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Adipose tissue concentrations of persistent organic pollutants and total cancer risk in an adult cohort from Southern Spain: Preliminary data from year 9 of the follow-up

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ABSTRACT

There is an increasing trend in the incidence of cancer worldwide, and it has been accepted that environmental factors account for an important proportion of the global burden. The present paper reports preliminary findings on the influence of the historical exposure to a group of persistent organic pollutants on total cancer risk, at year 9 in the follow-up of a cohort from Southern Spain.

A cohort of 368 participants (median age 51 years) was recruited in 2003. Their historical exposure was estimated by analyzing residues of persistent organic pollutants in adipose tissue. Estimation of cancer incidence was based on data from a population-based cancer registry. Statistical analyses were performed using multivariable Cox-regression models.

In males, PCB 153 concentrations were positively associated with total cancer risk, with an adjusted hazard ratio (95% confidence interval) of 1.20 (1.01–1.41) for an increment of 100 ng/g lipid.

Our preliminary findings suggest a potential relationship between the historical exposure to persistent organic pollutants and the risk of cancer in men. However, these results should be interpreted with caution and require verification during the future follow-up of this cohort.

1. Introduction

There is an increasing trend worldwide in the incidence of cancer, and predictions for 2030 include annual rates of 27 million incident cases of cancer and 17 million cancer-related deaths annually (Ferlay et al., 2010; International Agency for Research on Cancer, 2008). This trend cannot be solely explained by the improvement in diagnostics or by the aging of populations. In addition, wide disparities in the incidence of the most frequent types of cancer have been reported across the five continents, possibly due to complex interactions between genetic susceptibility and modifiable risk factors (Kamangar et al., 2006).

It has traditionally been accepted that environmental factors account for a large proportion of cancers, reaching up to 80–90% of the global burden of the disease (International Agency for Research on Cancer, 2008). Specifically, an estimated 6% of total cancer deaths is attributable to occupational or environmental exposure to carcinogenic agents, which appears to be a small percentage in comparison to other known causes, such as tobacco consumption, but translates into a large number of individuals in the general population (American Cancer Society, 2014). Furthermore, little is known about the health outcomes derived from exposure to complex mixtures of environmental pollutants that can interact with each other and with internal elements of the organism (American Cancer Society, 2014; Kortenkamp, 2006). In this regard, the European Code against Cancer emphasized the need to apply strict regulations aimed at preventing any exposure to known cancer-causing substances in order to decrease the cancer risk (Boyle et al., 1995).

Organochlorine pesticides (OCPs) and polychlorinated biphenyls (PCBs) are persistent organic pollutants (POPs), i.e., highly lipophilic

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chemicals that are very resistant to degradation and tend to accumulate and biomagnify in food chains, resulting in the considerable exposure of living organisms (UNEP, 2003). OCPs have been used in many agricultural activities and as vector control (Porta et al., 2002), while PCBs have been employed as dielectric and heat exchange fluids, among other commercial applications (WHO, 2000). Although the use of most OCPs and PCBs has been banned or severely restricted in most countries, these chemicals are still detected in virtually all human populations and environmental matrices, and diet (especially fatty food) has been reported to be the main route for human exposure (Bräuner et al., 2012a).

There is increasing scientific evidence that exposure to low levels of POPs, such as those occurring in the general population, is related to an increased risk of several adverse health effects, including some of the most prevalent types of cancer, e.g. hepatocellular carcinoma (Zhao et al., 2011), breast cancer (Salehi et al., 2008), cancer of the head and neck (Govett et al., 2011), non-Hodgkin lymphoma (Engel et al., 2007), prostate cancer (Xu et al., 2010), testicular germ cell tumors (McGlynn et al., 2008), or pancreatic cancer (Hardell et al., 2007). Additionally, long-term occupational exposure to PCBs has been associated with elevated melanoma mortality (Ruder et al., 2014). However, there have been conflicting reports on the relationship between exposure to low levels of POPs, such as those occurring in the general population, and cancer risk, with some studies reporting positive associations but many others finding no evidence to support a causal association (Cassidy et al., 2005; Charlier et al., 2003; Cohn et al., 2010; Gatto et al., 2007; Govett et al., 2011; Hardell et al., 2006; Hoyer et al., 2001; Laden et al., 2001; Lopez-Carrillo et al., 2002; Lopez-Cervantes et al., 2004; Mozzachio et al., 2008; Pavuk et al., 2004; Recio-Vega et al., 2011; Snedeker, 2001; Soto and Sonnenschein, 2010; Svensson et al., 1995; Ward et al., 2000; Wolff et al., 2000; Xu et al., 2010).

The present paper reports preliminary results on the influence of the historical exposure to a group of organochlorine pesticides and polychlorinated biphenyls on total cancer risk, at year 9 during the follow-up of an adult cohort from Southern Spain.

