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In ovo exposure to triclosan alters the hepatic proteome in chicken embryos

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ABSTRACT

The occurrence of triclosan (TCS) in the eggs of wild avian species is an emerging concern. We previously evaluated the effects of *in ovo* exposure to TCS on the liver transcriptome of chicken embryos and proposed adverse outcome pathways (AOPs). However, the key molecular events identified to be affected need to be verified at the protein level. Herein, we investigated the changes in the spectrum of hepatic proteins in TCS-treated chicken embryos by proteomic analysis to validate the key signaling pathways involved in the AOPs. We identified and quantified 894 unique proteins using matrix-assisted laser desorption/ionization time-of-flight/time-of-flight tandem mass spectrometry. In the 0.1 (low dose), 1 (median dose), and 10 μg triclosan/g egg (high dose) groups, TCS caused significant changes in the levels of 195, 233, and 233 proteins in males and 237, 188, and 156 proteins in females, respectively (fold changes > 1.3 or < 0.7). TCS exposure modulated the expression of proteins, predominantly involved in signaling pathways of lipid and energy metabolism in both genders. Among the proteins associated with TCS metabolism in the liver, phase I (e.g., CYP2C23a) and phase II (e.g., UGT1A1) enzymes mediated by chicken xenobiotic receptor, were only induced in males. In consonance with the malondialdehyde levels, which were increased upon TCS exposure in females in a dose-dependent manner, a battery of antioxidant enzymes, notably SOD2, GST, GSTz1, and PRDX1, was decreased and SOD1 and GSTK1 were increased in the embryos. Taken together, this proteome analysis complements the transcriptome profiling reported in our previous study and authenticates the AOPs proposed for chicken embryos *in ovo* exposed to TCS.

1. Introduction

Triclosan (TCS) is an antimicrobial agent used in a diverse range of personal care products. Owing to its widespread use and environmental fate (Dhillon et al., 2015), TCS is accumulated in terrestrial organisms, e.g. it was recently detected in the eggs of European starlings (*Sturnus vulgaris*) and American kestrels (*Falco sparveriu*) collected from Ada County of US with concentrations reaching up to 37.9 ng/g wet weight (Sherburne et al., 2016). The TCS exposure in birds might be more serious in the certain areas of Asia, considering the concentrations of TCS detected in the aquatic and terrestrial environments. For example, in the worst-case scenario, the maximum exposure to TCS in fish-eating birds was estimated to be 715 ng/g (Guo and Iwata, 2017). Given the persistence and bioaccumulation of TCS, concerns have been raised over the potential deleterious effects of this substance on birds.

We previously assessed the effects of *in ovo* exposure to TCS on a model avian species, chicken (*Gallus gallus*). Exposure to TCS increased the embryo mortality and attenuated tarsus length in both genders in a dose-dependent manner. At an environmentally relevant dose of 0.1 μg/g egg, TCS exclusively enhanced liver somatic index (LSI) in females (Guo et al., 2018). The liver is a vital organ with multiple roles in physiological processes, including detoxification, lipid and energy metabolism, and bile acid production (Chen and Xu, 2014). In mice, chronic exposure to TCS could increase hepatocyte proliferation and induce liver fibrosis, which may be due to the activation of constitutive androstane receptor (CAR; Yueh et al., 2014). Since CAR is suggested to be evolutionarily conserved in avian species, TCS might cause similar adverse outcomes in birds via the activation of its ortholog, chicken xenobiotic receptor (CXR; Handschin et al., 2000). By analyzing the transcriptome in the liver of TCS-treated chicken embryos, we found that the mRNA expression of phase I xenobiotic metabolizing enzymes, including three cytochrome P450 isozymes (*CYP2C23a*, *CYP2C45*, and *CYP3A37*), mediated by CXR, was significantly increased. Since these three CYP genes are orthologs of the mammalian *CYP2B* and *CYP3A* that are transcriptionally regulated by CAR, up-regulation of these genes suggested the involvement of CXR signaling in the effects induced by TCS (Guo et al., 2018). Surprisingly, transcripts encoding phase II enzymes such as sulfotransferase (SULT) and UDP-glucuronosyltransferase (UGT) that dominate the TCS metabolism in rodents have not been identified as differentially expressed genes (DEGs; Wu et al.,

