４ 研究内容

Effects of 2,3,7,8-tetrachlorodibenzo-p-dioxin on the thymus of chicken embryos

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Aim and procedure

Exposure to 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) is harmful to the immune system of mammalian model animals, affecting antibody production, cytotoxic T lymphocyte activity, and lymphocyte proliferation. TCDD exposure can cause thymus atrophy in mice, which is mediated by activated aryl hydrocarbon receptor (AHR) signaling. Studies in mice have also shown that TCDD can induce the altered differentiation of T cells, leading to the unbalanced development of regulatory T cells (Tregs) and T helper 17 cells (Th17), depending on the dose and the presence of other AHR ligands. For example, TCDD exposure induced differentiation of naïve T cells to regulatory T cells (Tregs), which suppressed experimental autoimmune encephalomyelitis in mice. However, there is still a lack of understanding of the effects of TCDD on the immune system of avian species. Therefore, investigating the relationship between AHR activation and the immune system in birds is crucial for risk assessment and evaluating the potential of AHR ligands as a modulator of the immune system.

To explore the mechanisms of TCDD-induced immunotoxicity in chickens, we treated chicken embryos with different concentrations of TCDD and measured the mRNA expression levels of thymic AHR and AHR nuclear translocator (ARNT) isoforms. We also investigated the effects of TCDD exposure on the differentiation of T cells in the chicken thymus and proposed mechanisms for TCDD-induced immunotoxicity in chicken embryos based on transcriptome and bioinformatics analyses.

Results

The thymus weight decreased in a dose-dependent manner, when exposed to 3.3 μM or higher concentrations of TCDD on day 0. The cytochrome P450 (CYP) 1A4 levels were increased up to 10-fold by ≥ 2.5 μM TCDD exposure on day 0. In contrast, there were much less changes in the CYP1A5 mRNA expression levels in any of the TCDD exposure groups. In the thymus of non-treated chicken embryos, *ck*AHR1 had the

highest expression levels, followed by *ck*AHR2, and *ck*AHR1β, whereas *ck*ARNT2 expression levels were higher than *ck*ARNT1. We also compared mRNA expression levels of *ck*AHR and *ck*ARNT isoforms in the thymus between the vehicle control and TCDD exposure groups. Embryos exposed to 2.5 μM TCDD on day 0, exhibited significantly lower expression levels of *ck*AHR1, *ck*ARNT1, and *ck*ARNT2.

The total mRNAs extracted from the thymus of chicken embryos exposed to TCDD on day 0 were analyzed and 6,520 genes were identified with next generation sequencing. Transcriptome data demonstrated that there were 196 statistically significant differentially expressed genes (DEGs). Among them, 97 DEGs were up-regulated and 99 DEGs were down-regulated in TCDD exposure groups compared to the vehicle control group. Pathway enrichment analysis and functional PPI network analysis using DEGs in the thymus of chicken embryos exposed to TCDD on day 0 showed that the cancer pathway, the PPAR signaling pathway, the neuroactive ligand-receptor interaction, the transcriptional misregulation in cancer, the extracellular matrix organization, and the jak-stat signaling pathway were affected by TCDD exposure. Furthermore, several pathways associated with immunotoxicity were also affected by TCDD exposure; Erb-B2 Receptor Tyrosine Kinase (ERBB)-, WNT-, and bone morphogenetic protein (BMP)-signaling pathways, cytokine-cytokine interaction network.

Perspectives in future

The results of the present study suggest that *ck*AHR1 and *ck*ARNT2 isoforms might contribute to the AHR-mediated effects on the thymus in chicken embryos treated with TCDD. The transcriptome analysis suggests that TCDD-induced thymus atrophy is triggered by the alteration of ERBB-, WNT-, and BMP-signaling pathways. Bioinformatics analysis of the DEGs also implied that immune suppression and thymus fibrosis may be due to the disruption of the cytokine-cytokine and extracellular matrix organization pathways. The present findings may render evidence that TCDD functions as a modulator of the immune response in chicken embryos. However, further efforts are necessary to elucidate the physiological function and role of AHR in the immune system of chicken embryos.