Abstract

Phthalates are the esters of phthalic acid used in production of lacquers, phthalic paints, glues and laminates. These chemicals are commonly added to the production of everyday items like foil, plastic bottles and food containers, toys, cosmetics and also medical devices. Phthalates are not permanently bound to plastic polymer structure, therefore they easily leach from the products to the environment under different factors like UV radiation and temperature. Due to their high-production volume, common use, and widespread environmental contamination, phthalates easily reach living organisms through ingestion, inhalation, and dermal exposure daily. The most common member of phthalates group is di(2-ethylhexyl) phthalate (DEHP). This plasticiser has an ability to cross a blood-placenta barrier and affect developing foetuses. Moreover, DEHP can also cross blood-brain barrier, which may cause a disturbance of proper function of nervous cells. Especially developing brains of children can experience a great harm from phthalates exposure.

Epidemiologic studies have shown the correlation between DEHP exposure and neurodevelopmental disorders in children including cognitive impairment, attention deficit hyperactivity disorder (ADHD) and autism. Studies have also shown a relationship between the exposure to DEHP and disorders in neurogenesis process. It was proven that DEHP inhibits the proliferation and differentiation of PC12 cells into neurons. However, until now, no experiments regarding the impact of DEHP on apoptosis and viability of neuronal and glial cells have been published. The effect of DEHP on AhR and PPARγ, receptors that are important in regulation of neurodevelopement and neuroregeneration, is also unknown.

The aim of this thesis was to investigate the impact of DEHP on neuronal and glial cells isolated from mouse cortex *in vitro*. The experiments were conducted on primary monocultures of neurons and astrocytes. The first part of the research focused on the impact of DEHP on viability, metabolism state, reactive oxygen species (ROS) generation and apoptosis process in the cells. The second part of the research focused on involvement of PPARγ receptor, MMP-2 and MMP-9 enzymes in DEHP mode of action. The third part of the research investigated the involvement of AhR, CYP1A1 and CYP1B1 in DEHP metabolism.

The presented studies reviled that DEHP causes apoptosis and is neurotoxic for primary cortical neurons. This compound decreases esterases activity and increases LDH leakage. Moreover, the increase in caspase-3 activity and apoptotic body formation was observed. In spite of the fact that DEHP stimulates activity of the mitochondrial enzymes in neurons, the expression of the marker of proliferation Ki-67 reminds the same. In contrast,

DEHP in the range of concentrations used in the experiments, does not evoke astrocytes cell death but stimulates astrocytes proliferation by increasing Ki-67 expression and activating mitochondrial enzymes. Even though DEHP increases the activity of caspase-3 in astrocytes, it does not generated apoptotic bodies formation. That observation may suggest a different, non-apoptotic role of caspase-3.

The second stage of the studies presented in this thesis investigated the ability of DEHP to activate PPARγ and to affect the expression of two metalloproteinases - MMP-2 and MMP-9 - enzymes engaged in the control of proliferation and reorganisation of nervous cells. Conducted experiments show that in neurons DEHP decreases the mRNA expression of *Pparg* and simultaneously increase the protein expression of this receptor. In the same time the protein expression of MMP-2 and MMP-9 decrease. In astrocytes DEHP decreases the protein expression of PPARγ and MMP-9 but increases MMP-2 expression.

Expression of MMP-2 and MMP-9 is not only regulated by PPARγ. AhR receptor has also an ability to affect metalloproteinases. AhR receptor is responsible for cellular response to toxic substances and is engaged in basic cellular processes like proliferation and apoptosis. Activation of AhR, which is also a transcription factor, cause the expression induction of enzymes from cytochrome P450 family, which activate the xenobiotic metabolism. The most significant are the cytochromes from the first group: CYP1A1 and CYP1B1. The experiments presented in this thesis show that in neurons DEHP causes a decrease of AHR expression correlated with the decrease of the mRNA and protein level of the CYP1A1 and CYP1B1. In the same time the activity of CYP1A1 increases, which suggest that DEHP may affect the activity independently from the AhR signalling pathway. Similarly, in astrocytes, DEHP inhibits the mRNA and protein expression of AHR with the decrease of protein level of CYP1A1 and CYP1B1. Likewise in neurons, the activity of CYP1A1 is higher in DEHP-treated astrocytes.

In this thesis it was demonstrated that the effects of DEHP are cell-depended. DEHP has a pro-apoptotic and cytotoxic impact on neurons. One of the probable mechanism of action of DEHP might be the induction of the oxidative stress. In astrocytes, in spite of the stimulation of ROS production, DEHP does not evoke the cell death but increases the proliferation process instead. Moreover, in both cell types: neurons and astrocytes, the mechanism of action of DEHP involves the transcription factors: PPARγ and AhR. Presented experiments prove that DEHP affects the function of these receptors on expression level. DEHP also affects the expression of metabolic enzymes responsible for detoxication and

xenobiotic metabolism: CYP1A1 and CYP1B1 as well as enzymes engaged in migration and cell differentiation: MMP-2 and MMP-9.

In this thesis, the experiments demonstrated that the exposure to DEHP might be dangerous for the correct brain function and development.

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