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Effects of halobenzoquinone and haloacetic acid water disinfection byproducts on human neural stem cells

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ABSTRACT

Human neural stem cells (hNSCs) are a useful tool to assess the developmental effects of various environmental contaminants; however, the application of hNSCs to evaluate water disinfection byproducts (DBPs) is scarce. Comprehensive toxicological results are essential to the prioritization of DBPs for further testing and regulation. Therefore, this study examines the effects of DBPs on the proliferation and differentiation of hNSCs. Prior to DBP treatment, characteristic protein markers of hNSCs from passages 3 to 6 were carefully examined and it was determined that hNSCs passaged 3 or 4 times maintained stem cell characteristics and can be used for DBP analysis. Two regulated DBPs, monobromoacetic acid (BAA) and monochloroacetic acid (CAA), and two emerging DBPs, 2,6-dibromo-1, 4-benzoquinone (2,6-DBBQ) and 2,6-dichloro-1,4-benzoquinone (2,6-DCBQ), were chosen for hNSC treatment. Both 2,6-DBBQ and 2,6-DCBQ induced cell cycle arrest at S-phase at concentrations up to 1 μmol/L. Comparatively, BAA and CAA at 0.5 μmol/L affected neural differentiation. These results suggest DBP-dependent effects on hNSC proliferation and differentiation. The DBP-induced cell cycle arrest and inhibition of normal hNSC differentiation demonstrate the need to assess the developmental neurotoxicity of DBPs.

Introduction

The human nervous system is particularly sensitive to toxic chemicals at early stages of development. Environmental chemicals are potentially more harmful to developing brains than adult brains due, in part, to the increased vulnerability during neuron growth and development during the early stages of life (Rice and Barone, 2000). Disruption of development may occur in different ways, including altered commitment of neural stem cells, proliferation of stem cells or neuronal progenitor cells, cell migration, neuron growth, apoptosis, neuronal communication, myelination, or development of the blood–brain-barrier (BBB) (Coecke et al., 2007). The adverse effects during exposure can manifest at any point after exposure. Much evidence has shown that the disruption of these neurodevelopmental pathways has been directly linked to neurodevelopmental diseases from environmental exposures (Grandjean and Landrigan, 2014; De Felice et al., 2015).

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The effects of drinking water disinfection byproducts (DBPs) on human neural development are unclear. Epidemiological studies have shown inconsistent correlations between a mother's exposure to treated water and adverse developmental effects in fetuses (Nieuwenhuijsen et al., 2009). However, associations between DBP exposure and an increased risk of adverse developmental outcomes, including stillbirth, spontaneous abortion, birth defects, and low birth weight, have been reported (Colman et al., 2011; Levallois et al., 2012; Grazuleviciene et al., 2013; Smith et al., 2016). Recently, a multigenerational *in vivo* experiment was conducted in rats, showing that exposure to a mixture of trihalomethanes (THMs) and haloacetic acids (HAAs) up to 2000 times regulated levels had no adverse effects on various reproductive endpoints (Narotsky et al., 2015). It was also proposed that using rats as an animal model might not be a representative model of developmental neurotoxicity experienced by humans. Thus, these results suggest two possible explanations: either (1) methods currently under use for assessment of human developmental neurotoxicity are not sensitive enough to detect DBP-induced effects, or (2) regulated DBPs are not the DBPs responsible for the observed effects.

Human stem cells are quickly becoming an established model in the field of drug discovery and development (Liu et al., 2013; Ko and Gelb, 2014). They are also a promising new model for evaluating the potential developmental effects of environmental chemicals (Liu et al., 2013; Mori and Hara, 2013). Many studies have used stem cell technology to characterize the toxicity of various environmental contaminants, such as bisphenol-A and tetrachloro-1,4-benzoquinone (Yin et al., 2015; Li et al., 2015, 2017). Stem cell models have the potential to proliferate into cells of all three germ layers (Thomson et al., 1998). In particular, human neural stem cells (hNSCs) are able to differentiate into all three phenotypes of the developing nervous system: neurons, oligodendrocytes, and astrocytes (Zhang et al., 2001). hNSCs can be grown as progenitor cells for several generations and can differentiate into different populations of neural cells when neural growth factor is removed from media. Thus, researchers can test cells at different neurodevelopmental stages, providing a distinct advantage over traditional cell-based models used for developmental neurotoxicity assessment. Furthermore, hNSCs can be analyzed by a variety of methods (colorimetric/fluorometric assays, immunostaining, image analysis) and are highly sensitive to low doses of toxins (Buzanska et al., 2009; Breier et al., 2010; Mori and Hara, 2013).

