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Influence of persistent organic pollutants on the complement system in a population-based human sample

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ABSTRACT

Background: Persistent organic pollutants (POPs) are toxic compounds generated through various industrial activities and have adverse effects on human health. Studies performed in cell cultures and animals have revealed that POPs can alter immune-system functioning. The complement system is part of innate immune system that helps to clear pathogens from the body. We performed a large-scale population-based study to find out associations between summary measures of different POPs and different complement system markers.
Methods: In this cross-sectional study, 16 polychlorinated biphenyls (PCBs), 3 organochlorine (OC) pesticides, octachloro-*p*-dibenzodioxin, and 2,2′,4,4′-tetrabromodiphenyl ether (BDE-47) were analyzed for their association with levels of protein complement 3 (C3), 3a (C3a), 4 (C4) and C3a/C3 ratio. A total of 992 individuals (all aged 70 years, 50% females) were recruited from the Prospective Investigation of the Vasculature in Uppsala Seniors cohort. Regression analysis adjusting for a variety of confounders was performed to study the associations of different POP exposures (total toxic equivalency value or TEQ and sum of 16 PCBs) with protein complements.
Results: The TEQ values were found to be positively associated with C3a ($\beta = 0.07$, 95% CI = 0.017–0.131, $p = 0.01$) and C3a/C3 ratio ($\beta = 0.07$, 95% CI = 0.015–0.126, $p = 0.01$) taking possible confounders into account. The association observed was mainly driven by PCB-126.
Conclusion: In this study involving 992 elderly individuals from the general population, we showed that POPs, mainly PCB-126, were associated with levels of complement system markers indicating that the association of these toxic compounds with downstream disease could be mediated by activation of immune system.

1. Introduction

Persistent organic pollutants (POPs) are synthetic compounds that, once released in the environment, can persist for a long time due to their resistance to degradation. They are lipophilic in nature and can bio-accumulate in the fat tissue of different organisms through the food chain. They may reach high concentrations in exposed organisms and can cause detrimental health effects. A wide range of compounds such as polychlorinated biphenyl (PCB) congeners, organochlorine (OC) pesticides (hexachlorobenzene or HCB, trans-nonachlordane or TNC and 2,2-bis (4-chlorophenyl)-1,1-dichloroethene or *p,p′*-DDE), polychlorinated dibenzo-*p*-dioxins, and brominated diphenyl ether (BDE) congeners are among the well-known POPs. During the industrial revolution, a large number of such chemicals were in use for commercial purposes. Due to their widespread use in the past, almost every individual has been exposed to POPs at some point in time (Hansen, 1998). The primary source of exposure is through a contaminated food that involves consuming meat, fish or dairy products (Johnson et al., 2010).

In order to measure relative toxic effect of different POPs, a concept of total toxic equivalency (TEQ) value was invented by Van den berg et al. that considers the toxicity of different POPs in comparison to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin, which thus has a toxic equivalency factor of 1 (Van den Berg et al., 1998). Different studies have confirmed the validity of using this approach in order to measure toxic effects of a mixture of different PCBs together (Bradlaw et al., 1980; Harris et al.,

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1993). Since human health can be influenced by exposure to different POPs, studies have already shown the association between POPs and disease caused by altered immune or inflammatory responses (Barrett, 2012; Gascon et al., 2013; Glynn et al., 2008; Noakes et al., 2006).

The immune system is a biological system that protects any organism from diseases. It can be divided into two main categories — the innate and the adaptive parts. The complement system is a major humoral component of innate immunity that forms the first line of defense and protects the body from infections (Ricklin et al., 2010).

The complement system is activated by binding recognition proteins to carbohydrates or antibodies present on foreign microbes in order to kill these pathogens. Studies involving transgenic as well as knockout mice have shown the importance of the complement system in maintaining health. Approximately 50 different proteins work together in the complement system, which provides a vital triage system that aids in rapid killing of foreign bodies by triggering a cascade of events. However, improper or uncontrolled activation of the complement system may lead to attack the host cells. Several studies have shown the adverse effects of exposure to various POPs on the immune system (Baccarelli et al., 2004; Davis and Safe, 1990; Kim et al., 2003; Lee et al., 2007b; Park et al., 2008; Vos and Moore, 1974). An early study by Vos and Moore showed that when pregnant rodents were administered with dioxins, the offspring were born with altered immune parameters (Vos and Moore, 1974). These effects may lead either to reduced capacity to fight infections or to the risk of developing autoimmune manifestation in later life. Complement 3 (C3) is the most abundant complement protein present in blood and plays a central role in the complement system. Enzyme complexes called C3-convertases cleave and activate multiple C3 molecules into the C3a and C3b fragments that lead to a cascade of further events. C3b is an opsonin that mediates phagocytosis and is also a part of a powerful amplification loop that promotes further activation of C3. C3a is an anaphylatoxin, which can bind to receptors on various inflammatory cells, and it is also the precursor of an adipokine called acylation-stimulating-protein that acts as mediator of inflammation and causes increased permeability of blood vessels. C4 is located upstream in the complement cascade and is predominately activated in response to antigen–antibody complexes. It is another major component of complement system that is highly homologous to C3 and is activated into an opsonin (C4b) and anaphylatoxin (C4a). C4a is less potent than C3a. C4b combines with another component called C2a to form C3-convertase. To the best of our knowledge, no effort has yet been made to understand the impact of various POPs on complement system in humans from the general population.

