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Serum polychlorinated biphenyls and leukocyte telomere length in a highly-exposed population: The Anniston Community Health Survey

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ABSTRACT

Background: Serum polychlorinated biphenyls (PCBs) have previously been associated with longer leukocyte telomere length (LTL) in most, but not all, of the few previous studies. PCBs were produced in Anniston, Alabama from 1929 to 1971 and participants of the Anniston Community Health Survey (ACHS) were highly exposed.
Objectives: We evaluated serum levels of 35 PCBs and relative telomere length in 559 ACHS participants.
Methods: Relative LTL was measured in DNA extracted from blood clots. We assessed PCBs individually, grouped by chlorination, and summed PCBs. We used linear regression to assess the association between each PCB metric while adjusting for pertinent covariates.
Results: Serum PCBs were associated with longer LTL among white participants and the oldest age group of black participants. Among white participants, compared with those in the first quartile of sum PCBs those in the third quartile of sum PCBs had 8.09% longer relative LTL (95% CI: 1.99; 14.55) and those in the fourth had 7.58% longer relative LTL (95%CI: − 0.01; 15.76) (p-quadratic = 0.05). Among African American participants, serum PCBs were associated with longer relative LTL among those over age 64 only. Tests for interaction were not statistically significant.
Conclusions: We observed a non-linear positive association between serum PCBs and LTL among white participants. Serum PCBs were associated with longer LTL in the oldest age group of African Americans. This association may provide insight into the cancers previously associated with exposure to PCBs, melanoma and non-Hodgkin lymphoma, which have been associated with long LTL in previous studies.

1. Introduction

Polychlorinated biphenyls (PCBs) are a class of 209 organic synthetic chemicals with one to ten chlorine atoms attached to the biphenyl ring, that were previously used in a variety of commercial applications (Warner et al., 2012). Manufacture of PCBs was banned in the United States in 1977. Because of their persistence in the environment PCBs can be detected in air, seawater, lake and river sediments (Li et al., 2009; Warner et al., 2012). The toxicity a PCB congener exerts is dictated by the number and positioning of chlorines atoms on the two phenyl rings. Non-*ortho* PCBs are congeners lacking chlorines at the 2, 6, 2′, and 6′ position that bind to the aryl-hydrocarbon receptor (AhR) to exert dioxin-like effects. PCBs containing one chlorine in an *ortho* position (mono-*ortho*) bind to the AhR, but with less affinity than non-*ortho* PCBs (Lauby-Secretan et al., 2016). It is established that AhR binding induces the expression of a number of genes that contribute to carcinogenesis via deregulation of several cell-cycle and signal transduction pathways (Wall et al., 2015). Adverse health effects caused by PCBs independent of AhR binding are active areas of investigation. Alternate mechanisms of PCB-induced toxicity include oxidative stress, genotoxic effects, immune suppression, inflammatory response and endocrine disruption (Lauby-Secretan et al., 2016). The degree to which these mechanisms are exerted is likely congener and pathway specific.

Telomeres are caps on the ends of chromosomes consisting of tandem nucleotide repeats and an associated protein complex called “shelterin”. Telomeres maintain genomic stability by preventing the fusion of chromosomal ends, nucleolytic decay, and atypical recombination (O'Sullivan and Karlseder, 2010). Loss of telomeric DNA during cell division prevents the loss of critical chromatin. Critically short telomeres are recognized as damaged DNA and activate the DNA

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damage response pathway leading to cellular senescence and apoptosis in normal cells (Sahin and DePinho, 2012). Aging, oxidative stress, and inflammation have been implicated in telomere shortening (Shin et al., 2010). Telomere shortening beyond critical lengths may lead to aberrant recombination, chromosomal fusion, and subsequent neoplasia (Wong et al., 2014). Alternatively, cells with longer telomeres may favor delayed senescence and thus have more potential to acquire genetic abnormalities and subsequent malignant transformation (Lan et al., 2009). Both short and long leukocyte telomere length (LTL) has been associated with increased risk of several types of cancer (Ma et al., 2011; Wentzensen et al., 2011). Short LTL has also been associated with increased risk of cardiovascular disease (Haycock et al., 2014) and type 2 diabetes (Willeit et al., 2014).

In vitro, immortalized human skin keratinocytes, hamster lung fibroblasts, immortalized lymphocytes, or human promyelocytic leukemia cells exposed to PCB 153, PCB 126, PCB 52, PCB 28, or 2-(4′-chlorophenyl)-1, 4-benzoquinone (PCB3pQ), have shortened telomeres (Jacobus et al., 2008; Senthilkumar et al., 2011; Senthilkumar et al., 2012; Xin et al., 2016; Ziegler et al., 2016). Conversely, PCB 138 and PCB 153 upregulate c-*myc* in vitro (Ghosh et al., 2007; Gribaldo et al., 1998). *C-myc* is a proto-oncogene that is involved in the reactivation of telomerase (Daniel et al., 2012). Exposure to PCBs is consistently associated with an increased risk of melanoma and to a lesser extent non-Hodgkin lymphoma (NHL) (reviewed by: (IARC et al., 2016)). Longer LTL has been associated with increased risk of melanoma (Caini et al., 2015) or NHL (Hosnijeh et al., 2014; Lan et al., 2009).

