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Environmental exposure to human carcinogens in teenagers and the association with DNA damage

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ABSTRACT

Background: We investigated whether human environmental exposure to chemicals that are labeled as (potential) carcinogens leads to increased (oxidative) damage to DNA in adolescents.

Material and methods: Six hundred 14–15-year-old youngsters were recruited all over Flanders (Belgium) and in two areas with important industrial activities. DNA damage was assessed by alkaline and formamidopyrimidine DNA glycosylase (Fpg) modified comet assays in peripheral blood cells and analysis of urinary 8-hydroxydeoxyguanosine (8-OHdG) levels. Personal exposure to potentially carcinogenic compounds was measured in urine, namely: chromium, cadmium, nickel, 1-hydroxypyrene as a proxy for exposure to other carcinogenic polycyclic aromatic hydrocarbons (PAHs), t,t-muconic acid as a metabolite of benzene, 2,5-dichlorophenol (2,5-DCP), organophosphate pesticide metabolites, and di(2-ethylhexyl) phthalate (DEHP) metabolites. In blood, arsenic, polychlorinated biphenyl (PCB) congeners 118 and 156, hexachlorobenzene (HCB), dichlorodiphenyltrichloroethane (DDT) and perfluorooctanoic acid (PFOA) were analyzed. Levels of methylmercury (MeHg) were measured in hair. Multiple linear regression models were used to establish exposure-response relationships.

Results: Biomarkers of exposure to PAHs and urinary chromium were associated with higher levels of both 8-OHdG in urine and DNA damage detected by the alkaline comet assay. Concentrations of 8-OHdG in urine increased in relation with increasing concentrations of urinary t,t-muconic acid, cadmium, nickel, 2,5-DCP, and

Abbreviations: 8-OHdG, 8-hydroxydeoxyguanosine; CALUX, Chemical-Activated LUciferase gene eXpression; CI, confidence interval; DAP, dialkyl phosphate; 2, 5-DCP, 2,5-dichlorophenol; DDT, dichlorodiphenyltrichloroethane; DEAP, diethylalkyl phosphate; DEHP, di(2-ethylhexyl) phthalate; DEP, diethyl phosphate; DETP, diethyl thiophosphate; DEDTP, diethyl dithiophosphate; DMAP, dimethylalkyl phosphate; DMP, dimethyl phosphate; DMTP, dimethyl thiophosphate; DMDTP, dimethyl dithiophosphate; DRC-ICP-MS, dynamic reaction cell inductively coupled plasma mass spectrometer; FLEHS, Flemish Environment and Health Study; Fpg, formamidopyrimidine DNA glycosylase; GM, geometric mean; HCB, hexachlorobenzene; HPLC, high performance liquid chromatography; HR-ICP-MS, high resolution inductively coupled plasma mass spectrometer; IARC, International Agency for Research on Cancer; IQR, interquartile range; LMPA, Low Melting Point Agarose; LOD, limit of detection; LOQ, limit of quantification; MeHg, methylmercury; MEHP, mono(2-ethylhexyl) phthalate; MEHHP, mono(2-ethyl-5-hydroxyhexyl) phthalate; MEOHP, mono(2-ethyl-5-oxohexyl) phthalate; PCB, polychlorinated biphenyl; PFOA, perfluorooctanoic acid; QA, quality assurance; QC, quality control; ROS, reactive oxygen species; SG, specific gravity; TEF, Toxicity Equivalency Factor; TRA, toxic relevant arsenic; UPLC, ultra-performance liquid chromatography

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DEHP metabolites. Increased concentrations of PFOA in blood were associated with higher levels of DNA damage measured by the alkaline comet assay, whereas DDT was associated in the same direction with the Fpg-modified comet assay. Inverse associations were observed between blood arsenic, hair MeHg, PCB 156 and HCB, and urinary 8-OHdG. The latter exposure biomarkers were also associated with higher fish intake. Urinary nickel and t,t-muconic acid were inversely associated with the alkaline comet assay.

Conclusion: This cross-sectional study found associations between current environmental exposure to (potential) human carcinogens in 14–15-year-old Flemish adolescents and short-term (oxidative) damage to DNA. Prospective follow-up will be required to investigate whether long-term effects may occur due to complex environmental exposures.

1. Introduction

Cancer is a leading cause of death worldwide, accounting for 8.2 million deaths in 2012 (Ferlay et al., 2015). Genomic instability is one of the most pervasive characteristics of almost all human cancers. It is probably the combined effect of DNA damage, tumor-specific DNA repair defects, and a failure to stop or halt the cell cycle before the damaged DNA is passed on to daughter cells (Lord and Ashworth, 2012; Negrini et al., 2010). DNA damage can arise from various sources: environmental factors, normal metabolic processes inside the cell but also errors in DNA replication and recombination may underlie genetic damage, leading to a range of nucleotide modifications and DNA breaks (Lindahl, 1993; Shiloh, 2003; Tomasetti and Vogelstein, 2015).