2. Material and methods

2.1. Study cohort

The present research is part of a hospital-based study that aimed to characterize the exposure to POPs of an adult cohort from Southern Spain and assess potential health outcomes. The study design, recruitment, and methods have been extensively described elsewhere (Arrebola et al., 2013a, 2010, 2009). In brief, Granada province covers an area of 12,635 km^2 in Southern Spain. Study subjects were recruited in two public hospitals, San Cecilio University Hospital in the city of Granada (240,000 inhabitants) and Santa Ana Hospital in the town of Motril (50 000 inhabitants).

Study participants were recruited between July 2003 and June 2004 among patients undergoing non-cancer-related surgery (47% inguinal hernia or abdominal surgery, 17% gall bladder surgery, 12% varicose vein surgery, and 24% other surgery). Inclusion criteria were: age over 16 years, absence of cancer, not undergoing hormone therapy, and residence in one of the study areas for ≥10 years. All subjects signed their informed consent to participate in the study, which was approved by the ethics committees of both hospitals. Out of 409 subjects who were contacted, 387 agreed to participate. A total of 19 subjects were excluded because of a previous diagnosis of cancer, leaving a final cohort of 368 participants. All of the participants were users of the public health system. No statistically significant differences in sex distribution or age were found between participants and non-participants (data not shown in tables). The characteristics of the study population are summarized in Table 1.

2.2. Exposure assessment

2.2.1. Sampling and chemical analyses

During surgery, samples of 5–10 g adipose tissue were collected, immediately coded, and stored at −80 °C until chemical analysis. The sample extraction and purification were previously described by Rivas et al. (2001). In brief, 200 mg of adipose tissue was extracted using n-hexane, and the solution was then purified through 2 g alumina in a glass column. All extracts were stored in glass tubes at −80 °C.

POPs were quantified by high-resolution gas chromatography with a mass spectrometry detector in tandem mode, using a system Saturn 2000 ion trap (Varian, Walnut Creek, CA). For the analysis, we used a 2 m × 0.25 mm silica capillary column (Bellefonte, PA) coupled with a 30 m × 0.25 mm analytical column (Factor FOUR VF-5MS, Varian Inc., Walnut Creek, CA). For all measured POPs, the limit of detection was set at 0.01 μg/L. Chromatographic concentrations below the limit of detection were assigned a random value between 0 and the limit of detection. In adipose tissue, residues of p,p′-dichlorodiphenyldichloroethylene (p,p′-DDE, the main metabolite of the pesticide dichlorodiphenyltrichloroethane [DDT]), hexachlorobenzene (HCB), β-hexachlorocyclohexane (β-HCH), and PCB congeners −138, −153 and −180 were quantified. Recoveries of the POPs from adipose tissue were studied to assess the extraction efficiency of the method used, and ranged from 90 to 98%.

Lipid content in adipose tissue samples was quantified gravimetrically as reported by Rivas et al. (2001), including a homogenization step of 100 mg adipose tissue with 5 mL of chloroform:methanol:hydrochloric acid (20:10:0.1) and acidification with hydrochloric acid 0.1 N before collecting and weighing the organic phase.

In adipose tissue samples, lipid-basis concentrations were calculated by dividing the crude adipose tissue concentrations by the total lipid content and were expressed in nanograms per gram lipid (ng/g lipid).

2.2.2. Total effective xenoestrogen burden (TEXB)

In order to calculate the overall estrogenicity of the adipose tissue extracts, samples were tested in the E-Screen bioassay, which measures the proliferative effect of xenoestrogens on MCF-7 breast cancer cells by comparing cell yield between cultures of MCF-7 cells treated with estradiol and those treated with different concentrations of xenobiotics or extracts (Soto et al., 1992). Each adipose tissue extract was resuspended in 5 mL Dulbecco's modified Eagle's medium without phenol red, supplemented with 10% charcoal dextran-treated human serum, and was then tested in the E-Screen bioassay for estrogenicity at dilutions of 1:1, 1:5, and 1:10, using a slight modification of the originally described technique. Each sample was assayed in triplicate with a negative (vehicle) and a positive (estradiol) control in each plate. The proliferative effect of the adipose tissue extract was referred to the maximal effect obtained with estradiol, transformed into estradiol equivalents (Eeq) units by reading from a dose–response curve, and expressed in Eeq units per gram of lipid.

In the present study, we quantified the estrogenicity of the whole adipose tissue extract, whereas previous studies of the TEXB in biological samples have measured the estrogenicity of two fractions of each extract, collected using a preparative normal-phase high performance liquid chromatography protocol: the alpha-fraction, which includes non-polar xenoestrogens (e.g. OCPs and PCBs); and the beta-fraction, which contains more polar xenoestrogens, sex steroids, and pharmaceutical estrogens (Fernandez et al., 2004). For the present study, no high performance liquid chromatography fractionation was performed and the overall estrogenicity of the whole extract was designated as TEXB-extract. We previously reported that the TEXB-extract yields information about the overall estrogenicity to which humans are exposed and may be useful to assess its potential contribution to health outcomes (Arrebola et al., 2012a).