The impact of air pollution in the Southern Bohemia Region on fetuses and newborns

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Abstract

Air pollution with increased concentrations of carcinogenic polycyclic aromatic hydrocarbons (c-PAHs, represented by benzo[a]pyrene, B[a]P) affects fetal development, reduces birth weights (LBW) of newborns, and increases intrauterine growth retardation (IUGR). The Southern Bohemia Region is believed to be one of the least air polluted regions in the Czech Republic. Monitoring air pollution in the city of Ceske Budejovice from 2011–2015, PM2.5 (particulate matter <2.5 μm) decreased from 20.3±14.5 μg/m³ to 14.3±8.6 μg/m³, but concentrations of B[a]P did not change between the years 2007–2015: 1.5±0.6 ng/m³ vs. 1.4±0.6 ng/m³. Higher B[a]P concentrations the winter induce genetic damage in newborns, increase frequency of micronuclei (chromosomal aberrations), deregulate genes for immunity in umbilical cord blood, and increase incidence of IUGR and LBW in newborns.

INTRODUCTION

The results of research in Europe and the USA have shown that long-term exposure to air pollution affects not only acute symptoms, but also contributes to the development of disease. The first study on the influence of air pollution on the results of sperm quality, the incidence of bronchitis in children as well as translocations in the genome were performed in the Czech Republic. Program Teplice, from the 1990s, which monitored the effect of air pollution on the health status of the exposed population, was based on air pollution in the Northern Bohemia Region in the 1970s and 1980s (Sram 2001).

MATERIALS AND METHODS

Importance of particulate matter

Particulate matter (PM) is a complex heterogenous group of compounds with specific composition (i.e., size of particle and chemical characterization). The composition of PM varies over time and depends on various sources of emission, chemical
processes in the air, and climate conditions. The WHO (2007) recommended using PM2.5 (particulate matter <2.5 μm) as an indicator of health risk. Available evidence shows an association between PM emitted from combustion sources, both mobile and stationary, affects the health status, and increases morbidity and mortality, relative to cardiovascular, respiratory diseases, and atopic reactions to external air. Carcinogenic polycyclic aromatic hydrocarbons (c-PAHs) compounds bind to the surface of PM2.5. c-PAHs in the air are formed from inadequate combustion or pyrolysis of organic material such as diesel, gasoline, natural gas, coal, and wood. The sources of airborne c-PAHs are industry, local heating, and traffic pollution. PM is a mixture of various chemicals, including c-PAHs, which can have negative impact on human health by inducing oxidative damage to DNA, lipids, and proteins (Sørensen et al. 2003; Knaapen et al. 2006).

**Polycyclic aromatic hydrocarbons**

Recent research has indicated the influence of prenatal exposure to carcinogenic PAH (c-PAHs) on fetal development (Sram et al. 2005) and the incidence of respiratory diseases in children (Herz-Picciotto et al. 2007). c-PAHs are one of the main carcinogens in the air. PAH are activated to form reactive derivatives via metabolic enzymes and can lead to chemical reactions that add extraneous chemical groups to DNA (called DNA adducts) and protein adducts. The presence of DNA adducts caused by c-PAHs can lead to mutations. High levels of DNA adducts are associated with an increased risk of carcinogenic diseases (Gammon et al. 2004).

c-PAHs also induce the formation of reactive compounds. The mutagenicity of these compounds is linked to the formation of reactive oxygen species (ROS) (Penning et al. 1996; Xue and Warshawsky 2005). The level of PAH-induced DNA adducts (the most commonly used marker of c-PAHs exposure) is most often analyzed using the 32P post-labelling method (Binkova et al. 1995).

It has been repeatedly shown that exposure to c-PAHs reduces the birth weight of newborns and increases intrauterine growth retardation (IUGR 10th percentile of birth weight for gestational age and gender). It can be assumed that c-PAHs are one of the most important groups of pollutants for human risk assessment.

Rubes et al. (2010) found increased DNA fragmentation in mature sperm when exposed to concentrations of B[a]P (benzo[a]pyrene) above 7.5 ng/m3 for 10 days, which could potentially cause infertility. DNA fragmentation is also linked to the lack of a specific gene, which is involved in detoxification (metabolism) of c-PAHs. Research results from Rubes et al. have also shown the impact of air pollution on male fertility.

Molecular epidemiology studies have shown that air concentrations of B[a]P greater than 1 ng/m3 in air causes DNA damage through increased frequency of translocations in genome (Sram et al. 2007), micro-nuclei in lymphocytes (Rossnerova et al. 2009), and DNA fragmentation in sperm (Rubes et al. 2010). DNA damage can be repaired, but if not, modified genetic information may lead to diseases that will not manifest for years or decades.

