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Investigating the role of the aryl hydrocarbon receptor in benzene-initiated toxicity *in vitro*☆

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Abstract

Chronic occupational exposure to benzene has been correlated with aplastic aneamia and acute myelogenous leukemia, however mechanisms behind benzene toxicity remain unknown. Interestingly, benzene-initiated hematotoxicity is absent in mice lacking the aryl hydrocarbon receptor (AhR) suggesting an imperative role for this receptor in benzene toxicities. This study investigated two potential roles for the AhR in benzene toxicity using hepa 1c1c7 wild type and AhR deficient cells. Considering that many toxic effects of AhR ligands are dependent on AhR activation, our first objective was to determine if benzene, hydroquinone (HQ) or benzoquinone (BQ) could activate the AhR. Secondly, because the AhR regulates a number of enzymes involved in oxidative stress pathways, we sought to determine if the AhR had a role in HQ and BQ induced production of reactive oxygen species (ROS). Dual luciferase assays measuring dioxin response element (DRE) activation showed no significant change in DRE activity after exposure to benzene, HQ or BQ for 24 h. Immunofluorescence staining showed cytosolic localization of the AhR after 2 h incubations with benzene, HQ or BQ. Western blot analysis of cells exposed to benzene, HQ or BQ for 1, 12 and 24 h did not demonstrate induction of CYP1A1 protein expression. Dichlorodihydrofluorescein staining of cells exposed to benzene, HQ or BQ revealed that the presence of the AhR did not affect BQ and HQ induced ROS production. These results indicate that the involvement of the AhR in benzene toxicity does not seem to be through classical activation of this receptor or through interference of oxidative stress pathways.
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1. Introduction

Benzene is a leukemogen that is widely used as an industrial solvent and is also a ubiquitous environmental pollutant. Occupational exposure to benzene occurs worldwide to workers in industries such as oil, shipping, automobile repair, and shoe manufacturing (Lan et al., 2004). Chronic occupational exposure to high concentrations of benzene (>10 ppm) has been correlated with bone marrow depression, aplastic aneamia and acute myelogenous leukemia (reviewed in Snyder, 2002). Furthermore, a recent study reported low white blood cell and platelet counts, and a significant decline in progenitor cell colony formation in workers exposed to benzene concentrations below 1 ppm raising concerns regarding health consequences of low exposure to benzene (Lan et al., 2004).

☆ A preliminary report of this research was presented at the Dioxin 2005/ISPAC 20 meeting (2005), the 38th Annual Symposium of the Society of Toxicology of Canada (2005), and at the 45th annual meeting of the Society of Toxicology (USA) (2006).

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Although the toxic effects of benzene have been known for many years, the mechanisms through which it exerts its hematotoxic effects remain unknown. However, it is generally accepted that the metabolism of benzene is essential for hematotoxic and leukemogenic effects to occur. Benzene is first metabolized in the liver by cytochrome P450's, particularly CYP2E1, to a number of reactive metabolites including phenol, catechol and hydroquinone (HQ) (Bauer et al., 2003; Rangan and Snyder, 1997). These metabolites can accumulate in the bone marrow where they can be further bioactivated by myeloperoxidases and quinone reductases to reactive quinones such as 1,4-benzoquinone (BQ). Both HQ and BQ have the potential to initiate redox cycling and produce reactive oxygen species (ROS), which can lead to oxidative stress. Evidence supporting ROS in benzene-initiated toxicity include studies showing that mice treated with benzene, phenol, catechol or HQ have elevated levels of oxidized DNA (Kolachana et al., 1993). It has also been shown that bone marrow cells from benzene exposed mice have increased activity of activator protein-1 (AP-1), a transcription factor that is a known target of oxidative stress (Ho and Witz, 1997). In addition, studies have demonstrated that benzene metabolites increase myeloid cell growth *in vitro* by the formation of ROS (Wiemels and Smith, 1999). Furthermore, studies in our laboratory have shown that *in vitro* exposure to HQ or BQ causes a significant increase in ROS (Wan et al., 2005). Together these data support a role for ROS in mediating benzene-initiated toxicity.

The aryl hydrocarbon receptor (AhR) is a ligand activated transcription factor that regulates the expression of a battery of genes in a diverse range of tissues and species. Identification of AhR ligands has primarily focused on the halogenated aromatic hydrocarbons (HAHs) and polycyclic aromatic hydrocarbons (PAHs). However, studies have shown that there are a number of structurally diverse chemicals that can act as ligands suggesting that the AhR has an indiscriminate ligand binding site (Denison et al., 1998). Many studies have shown that the toxic and biological effects of AhR ligands are dependent on AhR activation (Denison and Nagy, 2003). Once activated, the AhR translocates to the nucleus and binds to regions of DNA known as dioxin response elements (DREs) (Nebert et al., 2000; Pollenz et al., 1994). AhR binding to DREs leads to enhanced transcription and upregulation of a number of genes including cytochrome P450 1A1 (CYP1A1) (Denison and Nagy, 2003; Whitlock, 1999).

Interestingly, a study has shown that benzene-initiated hematotoxicity is absent in mice lacking both alleles for the AhR suggesting an essential role for this receptor in benzene toxicities (Yoon et al., 2002). These $AhR^{-/-}$ mice showed no changes in peripheral or bone marrow cellularity as well as no change in levels of bone marrow granulocyte-macrophage colony-forming units (Yoon et al., 2002). Yoon et al. (2002) postulated that the absence of hematotoxicity in the $AhR^{-/-}$ mice was due to a lack of expression of CYP2E1. However, there was not a significant difference in CYP2E1 expression between wild type and $AhR^{-/-}$ mice in this study. Therefore, the involvement of the AhR in mechanisms of benzene-initiated toxicity remains unclear and warrants further investigation. In the present study, we sought to elucidate the role of the AhR in benzene toxicity. Our first objective was to determine if benzene, HQ or BQ act through receptor mediated pathways by activating the AhR. Since it is possible that AhR deficient models may lack or underexpress AhR-dependent enzymes involved in oxidative stress pathways, we further hypothesized that the presence of the AhR may mediate ROS production in cells exposed to HQ or BQ. Our results suggest that AhR activation does not appear to play a role in modifying the immediate metabolism of benzene or its activation of ROS in liver cells.

2. Materials and methods

2.1. Chemicals

All tissue culture reagents (α-MEM media, FBS, penicillin/streptomycin solution, and trypsin) were purchased from Gibco Life Technologies (Burlington, ON). TCDD was purchased from AccuStandard Inc., New Haven, CT. Benzene was obtained from Fisher Scientific Ltd. Both hydroquinone and 1,4-benzoquinone as well as benzo(*a*)pyrene (B(*a*)P) were purchased from Sigma and were at least 97% pure.

2.2. Cell culture and treatment

Wild-type mouse hepatoma hepa 1c1c7 cells and TAO cells, an AhR deficient variant of the 1c1c7 line having only 10% of the AhR content (Miller et al., 1983) (cells obtained from Dr. Julio Herrera, University of Minnesota) were propagated in α-MEM (with L-glutamine and without ribonucleosides, deoxyribonucleosides and sodium bicarbonate) supplemented with 10% FBS, 100 units/ml penicillin/streptomycin solution and 2.2% weight/volume sodium bicarbonate. Cells were treated with non-cytotoxic concentrations of benzene (0–200 μM), HQ (0–50 μM) or BQ (0–50 μM) over periods of time ranging from 2 to 24 h. For the ROS study 1 mM H_2O_2 was used as a positive control. For all other studies, the known AhR agonists, TCDD (10 nM) and B(*a*)P (120 μM) were used as positive controls.

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