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Reduced gene expression levels after chronic exposure to high concentrations of air pollutants

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ABSTRACT

We analyzed the ability of particulate matter (PM) and chemicals adsorbed onto it to induce diverse gene expression profiles in subjects living in two regions of the Czech Republic differing in levels and sources of the air pollution. A total of 312 samples from polluted Ostrava region and 154 control samples from Prague were collected in winter 2009, summer 2009 and winter 2010. The highest concentrations of air pollutants were detected in winter 2010 when the subjects were exposed to: PM of aerodynamic diameter <2.5 μm (PM2.5) (70 vs. 44.9 μg/m^3); benzo[a]pyrene (9.02 vs. 2.56 ng/m^3) and benzene (10.2 vs. 5.5 μg/m^3) in Ostrava and Prague, respectively. Global gene expression analysis of total RNA extracted from leukocytes was performed using Illumina Expression BeadChips microarrays. The expression of selected genes was verified by quantitative real-time PCR (qRT-PCR). Gene expression profiles differed by locations and seasons. Despite lower concentrations of air pollutants a higher number of differentially expressed genes and affected KEGG (Kyoto Encyclopedia of Genes and Genomes) pathways was found in subjects from Prague. In both locations immune response pathways were affected, in Prague also neurodegenerative diseases-related pathways. Over-representation of the latter pathways was associated with the exposure to PM2.5. The qRT-PCR analysis showed a significant decrease in expression of *APEX*, *ATM*, *FAS*, *GSTM1*, *IL1B* and *RAD21* in subjects from Ostrava, in a comparison of winter 2010 and summer 2009. In Prague, an increase in gene expression was observed for *GADD45A* and *PTGS2*. In conclusion, high concentrations of pollutants in Ostrava were not associated with higher number of differentially expressed genes, affected KEGG pathways and expression levels of selected genes. This observation suggests that chronic exposure to air pollution may result in reduced gene expression response with possible negative health consequences.

1. Introduction

A significant number of inhabitants of both developed and developing countries are exposed to high concentrations of air pollutants. This has serious health consequences including increased incidence of cardiovascular and respiratory diseases, neurodegenerative disorders and cancer [1]. Air pollutants, which are often by-products of incomplete combustion of organic material (from traffic, local heating and industrial production), are adsorbed onto particulate matter (PM). The effect of PM on human health is determined by the size and chemical composition of the PM. Coarse particles of aerodynamic diameter <10 μm are deposited in the thoracic region of the lungs [2]. Fine particles of aerodynamic diameter <2.5 μm (PM2.5) can penetrate the lung alveoli and cause inflammation [3]. Ultrafine particles (<0.1 μm) can even enter cells and

Abbreviations: 8-oxodG, 8-oxo-7,8-dihydro-2′-deoxyguanosine; *APEX1*, apurinic/apyrimidinic endonuclease 1; *ATM*, ataxia-telangiectasia mutated; B[a]P, benzo[a]pyrene; c-PAHs, carcinogenic polycyclic aromatic hydrocarbons; *DMAP1*, DNA methyltransferase 1 associated protein 1; *FAS*, Fas cell surface death receptor; F_G/100, genomic frequency of translocations/100 cells; *GADD45A*, growth arrest and DNA-damage-inducible, alpha; *GSTM1*, glutathione S-transferase mu 1; *IL1B*, interleukin 1 beta; 15-F_{2t}-IsoP, 15-F2t-isoprostane; KEGG, Kyoto Encyclopedia of Genes and Genomes; MN/1000 BNC, micronuclei/1000 binucleated cells; PAHs, polycyclic aromatic hydrocarbons; PCA, principal component analysis; PM, particulate matter; PM2.5, particulate matter of aerodynamic diameter <2.5 μm; *PTGS2*, prostaglandin-endoperoxide synthase 2; qRT-PCR, quantitative real-time PCR; *RAD21*, RAD21 homolog (*S. pombe*).

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cause direct damage to macromolecules [4]. Chemicals adsorbed on the surface of fine PM that impact human health include e.g. polycyclic aromatic hydrocarbons (PAHs), transition metals and volatile organic compounds (VOCs). These chemicals are released from PM in the lungs and enter the organism via the bloodstream. Exposure to PAHs is particularly deleterious to human health. Some PAHs are classified as probably or possibly carcinogenic to humans (c-PAHs) [5] and benzo[a]pyrene (B[a]P), arguably the best known and most studied c-PAH, is a group 1 carcinogen [6]. A study on a model system indicates that in the lungs B[a]P is released from PM in two steps. First, a smaller fraction (about 30%) is rapidly released into the circulation and metabolized. The remaining B[a]P is desorbed slowly and 5.6 months after inhalation about one third of the compound is still present on the particles with reduced bioavailability [7].