In order to test the hypothesis that there might be associations between POP exposure and various components of complement system, we performed this large population-based cross-sectional study in Swedish men and women (all aged 70 years) from the Prospective Investigation of the Vasculature in Uppsala Seniors (PIVUS) cohort. We have recently demonstrated a strong correlation between risk factors for cardiovascular disease and plasma levels of C3, C3a and C4 in this cohort (Nilsson et al., 2014). Our primary aim here was to study associations of TEQ and summary measure of polychlorinated biphenyls (sum of PCBs) with complement components (C3, C3a, the C3a/C3 ratio and C4). Our secondary aim was to explore associations of different individual POPs with these complementary system components.

2. Materials and methods

2.1. Study participants

All individuals who were aged 70 years and living in the community of Uppsala, Sweden in 2001–2005 were invited to participate in the study. Approximately 50% (n = 1016) of the invitees agreed to participate in the study and were further investigated. All participants provided their written informed consent and study was approved by the Ethics Committee of Uppsala University, Uppsala. The participants were asked to fill out a detailed questionnaire about, among other things, smoking habits, medications and medical history. Standard laboratory techniques were employed to measure routine clinical variables like fasting blood glucose and different lipids following standard procedures. Only 992 individuals were employed for this study due to the availability of data about different POPs in these individuals. Further information about the recruitment and clinical characteristics has been published previously (Lind et al., 2005) and can be found on the Internet as well (http://www.medsci.uu.se/pivus/).

Large variations of population characteristics due to age as well as various lifestyle factors may influence outcomes. The prime motivation of selecting individuals of same age from general population was to control the confounding by age. Further, we have studied these elderly individuals of same age group where many of these lifestyle factors become attenuated and can be controlled for by extensive lifestyle data.

2.2. Measurement of POPs and quality control

A modified method by Sandau et al. was followed to measure POPs in the study participants (Sandau et al., 2003), and further details can be seen elsewhere (Salihovic et al., 2012). Following a method by Rylander et al., lipid content of plasma was considered to normalize the levels of POPs measured (Rylander et al., 2006). Quality control plasma samples as well as procedural blank samples were incorporated in every batch of 10 samples so as to ensure the quality. The blank samples had no target compounds at >5% of levels present in the samples except for *cis*-chlordane and *trans*-chlordane. In the samples analyzed, both *cis* and *trans*-chlordane were present below the detection limit in >90% of samples. The internal standard recovery was satisfactory, ranging from 60 to 110%. More details about the quality controls have been described in another article by our group (Salihovic et al., 2012). Samples with POP concentration falling below the limit of detection (LOD) were imputed and given value of $LOD/2^{-0.5}$.

2.3. Summary measures of POPs

Two different summary measures — TEQ and sum of PCBs were used as our primary exposure variables. Seven mono- and non-ortho-substituted dioxin-like PCBs with assigned TEF values (PCB-105, 118, 126, 156, 157, 169, 189) and OCDD were utilized to calculate the TEQ values as suggested by Van den Berg et al. (2006). The sum of PCBs was measured by adding concentration of 16 PCBs.

2.4. Measurement of complement system markers

Three different complement components (C3, C3a and C4) were considered in the present study. All components were analyzed in EDTA-plasma, which had been stored at −70 °C as reported. Briefly, C3 and C4 were measured by nephelometry (Immage, Beckman-Coulter Inc., CA, USA), and $C3aC3a_{desArg}$ was quantified by ELISA as described earlier (Nilsson Ekdahl et al., 1992). The ratio between C3a/C3 was also calculated since it indicates the degree to which complement protein C3 is proteolytically activated.

2.5. Statistical analysis

Variables were evaluated for their skewed distribution and if found to have a non-normal distribution, log-transformation was done in order to achieve normal distribution. The association between summary measures of POPs, i.e. TEQ, and sum of PCBs (independent variables) was analyzed for their association with complement system markers (dependent variables) by performing linear regression analysis. A number of potential confounders including age, sex, education (three levels), physical activity (four levels), waist circumference (cm), smoking