Neurotoxicology and Teratology 46 (2014) 40–48

Contents lists available at ScienceDirect

Neurotoxicology and Teratology

journal homepage: www.elsevier.com/locate/neutera

Neural alterations from lead exposure in zebrafish

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ARTICLE INFO

Article history:
Received 20 May 2014
Received in revised form 26 August 2014
Accepted 27 August 2014
Available online 19 September 2014

Keywords:
Zebrafish
Development
Lead
Neural
Vasculature

ABSTRACT

Lead was used extensively as a gas additive and pesticide, in paints, batteries, lead shot, pipes, canning and toy manufacturing. Although uses of lead have been restricted, lead persists in our environment especially in older homes, and generally in soil and water. Although extensive studies have determined that fetal and childhood exposures to lead have been associated with childhood and adolescent memory impairments and learning disabilities, there are limited studies investigating early neural and morphological effects that may lead to these behavioral and learning abnormalities. Here we utilize the zebrafish vertebrate model system to study early effects of lead exposure on the brain. We treat embryos with 0.2 mM lead for 24, 48 and 72 h and analyze neural structures through live imagery and transgenic approaches. We find structural abnormalities in the hindbrain region as well as changes in branchiomotor neuron development and altered neural vasculature. Additionally, we find areas of increased apoptosis. We conclude that lead is developmentally neurotoxic to a specific region of the brain, the hindbrain and is toxic to branchiomotor neurons residing in rhombomeres 2 through 7 of the hindbrain and hindbrain central artery vasculature.

1. Introduction

Although lead has been banned in the United States since the 1970s, lead is persistent in our environment (Tong et al., 2000). Soil remains contaminated with lead from old paint or past emissions of leaded gasoline. In addition, pollution from operating or abandoned industrial sites and smelters contributes to soil contamination. Leaded water pipes, found in many homes built before 1930 and soil erosion into water bodies contribute to water exposure (Philp, 2001; Tong et al., 2000). Although copper has replaced lead in newer construction, old leaded pipes are still found in homes and commercial buildings and soft water can cause increased leaching (Philp, 2001). In fact, a study performed in Washington DC schools in 2008 demonstrated that tap water in the school drinking water fountains contained up to 41% lead (Triantafyllidou et al., 2009). A number of activities and hobbies can expose humans to lead including pottery, stained glass and home repairs. Food crops may be contaminated by lead in agricultural water or soil. Because of the long term and widespread contamination of lead in the environment, lead exposure is a global concern (Tong et al., 2000).

Lead exposure is particularly toxic to the brain. Blood lead levels (BLLs) and the effects on learning and behavior have been studied (Needleman, 2004) and CNS (central nervous system) effects can occur at very low BLL. For children, an overall decrease in cognitive ability including reduced IQ (Intelligence Quotient), increased hyperactivity, ADHD (Attention Deficit Hyperactivity Disorder), and memory and behavioral disorders are among the main effects of lead neurotoxicity (Lidsky and Schneider, 2003, Needleman, 2004). Lead can easily pass through the placenta and affect the developing fetal brain and produce slow body growth. Based on a wealth of studies that demonstrated that even very low levels of lead can cause lifelong health effects, the Centers for Disease Control (CDC) changed its "blood lead level of concern" to 10 μg/dl in January 2012 (CDC, 2012).

Zebrafish are often used as a model of vertebrate development and toxicity studies (Dai et al., 2014, Hill et al., 2005, Parng et al., 2007, Teraoka et al., 2003). Sequencing of the zebrafish genome has shown that 70% of human genes have an analogous gene in the zebrafish (Howe et al., 2013). This similarity to humans and other vertebrates allows for the modeling of toxin and disease mechanisms. Details of the morphological and physiological characteristics are known for all stages of zebrafish development. Embryos develop ex utero and are transparent through the early stages of development allowing morphometric analysis (Padilla et al., 2012). The availability of fluorescent transgenic zebrafish, coupled with embryonic transparency, has allowed real time, in vivo analysis to be completed on toxin exposed embryos (Dai et al., 2014; Higashijima, 2008). This offers an advantage to traditional techniques like in situ hybridization or immunolocalizations where embryos must be fixed in time allowing only certain time windows of gene expression to be analyzed.

Previous studies in zebrafish found that a 72 hour lead exposure resulted in gene alterations associated with neurodevelopment (Peterson et al., 2011). Specifically lead exposed zebrafish embryos were analyzed for global transcriptional alterations at 72 and 120 hour post fertilization (hpf) time points using microarray approaches.

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http://dx.doi.org/10.1016/j.ntt.2014.08.008
0892-0362/

Comprehensive studies on specific alterations in neural morphology have been limited. However, lead mediated alterations in specific axon tracts and the role of axonogenesis related genes demonstrated decreased axonal density in axon tracts of the midbrain and forebrain concomitant with down-regulation of axonogenesis related genes before 24 hpf (Zhang et al., 2011). Dou and Zhang (2011) reported that a reduction in nerve cells due to increased apoptosis of neuron and glial cells leads to impaired neurogenesis (Dou and Zhang, 2011). Behaviorally, lead exposure has been linked to behavioral alterations, learning and memory deficits in adult zebrafish (Chen et al., 2012) as well as sensorimotor deficits (Rice et al., 2011).