Recently it was shown that according to the Environmental Performance Index (EPI), Belgium is the second-worst environmental performer in Europe, mainly due to air pollution, a result of high population density and industrial activity (Hsu, 2016). In the most polluted northern part of Belgium (Flanders), an environmental health surveillance program has been established. It is a cross-sectional large-scale human biomonitoring program and includes several population groups differing in environmental profiles and age, one of which are 14–15-year-old adolescents (Schoeters et al., 2012). A wide range of potential human carcinogens to which adolescents are daily exposed were measured in different biological matrices (blood, urine and hair) (Schoeters et al., 2011). We based our selection of potentially carcinogenic chemicals on the outcome of hazard evaluation by the International Agency for Research on Cancer (IARC): (i) known human carcinogens [IARC Group 1] included cadmium, chromium, arsenic, polychlorinated biphenyls (PCB) congeners 118 and 156 (based on the dioxin Toxicity Equivalency Factor (TEF) evaluation by the World Health Organization), benzene and carcinogenic polycyclic aromatic hydrocarbons (PAHs); (ii) probable human carcinogens [Group 2A] dichlorodiphenyltrichloroethane (DDT) and organophosphate pesticides; and (iii) possible human carcinogens [Group 2B] lead, nickel, methylmercury (MeHg), hexachlorobenzene (HCB), 2,5-dichlorophenol (2,5-DCP), di(2-ethylhexyl) phthalate (DEHP), and perfluorooctanoic acid (PFOA). The presence of these chemicals in low concentrations in the everyday environment, resulting in low-level lifetime exposure, and their potential contribution to the risk of cancer is a public concern (IARC, 2012).

A wide range of methods can be used to assess if low-level environmental exposure to (potential) human carcinogens is able to induce loss of DNA integrity or DNA damage. These include well-established biomarkers such as the alkaline comet assay on peripheral blood cells or urinary concentrations of 8-hydroxydeoxyguanosine (8-OHdG), both reflecting short-term (days) DNA damage, and the micronucleus test in peripheral blood lymphocytes as a measure of long-term damage (Andreazza et al., 2008; Fenech and Morley, 1986; Maluf and Erdtmann, 2000). It has been shown that DNA damage increases with age, with more damage in older people while at younger age DNA damage is more rapidly repaired (Gorbunova et al., 2007; Goukassian et al., 2000; Rossi et al., 2007). We assessed in this study whether, at adolescent age, DNA damage in relation to environmental chemicals can already be observed using sensitive assays of short-term DNA damage. The alkaline comet assay, originally developed by Ostling and Johanson (1984) and later adapted by Singh et al. (1988), detects a broad spectrum of DNA lesions including single and double strand breaks as well as single strand breaks associated with incomplete excision repair sites and alkali-labile sites (Collins et al., 2014; Tice et al., 2000). The comet assay can also be modified to allow assessment of oxidative and alkylated base damage using lesion-specific repair endonucleases (Collins et al., 2004; Speit et al., 2004). Formamidopyrimidine DNA glycosylase (Fpg) is recommended for the detection of oxidative DNA base damage, in particular, 8-OHdG (Collins et al., 2008). 8-OHdG, an oxidized nucleoside of DNA, is the most frequently detected and studied DNA lesion. Upon DNA repair or oxidation of the DNA pool present in the cell, 8-OHdG is excreted in urine (Cooke et al., 2008). Its urinary analysis is considered to be an important biomarker of oxidative stress and DNA repair. Both assays have been widely used in occupational and environmental biomonitoring studies with adults (Collins et al., 2014; Pilger and Rudiger, 2006). More recent environmental studies have demonstrated that these assays may also be used to assess background environmental or hot spot exposure to putative carcinogenic chemicals in children and adolescents (Ketelslegers et al., 2008; Koppen et al., 2007; Pelallo-Martinez et al., 2014; Sughis et al., 2012).

We investigated in this study if environmental exposure to (potential) human carcinogens in 14–15-year-old adolescents is associated with (oxidative) damage to DNA. Results were compared with molecular environmental and occupational epidemiological studies of the past 10 years, assessing the association between (potential) carcinogenic pollutants and (oxidative) damage to DNA evaluated by the comet assay or 8-OHdG.

2. Material and methods

2.1. Study population and field work

Adolescents aged 14–15 years were recruited from the general population of Flanders (reference population) (N=210; May 2008–July 2009) and from the industrial hot spot areas Genk-Zuid (stainless steel plant) (N=197; January 2010–November 2010) and Menen (shredder factory) (N=199; May 2010–February 2011). Participants of the reference population were recruited through a stratified, clustered two stage design. The number of participants from each of the five Flemish provinces was identified proportional to the population density at June 1st, 2007. In a first step, one or two schools (primary sampling unit) were randomly selected within each province taking into account that the selected schools should be located at least 20 km apart. In a second step, adolescents were randomly recruited in each selected school. As for the reference population, schools were used as primary sampling units for the recruitment in hot spot areas. To account for seasonal variation, recruitment in each area was spread over a one-year period. The adolescent group was stratified for sex and educational level (general, technical and vocational secondary school). Inclusion criteria were: 1) residing at least 10 years in Flanders, and living at least 5 years in the study area in case of the hot spot areas; 2)