To our knowledge, five cross-sectional studies and one longitudinal study have addressed the association between serum PCBs and LTL. The first was a study of 84 healthy Korean participants that reported a positive association between serum levels of PCBs and LTL. However, the four study participants with the highest levels of serum PCBs had shorter telomeres (Shin et al., 2010). Three analyses of National Health and Nutrition Survey (NHANES) participants have also identified a positive association between serum PCBs and longer LTL (Mitro et al., 2015; Patel et al., 2016; Scinicariello and Buser, 2015). In the largest NHANES analysis, comprising 2175 participants, those in the highest quartile of sum PCBs (> 142.80 ng/g) had 11.63% longer telomeres (95% CI: 6.18; 17.35) than those in the lowest quartile (Scinicariello and Buser, 2015). Conversely, in a study of 207 participants occupationally exposed to PCBs and 104 non-occupationally exposed controls, LTL in lymphocytes, but not granulocytes, was shorter among those exposed to PCBs (Ziegler et al., 2016). In the only longitudinal study to date, serum levels of PCB 153 were associated with shorter LTL after ten years of follow-up (Guzzardi et al., 2016).

The Anniston Community Health Survey (ACHS) is a cross-sectional study of residents of Anniston, Alabama, where PCBs were manufactured from 1929 to 1971. ACHS participants have serum levels of PCBs 1.5 to 3.5 times greater than those of NHANES participants of the same age and race (Pavuk et al., 2014a). To test the hypothesis that PCBs are associated with LTL, we assessed the association between serum levels of 35 PCBs and relative LTL in this highly exposed group.

2. Methods

2.1. The Anniston Community Health Survey (ACHS)

ACHS participants were recruited and completed active study participation between 2005 and 2007. Two-stage address-based random sampling was used to select 3320 households from a commercial list of residential sites within Anniston city limits. Addresses in west Anniston, where the former PCB manufacturing facility was located, were intentionally oversampled. Of the addresses identified, 489 were vacant or non-residential and 890 could not be reached after multiple attempts. In total, 1110 households agreed to participate (39% response rate; 57% participation rate). We used the survey responses to identify participants who reported that a doctor diagnosed them with diabetes, hypertension, or cancer; years of education; smoking status; and other pertinent covariates. Of those who completed the survey, 765 agreed to a clinic visit where they donated fasting blood samples. After centrifuging, 2 ml of serum from each participant was frozen at the ACHS study office in Anniston. The specimens, including blood clot samples used for LTL measurement, were sent on dry ice to CDC's National Center for Environmental Health (NCEH) laboratory (Atlanta, GA), where they were stored at −70 °C until chemical analysis. All ACHS participants provided informed consent and study protocols were approved by the University of Alabama at Birmingham and University at Buffalo's Institutional Review Boards.

2.2. Exposure assessment

Serum levels of 35 PCBs were measured by the Center for Disease Control (CDC) National Center for Environmental Health laboratory using high-resolution gas chromatography/isotope dilution high-resolution mass spectrometry, as previously reported (Pavuk et al., 2014a; Sjodin et al., 2004). These congeners were selected because of their relevance to human exposure assessment as previously described. The coefficient of variation for duplicate samples ranged between 2.4 and 11.2 for each congener (Pavuk et al., 2014a). We did not analyze congeners with ≥40% of individuals below the limit of detection, which were: PCB 18, 44, 49, 52, 87, 101, 110, 128, 149, and 151.

2.3. Outcome assessment

Blood clot samples were shipped from the Center for Disease Control (CDC) National Center for Environmental Health laboratory to the University at Buffalo on dry ice in 2015, where they were then stored in at −80 °C until DNA extraction. DNA was extracted from stored blood clot samples using the PureGene protocol and clotspin baskets by Qiagen. Purified DNA was then stored at −80 °C prior to telomere assays. Immediately before analysis experimental DNA samples were re-quantified using the NanoDrop spectrophotometer and diluted to 4 ng/μl using PCR-grade water. Of ACHS participants who donated a blood sample, 585 had blood clot samples available to measure relative LTL. DNA was successfully extracted and relative LTL was measured in 560 of these samples. We excluded one participant who reported their race as Native American.

Relative LTL was measured using monochrome multiplex quantitative polymerase chain reaction as described by Cawthon (2009). All assays were conducted using 96-well plates and the BioRad CFX-96 Touch real time PCR. Briefly, 6.6 μl of DNA were added to each well. The other reagents in the 25 μl PCR were 12.5 μl Sybr green master mix, 5 μl betaine, and 0.225 μl of the four primers. The four primers 5′-3′ were telg: ACA CTA AGG TTT GGG TTT GGG TTT GGG TTT GGG TTA GTG T, telc: TGT TAG GTA TCC CTA TCC CTA TCC CTA TCC CTA TCC CTA ACA, hbgu: CGG CGG CGG GCG GCG CGG GCT GGG CGG CTT CAT CCA CGT TCA CCT TG, and hbgd: GCC CGG CCC GCC GCG CCC GTC CCG CCG GAG GAG AAG TCT GCC GTT. The thermocycling profile was as follows: 1 cycle of 15 min at 95 °C; 2 cycles of 2 s at 98 °C and 30 s at 49 °C; 36 cycles of 2 s at 98 °C, 30 s at 59 °C, 15 s at 74 °C with signal acquisition, 30 s at 84 °C, and 15 s at 85 °C with signal acquisition. The signal acquisition at 74 °C provides the cycle thresholds for telomeres and the signal acquisition at 85 °C provides the cycle thresholds for the single copy gene human beta-globin (hbg). Five concentrations of pooled human genomic DNA (Promega) generated via three-fold serial dilution: 150, 50, 16.7, 5.5, and 1.85 ng were included in each plate in duplicate to generate standard curves.

BioRad CFX manager software was used to determine the telomere (T) and single copy gene (S) values for each experimental sample using the standard curve. These values were then used to generate a T/S ratio for each sample which was averaged. Samples with a relative LTL < 1.00 have an average LTL shorter than that of the reference DNA and samples with a relative LTL > 1.00 have an average LTL longer