DOCTORAL THESIS

Multi-omics Study of Triclosan-induced Dermal and Behavioral Effects

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ABSTRACT

Triclosan (TCS), an antiseptic, has been ubiquitously incorporated in consumer goods and personal care products, which raises people's concerns for its continuous detection in the environment as well as human biofluids and tissues. It has been well-reported that TCS-mediated toxicity includes disruption of hormone homeostasis, carcinogenicity, apoptosis, and inflammation. Recently, the occurrence of TCS in human brain attracts people's attention on its neurotoxicity. There are few studies on neurotoxicity related to TCS, therefore, more studies addressing this chemical-mediated effect on the central nervous system are urgently needed to fill in the data gaps. Dermal contact with TCS is one of the main human exposure ways. However, serious skin lesions were observed after repeated topical treatment of TCS in animals and TCS is considered as a moderate irritant. Hence, it is of importance to investigate the mechanisms of dermal toxicity related to TCS. Moreover, TCS and its chlorinated derivatives (CTDs) can be photochemically transformed in the surface water, yielding a range of polychlorodibenzo-p-dioxins (PCDDs) which are known as dioxins with toxicity and carcinogenicity. The generation of dioxins from TCS and CTDs is of obvious concern. Food chain and consumption are the main sources of dioxin exposure in human. Daily dioxin exposure is hard to explore, and the relevant toxicity is difficult to confirm. Mass spectrometry (MS)-based omics analysis is a credible tool to gain a landscape of biological changes after environmental exposure. In our study, we integrated liquid chromatography-mass spectrometry (LC-MS)

based metabolomics, lipidomics, isobaric tag for relative and absolute quantitation (iTRAQ) proteomics to explore the TCS-induced dermal and neuro effects with the hope to depict the underlying mechanisms. We also applied multi-omics approaches to investigate the molecular changes in human serum and to delineate the biomarkers and potential health risks associated with daily dioxin exposure.

TCS in acetone with concentrations of 0, 0.3%, and 1.5% *(w/v)* were topically administered on the shaved back of female ICR mice five times weekly for 20 weeks. We conducted behavior tests spanning sucrose preference test, elevated plus maze test and step through passive avoidance test to assess the TCS effects on neurofunctions in mice. Our results noted for the increased anxiety-like responses and impaired learning and memory ability. Quantification of TCS and its biotransformation products in serum, tissues and different brain regions were further conducted. It was noted that the levels of TCS are high in liver, thyroid, hypothalamus, hippocampus, and corpus striatum. Thinking of the functions of hippocampus and thyroid hormones (THs) in brain, we hypothesized potential reasons for the behavioral abnormality in the TCS-treated mice: one is that TCS affected the thyroid functions and disrupted circulating TH homeostasis leading to brain dysfunction mediated by the reduction of brain THs; the other is that TCS performed toxic effects on hippocampus directly.

To verify the hypothesis, we combined metabolomics and iTRAQ proteomics to investigate the TCS effects on mouse hippocampus. Our metabolomics results revealed

that TCS exposure increased energy demands in hippocampus, leading to disturbances in energy homeostasis and purine metabolism. We noted for the reconfiguration of energy metabolism from pentose phosphate pathway to glycolysis caused by the increased energy demands. The shift may impair the hippocampal ability in reducing reactive oxygen species (ROS), aggravating the oxidative stress which is extensively reported to induce anxiety and cognition impairment in hippocampus and associate with brain diseases. Additionally, we found that TCS is likely to disrupt long-term potentiation, reduce level of acetylcholine and disturb GABAergic neurotransmission, which are proved to be related to the impaired learning and memory ability and increased anxiety. Subsequently, global metabolic profiling and iTRAQ proteomics were conducted to investigate the TCS interferences on thyroid in mice topically exposed to TCS for 20 weeks. The levels of THs and thyroid stimulating hormone (TSH) in serum were measured to evaluate the TCS effects on TH homeostasis. We also quantified the levels of THs in different brain regions including cerebral cortex, hypothalamus, corpus striatum, hippocampus, middle brain, and cerebellum. The decreased levels of serum THs and the increased TSH levels were noted in both TCS treated groups, indicating the occurrence of hypothyroidism, which is usually a result of reduction of TH synthesis and release due to thyroid gland impairment. Based on the results of metabolomics and proteomics, our research revealed that TCS exposure disrupted the synthesis and the release of THs by affecting the uptake of iodine, the synthesis of thyroglobulin, the production and

reduction of H_2O_2 and the levels of lysosomal enzymes. As the dominating TH crossing the adult blood-brain barrier, thyroxine (3,5,3',5'-tetraiodothyronine; T_4) did not show significant changes in brain. While the active form triiodothyronine (3,5,3'-triiodothyronine; T_3) converted from T_4 via deiodinases, decreased significantly in hypothalamus, hippocampus, corpus striatum and middle brain, where also noted for the accumulation of TCS. Based on these results, we conjectured that TCS is likely to affect brain functions and induce behavioral disorders via affecting the deiodinases in brain instead of affecting the circulating TH homeostasis, which needs further confirmation.

We also conducted *in vitro* experiments integrating MS-based metabolomics and lipidomics analysis to explore how TCS causes dermal toxicity by interfering with metabolic pathways. The obtained data suggested that purine metabolism and glutathione metabolism were upregulated, amino acid metabolism was downregulated, and lipid metabolism were disturbed in keratinocytes. Our biological measurements pointed out that these disturbances led to the overproduction of ROS and accumulation of ammonia, resulting in increased oxidative stress in human keratinocytes. The levels of proinflammatory cytokines (IL-1β, IL-6, IL-8, IL-10, TFN-α) excreted from HaCaT cells were all significantly higher after exposed to TCS, indicating the increased oxidative stress triggered inflammation and apoptosis in human keratinocytes.

Finally, we integrated nontargeted metabolomics, nontargeted lipidomics and targeted analysis of acyl-CoAs and free fatty acids (FFAs) to study the global molecule changes

in human serum towards daily exposure of dioxins. Our results revealed the links of dioxin exposure with triglyceride accumulation, ceramide overproduction, glycerophospholipid remodeling, and FFA metabolism, providing the evidence for adverse health outcomes or health risks associated with dioxin exposure, such as cardiovascular diseases non-alcoholic fatty liver disease, inflammation, apoptosis and so on.