Halobenzoquinones (HBQs) are an emerging class of DBPs identified in drinking water in 2010 (Qin et al., 2010; Zhao et al., 2010). Specifically, four HBQs, 2,6-dichloro-1,4-benzoquinone (2,6-DCBQ), 2,6-dichloro-3-methyl-1,4-benzoquinone (DCMBQ), 2,3,6-trichloro-1,4-benzoquinone (TriCBQ), and 2,6-dibromo-1,4-benzoquinone (2,6-DBBQ), have been identified in tap water at nanogram per liter levels (Zhao et al., 2010). In addition to tap water, HBQs have also been identified in chlorinated recreational waters, presenting additional routes of human exposure (Wang et al., 2013). The toxicological significance of HBQs have been evaluated on the recommendation of quantitative structure toxicity relationship (QSTR) analyses that predicted HBQs have the potential to be up to 1000 times more toxic than regulated DBPs (Bull et al., 2006). Indeed, HBQs were found to increase both 8-hydroxydeoxyguanosine (8-OHdG) and protein carbonylation levels in T24 cells, indicating oxidative damage to genomic DNA and proteins (Du et al., 2013). Furthermore, the depletion of cellular glutathione (GSH) was found to sensitize cells to HBQs, and extracellular GSH supplementation could reduce HBQ-induced cytotoxicity, emphasizing the role of GSH-mediated and GSH-related enzyme-mediated detoxification of HBQs (Li et al., 2014). These findings are consistent with the reported toxic effects of quinone in organisms, particularly those involved in disrupting protein-handling systems (Xiong et al., 2014). However, the developmental effects of HBQs have yet to be examined.

Previous epidemiological and toxicological studies have shown that DBPs have the potential to affect neural development. hNSCs present a model which mimics the development of the human nervous system. The objective of our study is to examine the effects of regulated and non-regulated DBPs on hNSC proliferation and differentiation. Prior to DBP testing, we will first characterize the undifferentiated and differentiated hNSC cell populations for assessment of stem cell proliferation and differentiation. We have selected two regulated DBPs, monobromoacetic acid (BAA) and monochloroacetic acid (CAA), and two emerging DBPs, 2,6-DBBQ and 2,6-DCBQ, for analysis. BAA and CAA are HAA-DBPs, which are nearly ubiquitous in treated water. BAA and CAA have been demonstrated to be both cytotoxic and genotoxic (Plewa et al., 2010), and although they have been found to be developmental neurotoxins *in vitro* (Hunter et al., 1996), the *in vivo* and epidemiological evidence surrounding HAAs is unclear (Narotsky et al., 2015; Smith et al., 2016). Because comparative toxicity analysis of 2,6-DBBQ, 2,6-DCBQ, BAA, and CAA has revealed that these classes of DBPs preferentially influence different biological pathways (Procházka et al., 2015), these two classes of DBPs may also have different effects on neurodevelopment.

1. Materials and methods

1.1. Chemicals

2,6-DBBQ was purchased from Indofine Chemical Company (Hillsborough, NJ, USA). 2,6-DCBQ, BAA, and CAA were purchased from Sigma-Aldrich (Oakville, ON, Canada). Table S1 in Appendix A presents a list of the tested DBPs and their chemical structure.

1.2. Cell culture

Cryopreserved embryonic hNSCs were obtained from Gibco/Life Technologies (Carlsbad, CA, USA; Cat. No.: N7800100). Cell culture was established and maintained according to the manufacturer's instructions and incubated in a humidified chamber at 37°C and 5% CO_2. Undifferentiated hNSC culture was maintained with complete hNSC media containing KnockOut™ D-MEM/F-12 basal medium with 2 mmol/L GlutaMAX™-I supplement, 20 ng/mL fibroblast growth factor-basic (bFGF), 20 ng/mL epidermal growth factor (EGF), and 2% StemPro® Neural Supplement (ThermoFisher Scientific, Waltham, MA, USA). Media was refreshed 1 day after thawing

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