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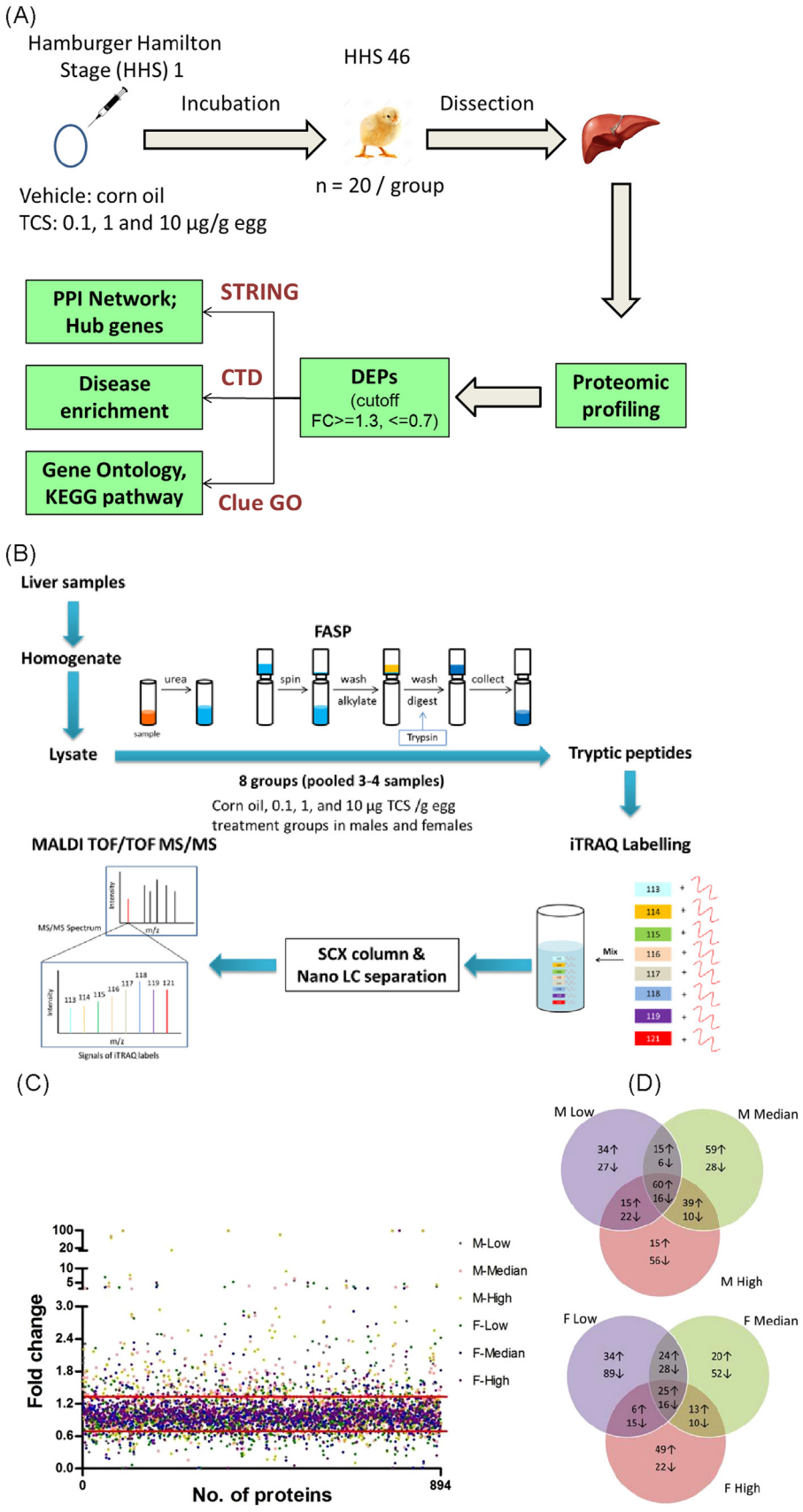

Fig. 1. Schematic of the strategy used for evaluating the effects of *in ovo* injection of triclosan (TCS) on chicken embryos. (A) Workflow of the exposure experiment, chemical analyses, proteomic profiling, and bioinformatics analyses; (B) Protocol for sample preparation and proteomic analysis of the liver; (C) Fold changes in the protein expression levels in the TCS-treated groups with respect to the control groups in male and female chicken embryos; proteins with fold change < 0.7 or > 1.3 were identified as DEPs. (D) Venn diagrams of the DEPs in male and female chicken embryos. DEPs: differentially expressed proteins; PPI: protein–protein interaction network. STRING: functional protein association networks; CTD: comparative toxicogenomics database; ClueGo: creates and visualizes a functionally grouped network of terms/pathways; FASP: filter-aided sample preparation; SCX: strong cation exchange.