Biomarkers of exposure, effect and susceptibility have been used to evaluate the effects of air pollution on human health for many years [discussed in [8]]. Although many studies have successfully demonstrated the suitability of biomarkers for monitoring the health effects, both in environmentally and occupationally exposed populations, some reports have failed to show changes in their levels [reviewed examples in [9–12]]. The lack of an effect of air pollutants on biomarkers may be partly explained by the complex interactions between the organism and the environment, which cannot be captured by the measurement of a single (or several) biomarker(s). Technological developments over the last decade have opened up new avenues in biomarker research and many researchers shifted their focus to studies of mechanisms of action of air pollutants using analyses of gene expression. These studies, conducted mostly on cell or animal models, showed a crucial role of PM in changes of expression of genes participating in inflammatory response, oxidative stress, vascular dysfunction or progression of atherosclerosis in various body parts including the brain and cardiovascular system [13–16]. These results are consistent with the proposed pathophysiological effects of air pollution exposure mentioned above. Currently, analyses of expression of single or several pre-selected genes are commonly replaced by genome-wide approaches based on microarrays and next-generation sequencing that allow obtaining a comprehensive assessment of the gene expression signatures associated with exposures [17]. Global gene expression analysis has become affordable for many laboratories and has recently resulted in the publication of transcriptomics data [18]. Many of the deregulated pathways identified in the studies on model systems included the genes detected in gene-specific experiments; these pathways were associated with e.g. antioxidant response, xenobiotic metabolism, inflammatory signaling, endothelial dysfunction or immune response [19–22].

Although studies that report results of gene expression profiling in human subjects exposed to polluted air have recently been published, they are usually small and deal with exposure to tobacco smoke or inhalation exposure to air pollutants, rather than real exposure to environmental PM [23–27]. Thus, a larger study that involves human subjects from a highly-polluted environment is still required [28].

Recently, we have conducted a biomarker study on subjects living and working in a heavily polluted industrial region of Ostrava, located in the northeastern part of the Czech Republic [29]. Repeat samples were collected during the winter and summer of 2009 and the winter of 2010. The biological samples were analyzed for a panel of traditional biomarkers, which included levels of bulky DNA adducts, oxidative stress markers [8-oxo-7,8-dihydro-2′-deoxyguanosine (8-oxodG), protein carbonyls and 15-F_{2t}-isoprostane (15-F_{2t}-IsoP)] and cytogenetic parameters (frequency of stable and unstable chromosomal aberrations). Unexpectedly, levels of most of these biomarkers did not reflect exposure to PM2.5 and B[a]P, although the concentration of PM2.5 reached 119 $\mu g/m^3$ and B[a]P reached 74.2 ng/m^3. The levels of biomarkers were lower or similar to the levels measured in the control subjects from Prague [29,30]. There were some exceptions: levels of B[a]P-like DNA adducts and protein carbonyls were higher in Ostrava than in Prague, but only in the winter of 2009. 15-F_{2t}-IsoP was the only parameter that corresponded to differences in air pollution in all seasons, although the difference between locations in the winter of 2010 was not significant. The results of biomarkers analyses in subjects enrolled in the present study are presented in Table 1 .

Here we aimed to explain these results by analysis of global gene expression in leukocytes from 466 blood samples. Our goal was to identify differentially expressed genes and signaling pathway differences between the regions. We further selected nine differentially expressed genes and verified their expression using quantitative real-time PCR (qRT-PCR). We hypothesized that analysis of global gene expression changes will help us to explain our previous data describing unexpected and minimal changes of biomarker levels in population exposed to high concentration of air pollutants in comparison to less exposed population. We further expected to observe gene expression changes related to DNA repair processes, cell cycle regulation, metabolism of xenobiotics and inflammatory response of the organism.

2. Materials and methods

2.1. Study subjects and exposure assessment

In this study the number of samples analyzed during individual sampling seasons (winter 2009, summer 2009 and winter 2010) varied from 47 to 58 (control subjects from Prague) and 84-133 (exposed subjects from the Ostrava region). Most of the subjects (85%) were sampled repeatedly either in all (94 subjects, corresponding to 282 individual samples) or in two sampling seasons (57 subjects, corresponding to 114 individual samples); however, 70 subjects (15%), particularly those from the Ostrava region, provided samples only in summer 2009 or winter 2010. For all analyses, the repeatedly sampled subjects were considered individual samples. The samples represented a subset of the population analyzed and described previously [29]. All study participants were healthy, non-smoking men. Smoking status was confirmed by analysis of urinary cotinine levels. Anyone who had undergone medical treatment, vaccination or radiography, up to three months before sampling, was excluded from the study to avoid potential effects on the parameters being analyzed. Prague volunteers were city policemen; subjects recruited in the Ostrava region were policemen who originated from Havirov and Karvina, smaller cities located south-east and north-east of Ostrava city, and office workers working in Ostrava city. In winter 2010 volunteers from Ostrava-Bartovice, a suburb of Ostrava located to the east of the biggest steelworks in the Ostrava region which is one of major sources of air pollution in the region also participated. The Ostrava region is characterized by a high concentration of heavy industry, particularly steelworks, coke ovens and coal mines. The region is infamous for being the most polluted part of the Czech Republic and, arguably, of the entire European Union [further details provided in [29,31].

The study participants provided informed consent and could cancel their participation at any time, in accordance with the Helsinki II declaration. The study was approved by the Ethical Committee of the Institute of Experimental Medicine AS CR, in Prague.

Blood samples were collected by venipuncture, into vacuettes containing ethylenediamine tetra acetic acid (EDTA). Leukocytes were isolated and stabilized using the LeukoLOCK™ Total RNA Stabilization and Isolation System (LeukoLOCK; Ambion, Austin, TX,