DELAYED SPERMATOGENESIS AND TRPM-2 UP-REGULATION IN THE TESTIS OF F1 OFFSPRING PERINATALLY EXPOSED TO 2,3,7,8-TETRACHRODIBENZO-p-DIOXIN

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Introduction

In recent years, a number of synthetic chemicals have been shown to be able to be mimic endogenous hormones[1,2]. It has been hypothesized that the hormone-like acting chemicals alter the normal pattern of development as well as the function of reproductive organs in wildlife and humans. Many studies have been shown that the chemicals cause reproductive dysfunction at environmentally relevant concentrations[3]. A variety of adverse effects have been reported in animal studies following exposure to TCDD. Among them, the reduction of sperm number and impairment of spermatogenesis were major effects induced by TCDD exposure in rat, but close histological and mechanistic studies on these impairments were so limited. TRPM-2 (Testosterone repressed prostate message-2), also known as clusterin or sulfated glycoprotein-2, was firstly isolated from ram rete testes fluid[4]. And, it is expressed in various organs and associated with a wide variety of physiological and pathological process, including tissue remodeling, lipid transport, complement regulation, apoptotic cell death, and reproduction[5]. In the male reproductive system, TRPM-2 is synthesized and secreted by Sertoli cells and epididymal epithelium and have a function associated with sperm. Disturbance of TRPM-2 homeostasis in male reproductive system may induce impairment of spermatogenesis. And, it has been known that TRPM-2 up-regulation in various organs including testis and epididymis was induced in testosterone deprived state[6]. The present study showed the relationship between impaired spermatogenesis and anti-androgenic effects induced by TCDD exposure with the inspection of TRPM-2 expression in testis.

Materials and Methods

Thirty female specific pathogen free (SPF) Sprague-Dawley (SD) rats were purchased from Biogenomics (Seoul, Korea). TCDD (GL Science Inc., Japan) was dissolved in DMSO (Sigma, USA) and diluted with corn oil (Sigma, USA). Pregnant dams were dosed orally with 1 or 2 μg TCDD /kg /day on gestation day 15 which is known as the onset of sexual differentiation and the gestational day used in other studies (Mably et al., 1992 a, b, c; Gray et al., 1995). The control group received the vehicle only. Postmortem examination and the numbers of spermatogonia, preleptotene spermatocytes, leptotene spermatocytes, zygotene spermatocytes, pachytene

spermatocytes, and elongated spermatids were counted at stages II-III, V, VII, and XII tubule were conducted on all male offspring at postnatal day 21, 49, 70, and 90. Daily sperm production in left testes of rats was examined at PND 90. Sperm number in caudal epididymis of 90-day male pups was examined. Total RNA was extracted from 50 mg of frozen samples of testis and prostate for examining the expressions of TRPM-2 and CYP1A1 genes by RT-PCR.

Results and Discussion

To study toxic effect and mechanism of TCDD on the male reproductive system, Sprague-Dawley rats after maternal exposure of single dose of 1 or 2 μg/kg-TCDD on Gestation Day 15 were examined. In adult rat, sperm number in the testis was appeared to decrease in TCDD-treatment groups without statistically significance. Interestingly, sperm number in caudal epididymis was significantly decreased in 2 μg/kg TCDD-exposed group. In accordance with the sperm number in epididymis, the number of round and elongated spermatid in seminiferous tubule (stageVII) were significantly decreased by TCDD treatment. And, some stageVIII-seminiferous tubuli showed delay of sperm release. Testicular expression of TRPM-2 in testis was dose-dependently increased during adult stage. In conclusion, Our findings on these studies demonstrated that in maternal single TCDD exposure resulted in permanent effects on their F1 male offspring. And, late stages of spermatogenesis and sperm maturation in male offspring were disturbed by maternal TCDD exposure and antiandrogenic effect of TCDD may be involved in reduction of sperm number and impairment of spermatogenesis induced by *in utero* and lactational exposure to TCDD.

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