DOCTORAL THESIS

Development of mass spectrometry-based omics for studying neurometabolic changes associated with exposure of polybrominated diphenyl ethers and its correlation with Parkinson's disease

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ABSTRACT

Polybrominated diphenyl ethers (PBDEs), as one typical persistent organic pollutants (POPs), are widely spread in the environment and pose potential adverse impacts on human health. As a predominant congener of PBDEs, 2,2',4,4'-tetrabromodiphenyl ether (BDE-47) has been reported to affect habituation capability, synaptic plasticity, and vesicular neurotransmitter release. As an important *in vivo* metabolite derived from BDE-47, 6-hydroxy-BDE-47 (6-OH-BDE-47) was also reported as a neurotoxin. However, the possible linkages between BDE-47/6-OH-BDE-47 exposure and typical neurodegenerative diseases such as Parkinson's disease (PD) are still unclear.

Mass spectrometry (MS) based omics integrated with bioinformatics is emerging as a powerful tool to evaluate metabolic changes occurred after different exposures. Here we developed non-targeted metabolomics, lipidomics, and isobaric tag for relative and absolute quantitation (iTRAQ) proteomics methods based on liquid chromatography-mass spectrometry (LC-MS) and gas chromatography-mass spectrometry (GC-MS) to depict BDE-47/6-OH-BDE-47 induced metabolic changes and to explore the possible contribution of their exposure to PD pathology/pathogenesis.

BDE-47 dissolved in corn oil (0, 1, 10 and 100 mg/kg bwt) was orally administered to adult male C57BL/6 mice for 30 consecutive days. Results of global metabolomics and lipidomics studies of PD-related brain regions based on LC-orbitrap MS revealed significant metabolite changes between the exposed and control groups in purine pathway, glutathione pathway, tryptophan pathway, phenylalanine pathway, alanine, aspartate and glutamate pathway, and lipid composition, mainly involved in oxidative stress and neurotransmitter production. By further quantifying metabolites involved in tryptophan and phenylalanine pathways in mice serum, colon and brain samples by using LC-triple quadrupole MS, dysregulation of PD linked neurotransmitters dopamine and serotonin were confirmed. iTRAQ proteomics study of the striatum, the part of the brain that is most intensively studied in PD pathogenesis, revealed that BDE-47 could induce neurotransmitter system disturbance, mitochondrial dysfunction, oxidative stress and abnormal phosphorylation. Oxygen consumption rate after BDE-47 treatment (0, 1 and 10 μM) in mouse neuroblastoma (N2a) cells was measured for the confirmation. BDE-47 was demonstrated to impair mitochondrial function.

We also investigated whether BDE-47 exposure could worsen PD situation by applying transgenic *Drosophila* (fly) model in which human α-synuclein (α-syn) was overexpressed in wide-type fly to simulate PD. BDE-47 (0, 2, 10 and 50 μM)

was fed to flies continuously for 30 days. Integrated LC-MS and GC-MS profiling indicated metabolic changes in tryptophan, phenylalanine, purine, and alanine, aspartate and glutamate pathways, similar to those from mouse experiment. After quantified metabolites of interest by LC-triple quadrupole MS, we confirmed the slowed-down formation of KYNA (kynurenic acid, a neuro-protector) and speeded-up formation of 3HKYN (3-hydroxykynurenine, a neurotoxin) in all BDE-47 exposed groups on the 20^{th} exposure day. The levels of SAM/SAH (methylation biomarker) and GSH/GSSG (oxidative stress biomarker) were found to decrease on the 30^{th} exposure day. Collectively, we propose that BDE-47 could induce imbalance of kynurenine metabolism, insufficient methylation and oxidative stress, which might contribute to the PD progression.

To further explore the underlying mechanism of 6-OH-BDE-47 induced neurotoxicity, we conducted omics study of metabolic changes induced by 6-OH-BDE-47 on N2a cells. Cells were exposed to 6-OH-BDE-47 (0, 0.5 and 1 μM) for 24 hours. Considerable metabolic changes in pyrimidine and purine metabolism were observed in high exposure condition while oxidative stress was appeared under low exposure condition. Moreover, 6-OH-BDE-47 was found to affect the dopamine production. iTRAQ proteomics was carried out and pinpointed the dysregulation of ribosome, proteasome, RNA metabolism,

aminoacyl-tRNA biosynthesis, vesicular trafficking, purine pathway, and mitochondria electron transport. Immunocytochemistry and Western blot analysis further confirmed that 6-OH-BDE-47 could inhibit autophagy flux, which might result in the aberrant protein aggregation, a pathological hallmark of PD.

We further investigated whether 6-OH-BDE-47 exposure could directly induce PD pathology in Sprague Dawley rat. 6-OH-BDE-47 (0.1, 1 and 10 μg) was stereotaxically injected into the right VTA and SNc regions in the midbrain of rat where there are abundant dopaminergic neurons. The apomorphine-induced rotation test indicated significant deterioration in motor function in the group receiving injection of 10 μg. Striatal dopamine was found to decline in a dose-dependent manner. Notably, 6-OH-BDE-47 also promoted the formation of α-syn aggregate, an important pathological hallmark of PD. Proteomics study revealed that protein degradation processes were crucial rather than oxidative stress in 6-OH-BDE-47 induced neurotoxicity *in vivo*. Mechanistic study based on Western blot further confirmed that 6-OH-BDE-47 could inhibit ubiquitination and autophagy. Collectively, the rat experiment demonstrated that 6-OH-BDE-47 administration could induce motor defect by impairing dopaminergic system and promote α-syn aggregation by inhibiting ubiquitination and autophagy, suggesting that 6-OH-BDE-47 could be a novel risk factor of PD.

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