MALE REPRODUCTIVE TOXICITY OF DIOXIN

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1. Introduction

In the past few years there has been growing concern that environmental exposure to complex mixtures of chemicals, both synthetic and naturally occurring, that disrupt endocrine systems may be adversely affecting male reproductive system development and function in animals and humans. One class of environmental endocrine disrupter that has the potential to cause such effects is the aryl hydrocarbon receptor (AhR) agonists, of which 2,3,7,8-tetrachlordibenzo-p-dioxin (TCDD, dioxin) is the prototype. TCDD binds the AhR which dimerizes with the AhR nuclear translocator (ARNT). This complex, a ligand activated transcription factor, binds to enhancer elements in the 5' regulatory region of genes and activates transcription. Although none of the TCDD-responsive genes identified to date has been directly linked to male reproductive toxicity, it is hypothesized that induction or repression of the transcription of genes, not yet identified, may play a key role in causing this toxicity.

2. Male Reproductive Toxicity Following Adult Exposure

In male rats, TCDD exposure during adulthood results in decreased testis, seminal vesicle and ventral prostate weights, altered testicular and epididymal morphology, increased incidence of epididymal sperm granulomas and decreased fertility[1]. These adverse reproductive effects are contributed to by TCDD-induced decreases in plasma androgen concentrations which are due, in part, to reduced Leydig cell number, volume and steroidogenic enzyme activity as well as impaired Leydig cell responsiveness to luteinizing hormone stimulation. The ED_{50} for the androgenic deficiency associated with adult TCDD exposure is approximately 15,000 ng/kg[1], a dose which is three orders of magnitude above the mean background body burden of TCDD equivalents (TEQs) in humans of 8-13 ng/kg in 1995[2]. Epidemiologic evidence demonstrates that the regulation of plasma testosterone, luteinizing hormone, and follicle stimulating hormone concentrations is disrupted in adult human males occupationally exposed to TCDD[3].

3. Male Reproductive Toxicity Following *In Utero* and Lactational Exposure

Although the male reproductive system can be adversely affected by exposure to TCDD *at any age, animals and humans* are most vulnerable during early development[1]. During

pregnancy, and to a greater extent during lactation, a portion of the maternal body burden of TCDD and related compounds is transferred to the fetus and neonate. Fetal and neonatal development is a time of intense growth and differentiation of male reproductive organs. Therefore, it is possible that exposure to TCDD during critical periods of early development could alter gene expression in male reproductive organs, either directly or indirectly, resulting in changes in cell proliferation, cytodifferentiation, and tissue morphogenesis which could alter function of male reproductive organs into adulthood.

Indeed, the male rat reproductive system has been shown to be extremely sensitive to *in utero* and lactational TCDD exposure. Male Holtzman rats born to dams given graded TCDD doses ranging from 64 to 1,000 ng/kg on gestation day 15 were studied from birth to sexual maturity. *In utero* and lactational TCDD exposure affected external indicators of androgenic status, decreased androgen-dependent accessory sex organ weights, decreased responsiveness of the adult ventral prostate to androgenic stimulation, decreased daily sperm production, cauda epididymal sperm numbers and ejaculated sperm numbers, partially demasculinized and partially feminized sexual behavior and partially feminized the regulation of luteinizing hormone secretion[1,4-10].

Several developmental male reproductive effects originally noted in Holtzman rats have also been observed in Long Evans rats, Sprague Dawley rats and golden Syrian hamsters following *in utero* and lactational exposure to TCDD[11,12]. In addition, similar effects including reduced reproductive capability have been demonstrated in Wistar rats exposed to certain PCB congeners that are AhR agonists[13,14]. So the spectrum of male reproductive effects caused by *in utero* and lactational TCDD exposure is not restricted to a single rodent species, rat strain, or AhR agonist.

