Perinatal Exposure to Indole-3-carbinol Alters Male Reproductive Development Similar to TCDD in Sprague-Dawley Rats

Clynn E. Wilker[1], Stephen H. Safe[2] and Larry Johnson[1,2]
Departments of [1]Veterinary Anatomy & Public Health and [2]Veterinary Physiology & Pharmacology, Texas A&M University, College Station, TX 77843-4466 USA

1. Introduction

The effects of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) on the adult male reproductive system in the rat requires relatively high doses (e.g. > 15 μg/kg) and is usually associated with decreases in feed intake and body weight [1)]. In contrast, fetal or neonatal exposure to low doses of TCDD can cause dramatic changes in the male reproductive system in the rat. For example, a single maternal dose of TCDD as low as 0.064 μg/kg administered on gestation day 15 to Holtzman rats results in a reduction in spermatogenesis, epididymal sperm reserves, and androgen-dependent organ weights in the male offspring [1-4)]. At a higher dose (1.0 μg/kg), the effects on the male reproductive system include: decreased anogenital distance (AGD), delayed testicular descent, decreased testicular parenchymal weight, demasculinization of sexual behavior, and feminization of luteinizing hormone (LH) regulation pattern [2-4)]. Similar effects on the male reproductive system have been reported in Long Evans Hooded rats at a TCDD dose of 1.0 μg/kg administered on day 15 of gestation [5)].

Indole-3-carbinol (I3C) is present in cruciferous vegetables and several studies have reported that I3C exhibits a broad spectrum of aryl hydrocarbon (Ah) receptor agonist activities including the induction of CYP1A-dependent enzyme activity in humans [6-8)]. I3C exhibits relatively low binding affinity for the Ah receptor; however, I3C undergoes acid-catalyzed self-condensation reactions to give polycyclic heteroaromatic compounds which are more potent Ah receptor agonists. Thus, the activity of I3C as an Ah receptor agonist or antagonist is dependent, in part, on the extent of conversion into more active compounds in animal species and their subsequent uptake into target organs. The effects of I3C and other structural classes of Ah receptor agonists on male reproductive development have not previously been investigated and this study reports the comparative effects of *in utero* exposure of female Sprague-Dawley rats to TCDD (1 μg/kg) and I3C (1 mg/kg) on reproductive development of male offspring using protocols which have previously been described [1-5)].

2. Materials and Methods

Chemicals. I3C was purchased from Sigma Chemical Co. (St. Louis, MO) and TCDD (> 98% pure) was synthesized in this laboratory.

Animal Studies. Timed-pregnant Sprague Dawley rats (5 per group) on day 15 of gestation were dosed by gavage with either corn oil, 1 μg/kg of TCDD in corn oil, or 1 mg/kg of I3C in corn oil. Following birth, the litter size was established at 10 pups. Anogenital distance was measured at 1, 4 and 5 days of age. Body weight was measured at 1 day of age and thereafter every 5 days. The male rats were terminated at 62 days of age. At termination, the weights of testes, epididymis, seminal vesicles, and prostate were obtained. Serum was collected from trunk blood.

3. Results

Perinatal exposure to TCDD reduced body weight and anogenital distance whereas I3C caused a reduction in body weight on days 5 and 15 (Table 1). TCDD exposure reduced body weight more severely during the early neonatal period than did I3C and, at 1 day of age, the pups from the I3C-exposed mothers were heavier than the control pups. However, the pups exposed to I3C grew at a slower rate from birth through day 15 of age compared to both the control and TCDD-treated pups (23.51 vs 27.74 or 26.63 g). The reduction in anogenital distance induced by TCDD was not significantly different from I3C-treated pups but was significantly lower than observed in control pups.

Table 1. Developmental effects of maternal exposure during the perinatal period to 1 μg/kg TCDD or 1 mg/kg I3C on day 15 of gestation on male offspring.