The developing child is increasingly receptive (sensitive) to DNA damage, especially during intrauterine development and later during their school age years.

The risk of c-PAHs exposure was summarized in a WHO document (2011): All the above data shows that exposure to c-PAHs pose a significant risk to human health. Since c-PAHs affect genetic material (via mutations), they can cause long-term damage to the body, which can manifest over a lifetime; additionally, this damage can be transferred to the next generation.

**Impact of c-PAHs on pregnancy outcome**

Dejemek et al. (2000) observed for the first time that c-PAHs in polluted air, at concentrations greater than 2.8 ng B[a]P/m3, during the first month of pregnancy increases the incidence of children with intrauterine growth retardation (IUGR) and low birth weight (LBW, <2500 g) (Baker 2006). The consequence of this functional immaturity in adulthood is an increased incidence of cardiovascular diseases, diabetes, renal dysfunction, and obesity. A higher incidence of IUGR is also seen by children of the next generation (Painter et al. 2008; Boekelheide et al. 2012). Today, the effect of the exposure of pregnant mothers to c-PAHs has been explained by the induction of DNA damage and histone modification (Perera et al. 2009a). PAH-DNA adducts were observed in the venous cord blood and mothers (Perera et al. 2005) as well as in the placentas (Topinka et al. 1997) after exposure to c-PAHs in polluted air. Exposure to c-PAHs during pregnancy (through inhalation of c-PAHs) is linked to its toxic effects on the fetus, which is expressed as IUGR, LBW (Dejemek et al. 2000; Choi et al. 2008b) as well as precocious delivery (Choi et al. 2008a). When these newborns are followed through school age, it has been observed that prenatal exposure to c-PAHs affects neurobehavioral development (Perera et al. 2009b) and also increases the incidence of bronchial asthma (Miller et al. 2004).

Epidemiologic studies observed that fetal growth is programmed from the very early stages of pregnancies; the result is a larger growth deficit as gestation continues (Neufeld et al. 1999; Smoth 2004; Milani et al. 2005). The consequence of these changes in children with IUGR or LBW is a greater risk of delayed mental development (Van Wassenenaer 2005), damage to respiratory functions (Lipsett et al. 2006), asthmatic symptoms in childhood (Nepomnyaschy and Reichman 2006), and cardiovascular disease in adulthood (Baker 2006), including hypertension, atherosclerosis, and diabetes (Martin-Gronert and Ozanne 2007).

A study by Choi et al. (2012) confirmed the data obtained by Dejemek et al. (2000) regarding the impact of increased concentrations of c-PAHs in polluted air...
being unfavorable to fetal development and the long-term effects of such burdens.

New information indicates that pregnancy outcomes as well as observed DNA damage are affected by the child's genotype and genetic polymorphisms (Sram et al. 2006). These results show that when exposed to increased c-PAHs concentrations in the air, some genotypes are more susceptible to DNA damage, which can lead to decreased birth weight (Sram et al. 2006). Therefore, it is reasonable to assume that the nature of the child's genome may affect morbidity via differential environmental burdens.

Miller et al. (2004) reported on the adverse effect of prenatal exposure to c-PAHs (at concentration 3.53±2.81 ng/m3) as manifested in respiratory problems of children aged 12–24 months, mainly asthma symptoms. Jedrychowski et al. (2005) observed the ability of perinatal c-PAHs exposure to influence an increase in respiratory symptoms such as coughing, wheezing, and ear infections in Kraków. The authors suggested that the immunotoxicity of c-PAHs can damage fetal immunity, which can increase the susceptibility of newborns and children to respiratory infections. These publications showed that the risk of respiratory problems caused by c-PAHs start at a very early age and low birth weight, associated with many respiratory problems, can increase the risk of inflammatory respiratory symptoms or respiratory hyper-reactivity.

Air pollution in Southern Bohemia

The Southern Bohemia Region is believed to be one of the least air polluted regions in the Czech Republic. This was why, at the time of the Teplice Research Project (Sram et al. 1996), the district of Prachatice was selected as the control district vs. the highly polluted district of Teplice.

A monitoring station using a VAPS (Versatile Air Pollutant Sampler, URG Corp., Chapel Hill, NC, USA) was installed in the town of Prachatice in 1993 to detect PM2.5 (particulate matter <2.5 μm) and c-PAHs. The concentration of PM2.5 from 1993–2009 was between 10–30 μg/m3. The concentration of PM2.5 from 1993–2009 was between 10–30 μg/m3, and in 2002 concentrations of B[a]P were lower than 1 ng/m3, and in 2002 they were 0.6 ng/m3. However, by 2009 the concentration had increased to 1.4 ng/m3, which was higher than the B[a]P concentration in Prague or Teplice. A probable reason for this change was the increase in natural gas prices that led to a significant number of citizens to return to the use of brown coal for home heating.

