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Impact of dioxins on antipyrine metabolism in firefighters

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HIGHLIGHTS

- Firefighter exposure to dioxins was assessed using a metabolic test.
- Current firefighters excreted metabolites consistent with CYP1A2 induction.
- Recent, but not former exposure, alters the metabolism of firefighters.

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ABSTRACT

Antipyrine (AP) metabolism was used to assess factors associated with the activity of hepatic oxidative enzymes in firefighters. Emphasis was placed on 3-hydroxymethylantipyrine (3HMAP), the metabolite with the greatest dependence on dioxin-inducible cytochrome P4501A2 (CYP1A2) activity. AP urinary metabolites were measured by HPLC in 38 male subjects from Eastern Siberia. Subjects were divided into three groups having similar ages and BMIs: current firefighters ($n = 11$); former firefighters ($n = 17$) and non-firefighters ($n = 10$). Multiple regression models were constructed using the three major AP metabolites as a dependent variable to assess the influence of age, smoking as urinary cotinine concentration, dioxin exposure (as either WHO-TEQ or body burden), group, and *CYP1A2*F* (-163C>A) genotypes. Models for the proportion of dose excreted as the metabolite 3HMAP produced the best fit (adjusted $R^2 = 0.46$, $p < 0.05$). When the models were restricted to current firefighters, only those based on 3HMAP were statistically significant (adjusted R^2 of 0.80 ($p < 0.002$)) due to contributions from urinary cotinine ($\beta = 0.56$, $p < 0.01$) and dioxin expressed as body burden ($\beta = 0.55$, $p = 0.014$). These results indicate that the antipyrine test can be used as metabolic probe of biological response to recent dioxin exposure provided the impact of smoking is carefully controlled.

1. Introduction

Dioxins are considered to be one of the most toxic classes of anthropogenic compounds because of their ability to produce a wide variety of alterations of human homeostasis and health. Dioxins are potent cellular dysregulators which have been extensively studied as carcinogens, developmental toxicants, and endocrine disruptors (White and Birnbaum, 2009). In particular dioxin-like compounds have been implicated in diabetes, cardiovascular disease, testicular cancer, prostate cancer and non-Hodgkin's Lymphoma (IARC, 2010) and other effects. Dioxins are typically formed when organic materials are burned or heated in the presence of chlorine as happens during waste combustion, metal or cement production, and electrical generation at coal power plants (Vikelsoe and Johansen, 2000; Dopico and Gómez, 2015). Some congeners such as 2,3,7,8-tetrachlorodibezo-*p*-dioxin (TCDD), and 2,3,4,7,8-pentachlordibenzodioxin (PeCDD) have biological half lives lasting for years; for example, 7 years for TCDD and 15.7 years for PeCDD (Flesch-Janys et al., 1996). Moreover, the biological half life of a congener can be influenced

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by age, percent body fat, smoking status and breast feeding (Milbrath et al., 2009).

Most of the effects of dioxins are mediated by the Ah-receptor (AhR) which plays a central role in the induction of the Phase I enzymes and acts as a modulator of cellular signaling pathways (Whitlock, 1993). Firefighter exposure to dioxins is usually accompanied by a complex mixture of other compounds (Edelman et al., 2003; Laitinen et al., 2012). High affinity dioxins may successfully compete for the AhR and in doing so may alter the toxicity of other agents such as polycyclic aromatic hydrocarbons whose conversion to active metabolites is dependent on CYP1 family (Denison and Nagy, 2003). The resultant mix of exposure agents means that some, but not all of the toxicity experienced by firefighters is mediated by the AhR pathway.

CYP1A2, along with CYP1A1 and CYP1B1, is an AhR-responsive oxidative enzyme whose dose dependent induction by dioxins occurs in both humans and rodents. In rodents, this induction results in the sequestration of TCDD and PeCDD in the liver (Diliberto et al., 1997). Furthermore, Grassman et al. (2002) found high and significant correlation to the total TEQ with the expression of CYP1A2 in human liver samples taken from post-mortem donors.

