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Dioxin induction of transgenerational inheritance of disease in zebrafish

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ABSTRACT

Dioxin (2,3,7,8-tetrachlorodibenzo-*p*-dioxin; TCDD) is an aryl hydrocarbon receptor (AHR) agonist, an endocrine disruptor, and a potent global pollutant. TCDD exposure is associated with diseases of almost every organ system, and its toxicity is highly conserved across vertebrates. While the acute developmental effects of dioxin exposure have been extensively studied, the ability of early sublethal exposure to produce toxicity in adulthood or subsequent generations is poorly understood. This type of question is difficult to study because of the time frame of the effects. With human subjects, such a study could span more than a lifetime. We have chosen zebrafish (*Danio rerio*) as a model because they are vertebrates with short generation times and consistent genetic backgrounds. Zebrafish have very modest housing needs, facilitating single and multigenerational studies with minimal time and expense. We have used this model to identify transgenerational effects of TCDD on skeletal development, sex ratio, and male-mediated decreases in reproductive capacity. Here we compare these findings with transgenerational effects described in laboratory rodent species. We propose that the zebrafish is a cost-effective model system for evaluating the transgenerational effects of toxic chemicals and their role in the fetal basis of adult disease.

1. Introduction

Mounting evidence suggests that environmental factors can alter developmental programming, resulting in the adult onset of latent diseases, including but not restricted to cancer, diabetes, cardiovascular disease and reproductive disorders (Gluckman and Hanson, 2004; Heindel, 2005; Lau and Rogers, 2004; Marczylo et al., 2012; Veenendaal et al., 2013). The etiology of some diseases is now linked to tissue- and developmental stage-specific epigenetic alterations in gene expression, resulting from nutritional deficits or exposure to contaminants *in utero*. Exposure to endocrine disruptors is of concern due to the roles that hormones play in regulating transient and irreversible developmental processes. Evidence is mounting that developmental exposure to chemicals, including endocrine disruptors, results in adult disease (Corrales et al., 2014b; Heindel, 2008).

TCDD is a toxic environmental contaminant that impacts growth and development in vertebrates and is associated with several diseases. It is the prototypical member of a family of dioxin-like compounds (DLCs), and is generally produced as a by-product of industrial processes and waste incineration. TCDD is stable in the environment, highly lipophilic and bioaccumulative, and human exposure comes mostly through dietary sources. TCDD acts primarily through activation of the AHR/ARNT transcriptional regulator to alter gene expression, but cross talk with other signal transduction systems is suspected (Poland and Bradfield, 1992; Puga et al., 2009; Schmidt and Bradfield, 1996; Swanson and Bradfield, 1993). AHR activation by TCDD leads to altered expression of hormone receptors, receptor activators and repressors, metabolic enzymes needed for metabolism of xenobiotics and hormone synthesis and degradation, and other gene products required for normal development and endocrine function (Abbott et al., 1994; Beischlag et al., 2008; Gierthy et al., 1996; Massaad et al., 2002; Safe et al., 1998).

Diseases in humans that have been associated with exposure to TCDD include cancer as well as chloracne, porphyria, and defects in the cardiovascular, skeletal, immune, central nervous system, hepatic and reproductive systems (Eskenazi et al., 2000; Guo et al., 2000; NAS-IOM, 2011; Pelclova et al., 2006; Warner et al., 2007, 2011). Recent epidemiologic evaluation following a major industrial release of TCDD revealed that exposure to TCDD *in utero* leads to reduced sperm quality, feminized sex ratio, and altered thyroid

Abbreviations: TCDD or dioxin, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin; DLC, dioxin-like compound; AHR, aryl hydrocarbon receptor; ARNT, aryl hydrocarbon receptor nuclear translocator.

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function in the offspring (Baccarelli et al., 2008; Mocarelli et al., 2000, 2011).

Laboratory studies confirm the potential for TCDD to cause disease later in life. Direct exposure to TCDD leads to infertility in many vertebrate species, including humans, and is associated with down-regulation of enzymes in the estrogen synthesis pathway, decreased egg release, increased number of atretic ovarian follicles, and decreased fertilization success (Baker et al., 2013; DeVito and Birnbaum, 1994; King-Heiden et al., 2006, 2012; Yoshizawa et al., 2009). Toxicity in adults following TCDD exposure during early development suggests that physiologic systems are being mis-programmed and that exposure to TCDD can potentially initiate irreversible and permanent modifications in gene expression and cell lineages. However, the molecular mechanisms that underlie latent and transgenerational disease caused by developmental exposure to TCDD are not well understood.

Our recent work has focused on studying the latent and transgenerational effects of TCDD exposure during critical periods of development, using zebrafish (*Danio rerio*) as a model system. In this review, we compare our findings with effects observed in rodent studies to highlight the usefulness of this model system for evaluating the potential for chemicals to cause disease in adults and subsequent generations.

