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香港浸會大學 HONG KONG BAPTIST UNIVERSITY

SKLEBA 環境與生物分析國家重點實驗室 State Key Laboratory of Environmental and Biological Analysis

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Mass spectrometry-based metabolomics reveals methoxychlor induced brain-metabolic changes in C57BL/6 mice

Fuyue Wang, Xiaoxiao Wang, Jiabin Wu, Zongwei Cai*

State Key Laboratory of Environmental and Biological Analysis, Department of Chemistry, Hong Kong Baptist University, Hong Kong SAR, China

Introduction

Methoxychlor (MXC), which is an organochlorine pesticide classified as a "Proposed Persistent Organic Pollutant" in the Stockholm Convention. There is ever increasing evidence that organochlorine pesticide exposure can cause damage to the dopamine system and is associated with increased risk of brain disease. However, the specific relationship between MXC and metabolites in the brain is still elusive. Here, we performed mass spectrometry-based metabolomics studies in C57BL/6 mice induced by MXC exposure to further explore its potential pathology mechanism in the brain.

Workflow

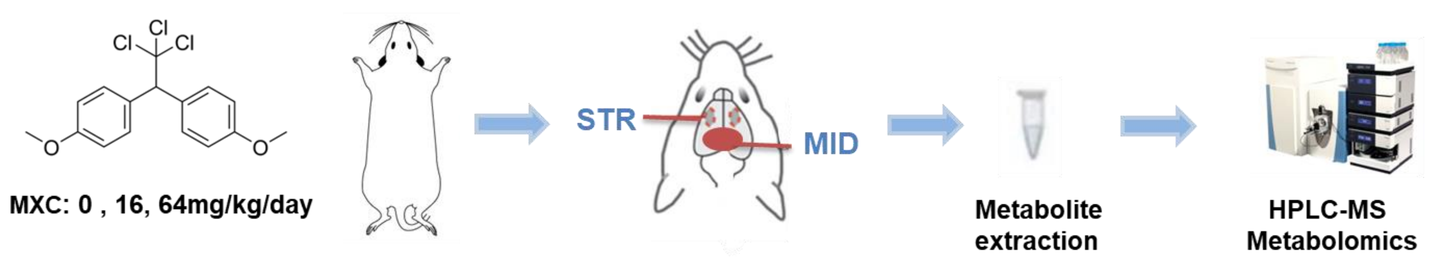

Figure1. Metabolomics analysis workflow of methoxychlor exposed C57BL/6 mice.

Animals and experimental design

Female C57BL/6 mice aged 4-6 weeks were randomly divided into three groups: control, low and high, with at least 6 mice in each group. MXC was administered to the above mice by gavage according to the dosage except the control group. These mice were sacrificed after 12 weeks of treatment, with midbrain and striatum being collected for subsequent metabolomics research. A Q-Exactive mass spectrometer (Thermo Scientific, headquarters), coupled with An Ultimate 3000 UHPLC system was employed for non-targeted metabolomics analysis.

Changes in body weight and dopamine levels

During the exposure period, the changes in body weight of the mice in each group were recorded every four weeks. Before the eighth week of exposure, there was a slow upward trend in the mice in each group, and after that it became stable. No significant changes were found in the body weight of mice in the treated groups (L and H) compared to the control group (Figure 2A).

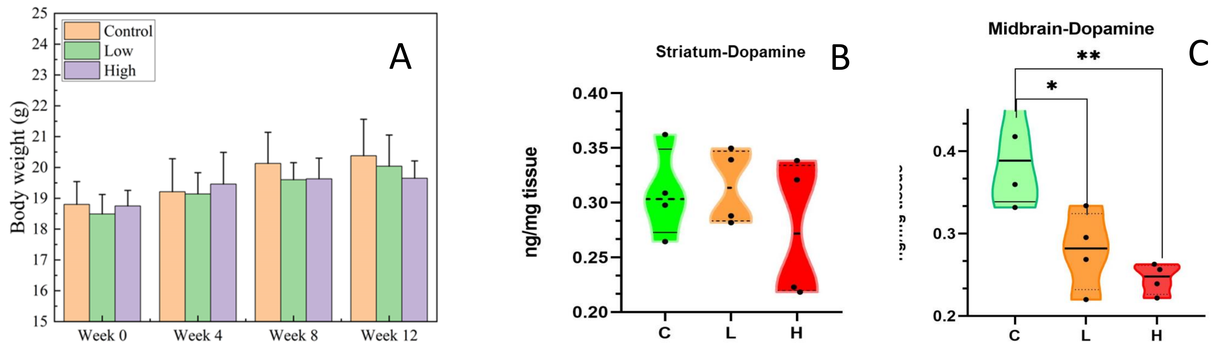

Figure 2. The weight changes of the mice were monitored every four weeks (A), MXC-induced dopamine changes in striatum (B) and midbrain (C).

After the mice were sacrificed, changes in dopamine levels in PD-related regions including the midbrain and striatum of mice after MXC exposure were investigated. As shown in Figure 2B, the two treatment groups (L and H) did not significantly decrease dopamine concentrations in the striatum compared with the control group, but a dose-dependent decrease was observed in the midbrain. This suggests that MXC exposure may interfere dopamine synthesis and transport-related pathways in the midbrain.