The ED_{50} for most of the effects observed in Holtzman rats was 160 ng/kg maternal body weight of TCDD, about 1/100th of the ED_{50} for the androgenic deficiency following adult exposure[4-6]. The LOAEL for the most sensitive effects (i.e., reduced ventral prostate weight, reduced daily sperm production, reduced cauda epididymal sperm numbers, and demasculinized sexual behavior) was 64 ng/kg[4-6]. Among these endpoints the decrease in cauda epididymal sperm numbers has been a consistent finding in our laboratory[6,7,10] and other laboratories[11,12]. The ED_{10} doses estimated for this effect on postnatal day 63 and 120 in Holtzman rats were 4.3 and 6.8 ng TCDD/kg maternal body weight, respectively[6,15]. These doses are less than the mean background body burden of 8-13 ng TEQ/kg estimated for humans in western industrialized countries in 1995[2]. Furthermore, nearly all effects of TCDD seen in response to *in utero* and lactational exposure in Holtzman rats occur in response to *in utero* exposure alone[10]. This finding is significant considering that only about 0.01% of the maternal TCDD dose is transferred to the fetal liver *in utero*, whereas 2-3% is transferred to the neonatal liver via lactation.[16]

There is no doubt that the male rodent reproductive system is very sensitive to developmental TCDD exposure. However, there is a paucity of epidemiological data linking developmental AhR agonist exposure to male reproductive effects in humans. Epidemiological studies of the boys born to women who had ingested PCB and PCDF contaminated rice oil during pregnancy (the Yu-Cheng cohort) have shown that sexual maturation was not delayed and that testicular and scrotal development was not altered[17]. However, the exposed boys exhibited significantly shorter penises than age-matched controls[17]. Because the rice oil was contaminated

with PCDFs and PCBs, only some of which are AhR agonists, and polychlorinated quaterphenyls, which are not AhR agonists, the observed effects in the prenatally exposed boys may have been contributed to by non-AhR agonists. Irrespective of this difficulty in interpretation, Yu-Cheng offspring are the best characterized cohort of humans prenatally exposed to AhR agonists and as such provide an invaluable resource for future studies which could include a wider breath of developmental male reproductive endpoints.

4. Potential Mechanisms of Developmental Male Reproductive Toxicity

The mechanism underlying the profile of male developmental reproductive responses caused by AhR agonists is poorly understood. Although TCDD is antiestrogenic in several model systems[1)] and some effects of *in utero* and lactational TCDD exposure in male rodent offspring are similar to those of developmental antiestrogen exposure (decreased testis, epididymis and accessory sex organ weights), other effects of antiestrogen exposure (spermatogenic arrest) do not match those of TCDD exposure. This makes it difficult to ascribe the entire spectrum of male developmental reproductive effects of TCDD to an antiestrogenic mechanism[18)].

On the other hand, the profile of developmental male reproductive effects caused by TCDD seems consistent with decreased testicular androgen production and/or circulating androgen concentrations. However, neither of these parameters is significantly affected, perinatally or at later times, by *in utero* and lactational TCDD exposure[8,11,18)]. The possibility remains, however, that the antiandrogen-like syndrome which follows perinatal TCDD exposure could result from interference with the androgen signalling pathway at some point distal to testicular androgen synthesis[18)]. However, as with antiestrogen exposure, the spectrum of effects following developmental TCDD exposure is similar to but not identical to the spectrum of effects following developmental exposure to antiandrogens or inhibitors of 5α-reductase (the enzyme which converts testosterone to the more potent androgen, dihydrotestosterone)[18)].

4. Conclusion

Because the mechanisms involved in mediating developmental male reproductive toxicity of TCDD are not understood[18)], and because there is a great disparity in the number of developmental male reproductive endpoints of TCDD exposure reported in laboratory animal studies versus those detected in human epidemiological studies, extrapolation of laboratory results to human health risk is difficult. By determining whether nonpersistent AhR agonists cause signs of TCDD-like developmental male reproductive toxicity[12)] and by assessing responses to complex mixtures of AhR agonists at doses that bracket the background human body burden of TEQs, a more accurate evaluation of human male health risks caused by exposure to environmentally relevant body burdens of AhR agonists could be made. Also by demonstrating a role for the AhR in TCDD-induced developmental male reproductive toxicity, defining the molecular and cellular events that underlie the most sensitive TCDD-induced developmental male reproductive endpoints, and collecting human epidemiological data on male reproductive endpoints similar to those evaluated in laboratory rodent studies, the risk that *in utero* and lactational exposure to TCDD and related compounds poses to human male reproductive system development and function could, in the future, be assessed with less uncertainty.

5. Acknowledgement

Supported by NIH grant ESO1332.

6. References

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