The Czech Hydrometeorology Institute started to monitor PM2.5 in the city of Ceske Budejovice in 2004 and B[a]P in 2006. It seems that during the last 5 years the concentration of PM2.5 decreased from 20.3±14.5 μg/m3 in 2011 to 14.3±8.6 μg/m3 in 2015. The concentration of B[a]P remained more the same from 2007 to 2015 (1.5±0.6 ng/m3 vs. 1.4±0.6 ng/m3 (CHMI 2016)).

Impact of air pollution on genetic damage in newborns

We tested the hypothesis that the level of genetic damage, expressed as the level of DNA adducts, the frequency of micronuclei, and changes in the transcriptome varies depending on levels of air pollution. The study was focused on two different locations: Ceske Budejovice (CB) – a regional city in the center of South Bohemia, surrounded by villages and a mostly rural countryside, and Prague (P) – the capital city of the Czech Republic with high, traffic-related, air pollution (Sram et al. 2013). South Bohemia is traditionally thought to be a region with moderately clean air (Sram et al. 1996).

The study populations were recruited from the Ceske Budejovice Hospital, a.s. and the Motol University Hospital, in Prague. One-hundred and seventy-eight mothers and their newborns, from both hospitals, all of Caucasian ethnicity, were included. Mothers were aged 30.9±4.1 years [median (range): 31.2 (18.3–49.0) years]. Newborns had an average birth weight of 3435±463 g [median (range): 3430 (2270–5150) g]. Information on the demographic and medical history of mothers was obtained through interviews. The study was approved by the IRB of the Institute of Experimental Medicine AS CR.

Data from Ceske Budejovice were collected between 15 January and 31 March 2009 (92 deliveries). The group was split into mothers living in Ceske Budejovice (40 deliveries) and mothers living in villages and small towns (52 deliveries). All births in both groups were vaginal, from uncomplicated pregnancies.

The levels of B[a]P and benzene, a representative c-PAHs and VOC and PM2.5, for both locations in 2008 and 2009, were obtained from the Czech Hydrometeorological Institute. The mean concentrations of B[a]P, PM2.5, and benzene for the three months before delivery were calculated to estimate the individual exposure of each mother-newborn pair: the B[a]P concentration in Ceske Budejovice was 3.2±0.2 ng/m3 vs. in Prague 1.9±0.5 ng/m3 (p<0.001), CB PM2.5 24.5±0.7 μg/m3 vs. P 27.0±2.5 μg/m3 (p<0.001), and CB benzene 2.1±0.1 μg/m3 vs. P 2.5±0.5 μg/m3 (Rossnerova et al. 2009).

Biomarkers of exposure were determined in the umbilical cord blood (UCB) as DNA adducts using 32P post-labelling (Binkova et al. 2007) (Table 1) and biomarkers of effect were determined as micronuclei using automated image analysis (Rossnerova et al. 2009) (Table 1). DNA adducts were analyzed as B[a]P-like adducts and total adducts, both categories were significantly higher in Ceske Budejovice vs. Prague (p<0.001) (Sram et al. 2013). Similarly, the frequency of micronuclei in newborns in Ceske Budejovice vs. Prague (p<0.001) was higher. Multivariate logistic regression on the frequency of micronuclei showed a significant impact at the 3-month mean B[a]P before delivery in newborns (OR=3.07 (1.63–5.77), p<0.001). The comparison between data coming from cities and villages around Ceske Budejovice and Ceske Budejov-
ice itself, showed that there were some differences, but they were not significant (Rossnerova et al. 2011).

Can polluted air induce any changes in transcriptome? To answer this question, we used our UCB samples for a pilot study (Sram et al. 2013). Non-smoking mothers without exposure to ETS (environmental tobacco smoke) were put into groups, 52 from Ceske Budejovice and 35 from Prague. Total RNA was isolated from leukocytes and gene expression profiles were determined using HumanRef-8 Expression BeadChips (Illumina, San Diego, CA, USA) containing 24,526 transcript probes. Genes with logFC >0.58 (binary logarithm of fold change) and P<0.01 were considered as differentially expressed between Ceske Budejovice and Prague. Leukocytes from newborns showed different expression in 104 genes (37 up-regulated and 67 down-regulated genes). Among the down-regulated biological processes were biological processes for the immune response (PRF1, NR4A2, GZMB, TNFAIP3, PP2R2B, DDIT4, SERPINA1), apoptosis (RIR2DL3, KIR3DL3, KIR3DL4, KIR2DS5, KLRC3, CLA4), negative regulation of proliferation (CNDKN1A, CTLA4, TGFB1), response to oxygen levels, cell migration, organ regeneration, signal transduction (RG51, SOCS1, THBS), cell differentiation (FLT3, ZBTB16), and the up-regulated gene encoding SERPINA1 (which is considered a biomarker of exposure to genotoxic agents). Among biological pathways, natural killer cell mediated cytotoxicity and autoimmune thyroid disease were down-regulated, and the MAPK signalling pathway was up-regulated.