Metabolic profiles

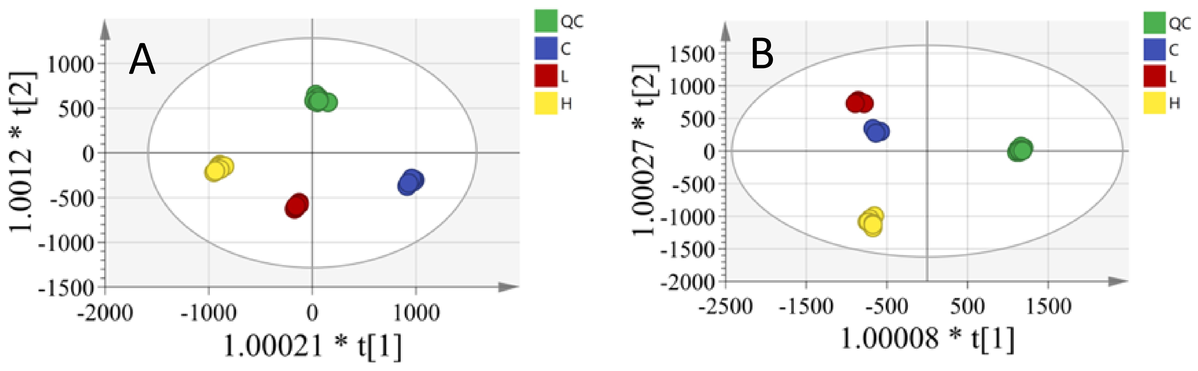

Figure.3 OPLS-DA score plots of midbrain from MXC exposed mice in negative (A) and positive (B) ion modes respectively, showing separation of H, L groups from C group.

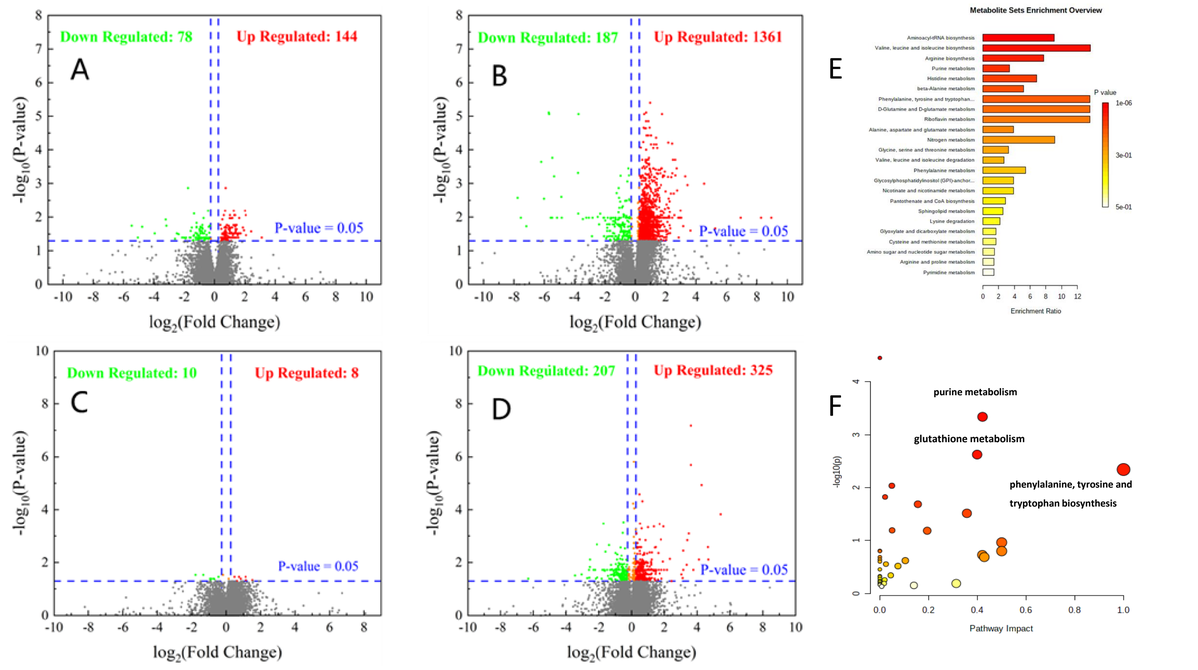

Figure.4 Volcano plot analysis of differential metabolites in mice midbrain negative ion modes L group compared to C group (A), H group compared to C group (B) and correspondingly positive ion modes (C), (D). Metabolites enrichment analysis (E) and metabolic pathway analysis (F) in the midbrain of MXC-exposure C57BL/6 mice.

Conclusion

- Metabolites changes in the midbrain were investigated in our study. We can see that metabolites changes were very significant in the midbrain after MXC exposure from OPLS-DA (Figure 3).
- Volcano plots shows significant up-regulation and down-regulation of relevant features in H and L groups compared to C groups (Figure. 4A-D). As expected, far fewer perturbed metabolites were found in L groups than in H groups.
- Through metabolites enrichment analysis (Figure. 4E) and metabolic pathway analysis (Figure. 4F), purine metabolism, glutathione metabolism, phenylalanine, tyrosine, and tryptophan biosynthesis pathway were highlighted, further revealing possible links between MXC-induced brain-metabolite changes and related brain disease pathologies.

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Contact information

WANG Fuyue (20482477@life.hkbu.edu.hk)