CYP1A2 is predominantly a hepatic enzyme and as such, CYP1A2 mRNA is not detectable in lymphocytes (Krovat et al., 2000). Because of this, expression of the enzyme is assessed indirectly through the measurement of the products of CYP1A2-dependent metabolism. Both caffeine (Halperin et al., 1995; Abraham et al., 2002) and antipyrine (AP) (Ostashevsky et al., 1994) have been used as metabolic probes in dioxin exposed populations. AP was used to evaluate CYP1A2-dependent activity in residents of South Vietnam living in regions that had been sprayed with Agent Orange (Ostashevsky et al., 1994). Although dioxin body burdens were not measured, a significant correlation between the pattern of urinary AP metabolites and CYP1A1 activity (benzopyrene-hydroxylase) in blood lymphocytes of humans was found. Halperin et al. (1995) examined the effect of occupational exposure to chemicals contaminated with 2,3,7,8-TCDD on CYP1A2 activity by using caffeine as a substrate and subsequently measuring the caffeine metabolite ratio (CMR). The authors did not find a significant association between the CYP1A2 activity and serum TCDD, whereas cigarette smoking induced CYP1A2. Abraham et al. (2002) measured caffeine demethylation in two individuals who were highly exposed to TCDD. Eighteen months after the presumed time of intoxication, they found a more than 10-fold induction of hepatic CYP1A2 enzymes. Specifically, the two poisoned individuals had CMRs of 39.3 and 29.8, compared with a mean CMR of 3.65 in 30 nonsmokers. A positive association between the serum concentration of PCB-105 (one of three dioxin-like congeners that were investigated) and CMR was found in a study by Petersen et al. (2006). Lambert et al. (2006) used the caffeine breath test to measure CYP1A2 activity in members of the Yucheng cohort who were exposed to PCBs at levels far above those measured by Petersen and team when they consumed contaminated rice oil. After 16–17 years of exposure, they had levels of CYP1A2 activity that were double the levels measured in breath samples and were correlated with serum TEQ, consisting predominantly of PCDFs and PCBs (Lambert et al., 2006).

Current evidence demonstrates that firefighters are occupationally exposed to dioxins (Kelly et al., 2002; Schecter et al., 2002; Edelman et al., 2003; Hsu et al., 2011; Chernyak et al., 2012; Shaw et al., 2013). Consequently, there is a need for methods that measure the biological response of workers, which is determined not only by the toxicant dose, but also by the activity of enzymes involved in its biotransformation. The purpose of this study is to employ antipyrine metabolism as a metabolic probe to measure factors that influence the activity of hepatic oxidative enzymes, predominantly CYP1A2, in current, former, and non-firefighters.

2. Materials and methods

2.1. Selection of the cohort and blood donors

In 2009–2010 we examined dioxin levels in 40 men, thirty of whom were recruited from a cohort of 165 firefighters originally assembled in 2003 to study dioxin exposure and health effects following the 1992 Shelekhov fire (Chernyak et al., 2004). This examination included an additional 10 men who were recruited to serve as non-firefighter controls. The thirty firefighters were grouped according to their status as current or former firefighters. The formation of the firefighter groups was complicated due to the limited number and inaccessibility of candidates for a variety of reasons, including change of residence and death. Approximately 20 of those contacted declined to participate. Men were screened to ensure their body mass index (BMI) and ages were comparable. All participants are from similar economic strata within Irkutsk Oblast. Information on demographic, familial, occupational, and personal characteristics including smoking habits, diet, hobbies, and illnesses was obtained through an oral questionnaire. Informed consent, which included authorization for blood and urine sampling and banking, was provided by all participants. The study protocol was approved by the Biomedical Ethics Committee of East-Siberian Scientific Center of Siberian Branch of Russian Academy of Medical Sciences in Irkutsk and the Brooklyn College-CUNY Institutional Review Board.

2.2. Measurement of serum dioxin concentrations

After overnight fasts, each participant provided 40–50 ml of blood from which serum was obtained using a standard procedure. Seven polychlorinated dibenzo-*p*-dioxin (PCDD), 10 polychlorinated dibenzofuran (PCDF), and 12 polychlorinated biphenyl (PCB) congeners were analyzed by gas chromatography/high-resolution mass spectrometry in each of the samples at the A.N. Severtsov Institute of Ecology and Evolution (Moscow) according to the protocol previously described (Chernyak et al., 2012). The system of toxicity equivalence factors developed by the WHO in 2005 was used to calculate the total toxicity equivalent (TEQ) (Van den Berg et al., 2006). Measurements below the limit of detection (LOD) were assigned a value representing the level of detection divided by the square root of 2 as recommended by the US Centers for Disease Control and Prevention (Hornung and Reed, 1990). Results are expressed as WHO-TEQ and as body burden. Dioxin exposure as body burden was calculated using the measured TEQ and the following formula for percent of lipids in the body: % lipids = 495/(1.0324–0.19077(log(waist–neck)) + 0.15456(log(height)) – 450) (Hodgdon and Beckett, 1984).

2.3. Antipyrine test

The current study used antipyrine (AP) (pharmaceutical purity, Fluka Chemical Co., Milwaukee, WI, USA) as a metabolic probe to measure the activity of hepatic oxidative enzymes. Because AP has not been used as an analgesic in recent years, there was no possibility that participants were exposed outside of the study. AP metabolism was assessed by HPLC performed on urine samples obtained for 38 from 40 participants, two individuals from the current firefighters group were excluded because of medical conditions.

The subjects were instructed to abstain from ingesting medication 48 h before the AP dosage until the end of the urine collection. After voiding the bladder, urine was collected for 24 h