DOCTORAL THESIS

Environmental screening of endocrine-disrupting chemicals and biological characterization of their effects on reproductive health

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Environmental Screening of Endocrine-Disrupting Chemicals and Biological Characterization of their Effects on Reproductive Health

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Abstract

Exposure to environmental pollutants is one of the culprits to reproductive problems worldwide. This relationship is based on studies that reported the association of body loading of pollutants with reproductive dysfunction. We hypothesize that the dietary route is the major source of animal intake of endocrine-disrupting pollutants. Fish consumption is one of the major sources of pollutant intake in people living in coastal environment. The endocrine disrupting pollutants can interfere with cell signaling system and epigenetic reprogramming, which is critical during the process of embryonic development. In the first part of the study (Chapter 2-4), the concentrations of dioxins and bisphenol A (BPA) in the extracts of fish samples were revealed using instrumental analysis, and three biomarker assays (i.e. 7-ethoxyresorufin-O-deethylase (EROD), Dioxin-responsive-element (DRE) and estrogen-responsive (ER) luciferase reporter). Concentrations of polychlorinated dibenzo-p-dioxins (PCDDs), polychlorinated dibenzofurans (PCDFs), and dioxin-like polychlorinated biphenyls (PCBs) were measured in 20 common fish species purchased at local markets. Concentrations of total dioxins in fish ranged from 0.481 to 9.05 pg TEQ/g ww. Bisphenol A (BPA) was detected in 19 species of fish at concentrations, ranging from 1.7 to $2.2x10^{1}$ ng/g lipid. Average daily BPA intake per person ranged from $1.1x10^{2}$ ng/day for marine fish and $1.8x10^{2}$ ng/day for freshwater fish. The mean values of the detected dioxin- and estrogen-like activities in the freshwater fishes were 25.3 pg TEQ/g ww and 102.3 pM EEQ/g ww whereas in the seawater fishes, the values were 46.2 pg TEQ/g ww and 118.8 pM EEQ/g ww. The data highlighted the risk of dietary intake of dioxin-like and estrogenic-like pollutants.

On the basis of these findings, potential reproductive health risks of Endocrine Disruptors (EDs) from fish consumption in our region were estimated using in vivo animal experiments. In Chapter 5, a murine model was used to study potential effects of BPA

exposure during perinatal and postnatal periods on endocrine functions of hypothalamic-pituitary-gonadal (HPG)-axis. At the hypothalamic-pituitary level, BPA exposure resulted in the up-regulation of the expression levels of KiSS-1, GnRH and FSH mRNA in both male and female pups. At the gonadal levels, BPA caused inhibition in the expressions of testicular steroidogenic enzymes and the synthesis of testosterone in the male pups. Conversely exposure to BPA resulted in a greater aromatase expression level and the synthesis of estrogen in the female pups. The effects of BPA on reproductive dysfunction may be due to its actions on gonadal steroidogenesis and so the anomalous releases of endogenous steroid hormones. This non-ER-mediated effect is more potent in affecting the feedback regulatory circuits in the HPG-axis. In Chapter 6, we investigated the effects of maternal transfer of mixture of BPA and Di(2-ethylhexyl) phthalate (DEHP), during gestational and weaning periods on gonadal development and fertility of the male offspring. The overall negative effects on the development and function on the male reproductive system of the exposed pups were prominent. The exposure significantly lowered the male-to-female sex ratio and the sizes of gonads of the male pups. The testes of the perinatal exposed male pups were atrophied and the expression levels of testicular anti-mullerian hormone (AMH), androgen receptor, cyclin A and StAR were significantly lesser than the control pups. The sub-function of the atrophied testes was correlated with significant reductions in the mRNA expression levels of GnRH and FSH at the hypothalamus-pituitary levels. The negative effects of the perinatal BPA+DEHP exposure were found to be persistent in the sexually mature pups. Significant lower testicular weights, epididymal sperm counts, serum testosterone and FSH levels were observed in the mature pups. The study illustrates the negative impact of perinatal BPA+DEHP exposure on the reproductive development and function of the male mice. Collectively the present study has highlighted the risk of fish consumption in this region and has demonstrated the negative effects of BPA and BPA+DEHP exposure on reproductive dysfunction, particularly in male.

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