLE INFO

Article history:
Received 2 July 2015
Received in revised form
16 October 2015
Accepted 17 October 2015
Available online 6 November 2015

Keywords:
Cytochrome P450 1A2
Benzo(a)pyrene (BaP)
Exposure biomarker
Caffeine Breath Test (CBT)

ABSTRACT

Cytochrome P450 1A2 (CYP1A2) is an enzyme involved in the metabolic activation of certain carcinogens, and inducible by toxic substrates. To date, few studies have investigated *in vivo* CYP1A2 induction in humans and its relationship to polycylic aromatic hydrocarbons (PAHs) like benzo(a)pyrene (BaP). Non-smoking healthy male coke-oven workers (n = 30) were recruited as 'exposure' group, and non-smoking healthy office workers in the same city (n = 10) were selected as 'control' group, to test whether high inhalation exposure to PAHs can induce CYP1A2 activity in human livers. Significantly higher inhalation exposure of PAHs were found among the exposure group compared to the control. Inhalation BaP exposure concentration in the exposure group was more than 30 times higher than the control group ($p < 0.001$). However, the exposure group did not exhale significant higher levels of $^{13}CO_2/^{12}CO_2$ in breath samples ($p = 0.81$), and no significant relationship was found between the inhaled BaP concentration and the $^{13}CO_2/^{12}CO_2$ ratio ($p = 0.91$). A significant association was found between the $^{13}CO_2/^{12}CO_2$ exhalation and dietary BaP intake level. Hepatic CYP1A2 activity/induction level was not effected by inhaled BaP but was altered by ingestion of BaP.

1. Introduction

Cytochrome P450 1A2 (CYP1A2) is one of the major members of cytochrome P450 enzyme family in human liver. This enzyme is involved in the metabolic activation of certain chemicals including carcinogens (Rendic, 2002; Shimada et al., 1994) which is an important initial step in the formation of many diseases like cancer in human (Ma and Lu, 2007; Zhou et al., 2009). Since CYP1A2 is mainly in human liver and cannot be assayed from peripheral blood samples, direct measurements in epidemiological studies seem less possible (Lambert et al., 2006). The activity of CYP in humans can be measured by using *in vivo* methods using some model substrates like phenacetin, caffeine and theophylline (Faber et al., 2005). The Caffeine Breath Test (CBT) is a well-established, non-invasive technique that involves the analysis of human expired breath. This technique has been used to measure inducible CYP1A2 robustly in a number of previous studies (Feyk et al., 2000; Lambert et al., 2006; Oshikoya et al., 2015; Park et al., 2003; Konstantinou et al., 2014).

Like other enzymes in the P450 super family, CYP1A2 is inducible, and many inducers are substrates of the enzymes (Fitzgerald et al., 2005; Ma and Lu, 2007). For instance, by using CBT method, it was reported that people exposed to some polychlorinated biphenyls (PCBs) and dibenzofurans (PCDFs) due to accidental ingestion of contaminated rice oil, had elevated CYP1A2 activity at more than 2-fold compared to the CYP1A2 level in unexposed controls (Fitzgerald et al., 2005; Lambert et al., 2006). With similar chemical structures to PCBs, PCDFs, and TCDD, some polycyclic aromatic hydrocarbons (PAHs) like benzo(a)pyrene (BaP)

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http://dx.doi.org/10.1016/j.envpol.2015.10.023
0269-7491/

may be able to bind to the aryl hydrocarbon (Ah) receptor and potentially induce liver P450 1A2 activity in humans (Larsen and Brosen, 2005; Oziolor et al., 2014). Since the enzyme catalyzes an important step in the bioactivation of these carcinogens, the induction of enzyme is detrimental in human exposure to high levels of these toxic pollutants (Shimada and Fujii-Kuriyama, 2004). Humans may be exposed to PAHs through various routes including inhalation, ingestion and dermal exposure. For most people, the first two routes are main exposure pathways. The relative contributions of inhalation and ingestion exposure pathway may vary among population and time. China is one country with severe PAH pollution in ambient air. It was reported that, the overall population attributable fraction (PAF) caused by inhalation exposure to 16 U.S. EPA priority PAHs was as high as 1.6% (0.91–2.6%) (Zhang et al., 2009). However, to date, few studies investigated the *in vivo* CYP1A2 induction level due to PAH exposure.

The main objective of this study is to test the hypothesis that high inhalation exposure to PAHs can induce CYP1A2 activity as measured by the CBT technology, and to examine the relationship between *in vivo* CYP1A2 activity and the current-day PAHs inhalation exposure.

2. Materials and methods

2.1. Subjects

To test the induction of inhalation PAHs exposure, we selected two groups of subjects with potential distinguished inhalation exposure to PAH in a typical Northeast city in China. “Exposure group” were 30 subjects who worked in highly exposed PAH areas of a coke factory. “Control group” were 10 subjects from an area in the same city that had the lowest level of air pollution. All the 40 subjects were healthy non-smoking male adults with no chronic disease medical record and had not taken any medication during the 2 weeks prior to sample collection. They were all free from dermal exposure sources of PAHs (e.g., chimney soot, engine oil). The demographic information (age, body weight and body mass index) for the exposure and control groups were similar without significant differences (Table S1). The study protocol was approved by the University of Medicine and Dentistry of New Jersey's Institutional Review Board (IRB); and written informed consent was obtained from each subject.