**DISCUSSION**

These results were surprising, since air pollution in Prague was originally thought to be worse than in Ceske Budejovice. Results of analysis of DNA adducts, micronuclei, and the transcriptome indicate, in the same direction, the significance of exposure to B[a]P in Ceske Budejovice to induce genetic damage in newborns, even when PM2.5 and benzene were higher in Prague. It seems to be a coincidence that the changes observed in Ceske Budejovice were observed when exposure to B[a]P was 3.2 ng/m³, which exceeds the exposure capable of inducing IUGR (2.8 ng/m³) (Dejmek et al. 2000).

This should lead us to re-evaluate risk of air pollution in Southern Bohemia.

According to the WHO (2011), concentrations of 1 ng/m³ B[a]P in ambient air can induce genetic damage, which has been demonstrated with several biomarkers. The European Union standard is 1 ng/m³ B[a]P/year. The concentration of B[a]P in the city of Ceske Budejovice in the year 2015 was, as a yearly average, 1.4±0.6 ng/m³. Specifically, January, February and December had a B[a]P of 2.7–3.4 ng/m³, however, from May to September the value fell to 0.0–0.5 ng/m³. This indicates that B[a]P concentrations in winter reach levels that can induce adverse reproductive outcomes such as IUGR and LBW.

The most likely explanation for this is local heating during the winter, when people burn brown coal and sometimes also waste. Since 2007, this situation seems to be the result of economic policies, which increased the price of natural gas. We can see this effect in the form of higher frequencies of micronuclei in rural villages compared to the city of Ceske Budejovice.

Our results on B[a]P pollution in the winter air of Southern Bohemia showed that B[a]P concentrations during the winter period represent a significant health risk, which could affect pregnancy outcomes and child morbidity.

What are the solutions? The higher concentrations of B[a]P in Ceske Budejovice may partially be caused by outdated central heating systems, which are expected to be continuously modernized, starting this year. Certainly, the most important source of pollution is local heating with brown coal. This situation may improve in the future through actions of the Czech Ministry of the Environment that is financially supporting more effective heating with modern systems. But undoubtedly, the best solution would be the increased use of natural gas for heating.

Adverse pregnancy outcomes can also be affected by lifestyle. The effect of active smoking as well as passive smoking is well known (Dejmek et al. 2002). There is no question and it is well understood by the Czech population that pregnant mothers should not smoke. Although, the habit still exists, it can be affected by education and social standards.
Another marker of a healthy lifestyle is diet. A recent study on the quality of diet among pregnant mothers in the Ceske Budejovice showed that the nutritional quality of typically consumed food was poor; with a low intake of vegetables – 22.8% of the recommended daily amount (RDA), fruits 61.8% of the RDA, and milk 30.2% of the RDA. These data came from samples of real of diets of mothers, 10 in summer 2013 and 10 in winter 2014; with 25% of daily food intake being collected for the 7 to 14 days before the expected delivery date. The quality of the mothers’ diet and intake of vegetables were negatively correlated with DNA adducts of the newborns (Honkova et al. 2015). These results confirmed that sufficient intake of antioxidants can improve PAH detoxification in pregnant mothers (Sram et al. 2013).

Southern Bohemia is often described a beautiful countryside, with many fish ponds, forests and old castles – an ideal place to relax. We are sorry to say that our data indicates that air pollution in this region during the winter period may represent a risk to fetal development, which may affect the health of newborns and later their morbidity. We are in the early stages in understanding the health effects produced by fetal programming and how these affects manifest later in life. Clearly, it would be of great value to follow newborns from the Ceske Budejovice region for an extended period of times in an effort to study not only the chronic effects of air pollution, but to also see if decreases in pollution and changes in lifestyle can simultaneously reverse or mitigate the genetic damage observed in today’s newborns.

CONCLUSION

This overview of air pollution in the Southern Bohemia Region indicates that from 2007–2015 the concentrations of the carcinogenic pollutant benzo[a]pyrene was significantly higher than the EU standard, especially during the winter months.

The higher concentrations of B[a]P induce genetic damage in newborns such as DNA adducts or chromosomal aberrations (micronuclei), and deregulated genes needed for immunity, as well as increase the incidence of IUGR and LBW.

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