However, other than the few studies mentioned, lead developmental toxicity has not been fully characterized in zebrafish. Early acute lead exposure has been studied in rat models and resulted in impaired cognitive function, learning impairments and behavioral abnormalities (Struzynska et al., 2001). Impaired neuronal morphogenesis has been noted in tadpoles (Cline et al., 1996). Many questions remain on how lead affects the brain, specifically in the early neural developmental stages. In the lab setting, zebrafish embryos are commonly exposed to chemicals at high concentrations during early life stages to screen for potential toxicity (Hill et al., 2005). In fact, the EPA (Environmental Protection Agency) has developed the ToxCast™ program which treats embryos at early developmental stages (6–8 h) with toxic chemicals at relatively high doses to assess overt and organismal toxicity (Padilla et al., 2012) to support predictive human modeling of developmental toxicity. Because zebrafish follow the vertebrate developmental plan and share the same developmental signaling pathways as higher order mammals, including humans, they can provide information relevant to overt and organismal toxicity (Padilla et al., 2012). Furthermore, in vivo toxicity can be assessed because zebrafish develop metabolic

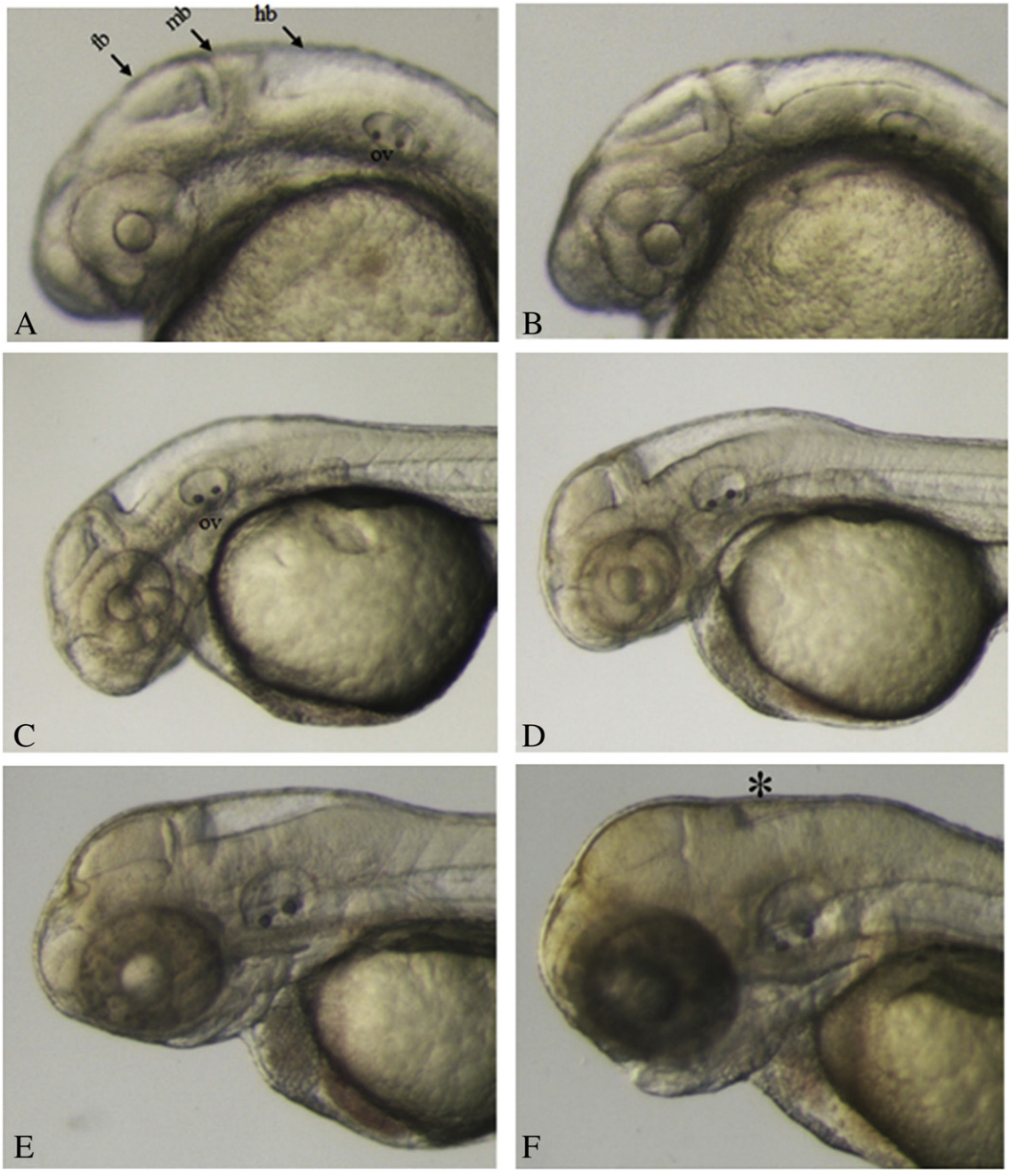

Fig. 1. Developmental time course of lead exposure in live embryos. (A, C, E) Control and (B, D, F) lead treated shown in lateral views. (A, B) 24 h, (C, D) 48 h and (E, F) 72 h. fb: forebrain, mb: midbrain, hb: hindbrain, ov: otic vesicle. Asterisk denotes lead treatment value of less than 0.05.