2.2. Exposure to PAHs

Personal inhalation samples were collected in each subject during 24 h period. A quartz fiber filter and a polyurethane foam (PUF) were used to collect particulate phase and gaseous PAHs, respectively. After the sample collection, the filter and the PUF were placed in the Petri dishes and glass jars respectively, wrapped with aluminum foil to avoid light exposure. The samples were extracted with a Soxhlet extraction using 150 mL of dichloromethane for at least 16 h at 60 °C. The extract was concentrated to about 1 mL under reduced pressure at 40 °C. The concentrated solution was washed with total of 3 mL dichloromethane for 3 times; and all the liquids were transferred to a 10 mL graduated KD-concentrator, and then blown down to 0.1 mL with high-purity air at 37 °C. Acetonitrile was added into the solution to make a 1 mL final solution that was subsequently filtered with a 0.2 μm PVDF Liquid Filter before HPLC analysis.

Food samples were collected as “duplicate plate” method. Food consumed within the same 24-h air monitoring period was collected using the so-called, i.e., subjects were asked to prepare two identical plates of meal (including snacks and excluding drinks), one for actual consumption, and the other for sample collection. These samples were placed inside a cooler with icepacks until returned to the laboratory. After each meal/snack sample had been weighed, all the samples within the 24-h period were combined and blended homogeneously to form a composite sample. An aliquot (100 g) of composite sample was placed into a 350-mL Erlenmeyer flask containing 12.5 g of KOH in 200 mL of 95% ethanol and a mixing stirrer. After the flask was placed on a hot plate at 60 °C for 4 h, the entire content of the flask was filtered through a filter paper (0.45 μm pore size); and the filtrate was transferred into a 2-L seperator funnel where a liquid–liquid extraction was performed using 60 mL of isooctane for 10 min and then repeated to separate PAHs from ethanol. The isooctane phase was transferred to a 350 mL amber round flask and concentrated to 5 mL using a vacuum rotary evaporator at 60 °C. If visible solid-phase materials still occurred in the condensed extract, centrifugation was used to remove the solid residues. Sample enrichment and purification was completed using a Sep-Pak Florisil cartridge (Waters051960, MA). The cartridge was first primed with 5 mL isooctane. After the condensed sample extract passed through the cartridge at a flow rate of 1 mL/min, 10 mL of benzene was introduced into the cartridge to extract PAH from the cartridge coating material. This benzene solution was then evaporated to 0.1 mL using a gentle nitrogen gas flow. Acetonitrile was added to make a 1 mL final solution that was further filtered through a 0.2 μm PVDF Liquid Filter before HPLC analysis.

The final solutions of air and food samples were analyzed using a HPLC system (Waters 600E, MA) equipped with a programmable fluorescence detector (LDC 4100) and a. LC-PAH column (250 × 4.6 mm, 5 μm, Supelco Co., PA) at 30 °C. A mobile phase gradient program was used. Flow rate was sustained at 1.0 mL/min. Helium gas was used for solvent degassing. The fluorescence wavelength program used in the analysis was $270_{ex}/350_{em}$ nm in 0–32.8 min; $250_{ex}/400_{em}$ nm in 32.8–47 min; and $280_{ex}/425_{em}$ nm in 47–70 min. The recovery rate, precision and method detection limit (MDL) of BaP were about 85%, 15%, 0.7 ng/m^3 in inhalation samples and 80%, 10%, 0.04 ng/g in ingestion samples, respectively. The recovery rate, precision and MDL of other PAHs were also at acceptable levels.

2.3. The Caffeine Breath Test (CBT) for CYP1A2 induction

This technique is based on the principle that the production rate of caffeine metabolites through 3-N-demethylation is a function of CYP1A2 activity (Lambert et al., 1983; Schmilovitz-Weiss et al., 2008). Because one of the ultimate products of 3-N-demethylation of the caffeine molecule is carbon dioxide (CO_2) that is subsequently excreted in the expired breath, the CBT method was designed to measure the production rate of CO_2 due to this specific metabolic pathway. To differentiate CO_2 produced by CYP1A2-catalyzed caffeine metabolism from the bulk of CO_2 in the expired breath, the CBT method requires study subjects to ingest caffeine with carbon atom in the 3-N-methyl group labeled with carbon-13 (^{13}C). The labeled CO_2 production rate, expressed as percent increase of ^{13}C per hour, is calculated from breath samples collected before and after ingestion of the ^{13}C-labled caffeine. It has notable advantages compared to the measure of metabolic ratios in plasma and/or urine (Fitzgerald et al., 2005).

According to the method requirement, all the subjects had fasted for at least 6 h before a CBT. If a subject could not tolerate drinking a reasonable amount of coffee, or had a diastolic blood pressure >90 mmHg, or a systolic pressure >140 mmHg, or resulting pulse >80 b/min, the subject was excluded from the study. All subjects meet the criteria for the study. Each subject sat quietly for at least 5 min before ingesting 3 mg/(kg body weight) (up to a maximum of 200 mg) of ^{13}C-labeled caffeine dissolved in 20 mL of