2. Zebrafish as a model for multigenerational studies

To study transgenerational effects, we need a vertebrate model that has a short time to sexual maturity so that we can study successive generations. From this perspective, humans are not ideal subjects for study (Heindel, 2007; Skogen and Overland, 2012). In addition, the diverse genetics of the human population, confounded by individual variations in exposures make studies with human subjects difficult. The zebrafish is well established as a model for investigating human disease, especially as it pertains to altered development. Attributes that make the zebrafish outstanding in this arena are: short time to sexual maturity (about 3–4 months), transparent embryos that allow observation of organ development without disturbing the embryo, the ability to obtain large groups of synchronously developing embryos, low cost for exposure chemicals since volumes are small, and the ease of housing multiple generations of fish. This last point means that one can expose the first F_0 generation and maintain offspring across many generations inexpensively and compactly.

While small rodent models are more common than small fish models for studying human disease, rodents have a number of disadvantages for studying the fetal basis of adult disease. Rats and mice for example have far fewer offspring per pair, and maintenance costs are considerably greater. While zebrafish reach sexual maturity in a similar timeframe to some rodents, their small size allows for the ability to house and maintain large groups of synchronously developing fish over multiple generations inexpensively and compactly. Similar to human populations, laboratory zebrafish are less isogenic than laboratory rodent strains, which decreases inbreeding effects when studying changes in the zebrafish genome/epigenome. Zebrafish developmental processes are well characterized, and many organs and cell types have been marked with fluorescent reporters in transgenic lines. Due to complete sequencing of the zebrafish genome, technologies that include specific antibodies, genetic/epigenetic markers, and high throughput sequencing also can be readily utilized. MicroRNAs may be involved in the transgenerational inheritance of disease (Grandjean et al., 2009; Wagner et al., 2008) and there is a rapidly growing microRNA literature in zebrafish. Finally, developing zebrafish are very small and transparent, so development can be readily followed with microscopy and automated screening techniques (Kaufman et al., 2009; Westhoff et al., 2013; Wittmann et al., 2012).

Even though zebrafish are oviparous, the reproductive systems of fish and mammals are similar. The testis and ovary in zebrafish contain the same germ cells that are found in mammals, and hormonal regulation of spermatogenesis and oogenesis is highly conserved across vertebrates, occurring via the hypothalamic–pituitary–gonadal axis (Liu et al., 2011; Lohr and Hammerschmidt, 2011; Segner, 2009).

3. Defining transgenerational toxicity: zebrafish vs. rodents

Chemical exposures that affect subsequent generations are now well documented. An epigenetic mechanism is likely for cases of multigenerational disease, in which neither the parent nor the offspring has been directly exposed. This is a transgenerational effect because there is no direct connection to chemical exposure (Skinner, 2008). In rodent models, exposure during early development requires prenatal exposure in an F_0 generation mother during pregnancy (Fig. 1, left column). This leads to F_1 offspring that developed in an exposed environment. The F_2 offspring then develop in parents that were exposed *in utero*, so only effects in the F_3 generation can be due to epigenetic alterations in gametes.

In contrast, zebrafish eggs are fertilized in water, embryos develop externally, and are subsequently exposed at the juvenile stage of development (Fig. 1, right column). Thus, F_0 fish are equivalent to F_1 mice because they develop in an exposed environment. The F_1 zebrafish generation originates from gametes produced by exposed fish, similar to the F_2 mouse generation. The gametes producing the F_2 zebrafish generation have not been exposed so the effects seen in F_2 zebrafish are transgenerational. Thus, the F_2 zebrafish is equivalent to the exposure-free F_3 mouse.

4. Using zebrafish to identify transgenerational effects of TCDD

Sublethal TCDD exposure *in utero* and in early development leads to adverse health effects in adulthood and subsequent generations. Adverse effects have included increased congenital abnormalities, decreased survival, differences in sex ratios of offspring, and decreased reproductive function and fertility in both males and females (Ding et al., 2011; Ikeda et al., 2005a, 2005b; King-Heiden et al., 2009; Nomura et al., 2004; Wolf et al., 1999). Transgenerational effects of TCDD exposure have now been observed in mice, rats and zebrafish (Baker et al., 2014; Bruner-Tran and Osteen, 2011; Manikkam et al., 2012a, 2012b; Nilsson et al., 2012). The TCDD-induced transgenerational defects identified in these species involve skeletal development, sex ratio, ovary, and reproductive success and are summarized in Table 1.

5. Skeletal development

Direct TCDD exposure causes skeletal, cartilage, and bone abnormalities in several animal models (Bursian et al., 2013; Hornung et al., 1999; Peterson et al., 1993; Xiong et al., 2008). *Spina bifida*, a developmental abnormality that is caused by incomplete closing of the neural tube and malformed vertebrae, occurs in human offspring following exposure to Agent Orange, a TCDD-contaminated herbicide (NAS-IOM, 2011). In mink, skeletal abnormalities were observed in F_1 offspring of TCDD-exposed adults (Bursian et al., 2013). TCDD exposure during development altered craniofacial structures in adult zebrafish, and produced scoliosis-like kinks in the axial skeletons of adult F_0 parents as well as in F_1 and F_2 offspring (Table 1; Baker et al., 2013, 2014; King-Heiden et al., 2009). Transgenerational skeletal abnormalities have not yet been reported in mammals. This response might be idiopathic to fish, but the effects may also be easier to observe in zebrafish. It is also