Parameter	Control	TCDD	I3C
Body wt (g) - day 1	7.04 ± 0.13	5.79 ± 0.12*	7.61 ± 0.13
AGD (mm) - day 1	4.5 ± 0.1	4.0 ± 0.1*	4.3 ± 0.1
Body wt (g) - day 5	12.84 ± 0.24	10.19 ± 0.31*	11.72 ± 0.15*
AGD (mm) - day 5	6.5 ± 1.0	5.9 ± 0.1*	6.2 ± 0.1
Body wt (g) - day 15	34.78 ± 0.51	32.42 ± 0.45*	31.12 ± 0.57*

Means ± SEM; * significantly lower ($p < 0.01$) than observed in control animals.

The retardation in growth induced by perinatal exposure to I3C continued until termination (Table 2). I3C and TCDD significantly decreased body weights and weights of the prostate and seminal vesicles. TCDD, but not I3C, significantly decreased weights of the epididymis and testis in the perinatally exposed 62-day old male rats.

Table 2. Effects of exposure to 1 μg/kg TCDD or 1 mg/kg I3C on day 15 of gestation on body weight and reproductive organ weights in male Sprague-Dawley rats.

Parameter	Control	TCDD	I3C
Body wt (g) - day 62	358.42 ± 5.6	309.87 ± 4.47*	328.73 ± 6.48*
Testis wt (g)	1.51 ± 0.02	1.44+0.03*	1.50 ± 0.02
Epid wt (g)	0.37 ± 0.04	0.31 ± 0.01*	0.35 ± 0.01
Sem Ves wt (g)	0.50 ± 0.01	0.37 ± 0.02*	0.44 ± 0.02*
Prostate wt (g)	0.82 ± 0.02	0.57 ± 10.02*	0.66 ± 0.02*

Means ± SEM; * significantly lower ($p < 0.01$) than observed in control animals.

4. Discussion

Previous studies have demonstrated that after maternal exposure of pregnant Holtzman and Long Evans rats to TCDD, there were significant developmental deficits in the male offspring [1-5]. The results summarized in Tables 1 and 2 indicate that TCDD-induced developmental deficits in male Sprague-Dawley rats exposed *in utero* to 1 μg/kg TCDD included decreased body (day 1, 5 and 15) decreased anogenital distance (days 1 and 5). Many of the other parameters associated with impairment of male reproductive development were also decreased in the 62 day old male rats (Table 2) and these data are consistent with results of previous studies with TCDD [1-5] in Holtzman and Long Evans rats.

The effects of I3C (1 mg/kg) on male reproductive development were surprising since ongoing studies in B6C3F1 mice and mammalian cells suggested that I3C was a weak Ah receptor agonist and, in animals/cells cotreated with TCDD plus I3C, there was a significant decrease in TCDD-induced immunotoxicity and CYP1A1-dependent activity. In contrast, perinatal exposure to 1 mg/kg I3C resulted in the significant reduction of at least 2 parameters associated with male reproductive development, namely, decreased seminal vesicle and prostate weights. These results show that another Ah receptor agonist also causes male reproductive problems in rats.

Human populations in industrialized countries are exposed to low levels of PCDDs/PCDFs in food and the estimated dioxin or toxic equivalents (TEQs) of these compounds in the diet range from 80 to 120 pg/day. Levels of I3C in the diet are approximately 735,000,000 pg/day [8] and dietary levels of other "endodioxins" such as polynuclear aromatic hydrocarbons (PAHs) and aromatic amines in cooked foods are also relatively high (12-50 x 10^6 and 11 x 10^6 pg/day, respectively). The relative contribution of HAHs (exodioxins) and endodioxins to the total TEQs in the diet are dependent on the relative potency or toxic equivalency factors (TEFs) which are assigned to the various classes of compounds. Since the TEF for I3C in the rat reproductive model would be in the 0.001 to 0.0001 range, then the overall dietary TEQ for I3C derived from this single response would be higher than the corresponding value for TCDD and related compounds. The relevancy of this specific Ah receptor-mediated toxic response in rats for human risk assessment is unknown. Nevertheless, the results of this study suggest that health assessment of background exposure to TCDD and related HAHs on human populations should take into account the overall dietary exposures to both exodioxins and endodioxins.

5. References

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