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Benzo[*ghi*]perylene activates the AHR pathway to exert biological effects on the NL-20 human bronchial cell line

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ABSTRACT

Polycyclic aromatic hydrocarbons (PAH) are produced by incomplete combustion of organic material. In the Mexico City atmosphere, the most abundant PAH is benzo[*ghi*]perylene (B*ghi*P), a gasoline combustion marker. At present, there are no reports of the effects of B*ghi*P on human bronchial cells, so the aim of the study was to evaluate the effects *in vitro* of B*ghi*P on the NL-20 cell line. Results showed that B*ghi*P induced the formation of small vesicles throughout the cytoplasm, with absence of nuclear fragmentation. At 48 h exposition, damage in cell membrane increased significantly at 1.24 μg/mL of B*ghi*P ($p < 0.05$). Immunocytochemistry revealed that B*ghi*P provokes nuclear translocation of AhR receptor, which indicates that this compound can induce transcription of genes via receptor binding (AhR pathway activation). B*ghi*P induced a two-fold increase ($p < 0.05$) in the expression of AhR and CYP4B1 (a lung-specific pathway effector). In the presence of the receptor antagonist CH-223191, the loss of viability, the nuclear translocation and the overexpression of genes decreased, though this did not prevent the formation of vesicles. B*ghi*P induced oxidative stress and in presence of the receptor antagonist this increased significantly. In conclusion, B*ghi*P can activate the overexpression of AhR and CYP4B1, and the effects are abated by the AhR receptor antagonist. This is the first report to prove that B*ghi*P utilizes the AhR pathway to exert its toxic effects on the NL-20 human bronchial cell line .

1. Introduction

Polycyclic aromatic hydrocarbons (PAH) are a class of structurally different organic molecules composed of two or more fused aromatic rings. They are widely distributed environmental contaminants formed by the incomplete combustion of virtually any type of organic material. While they can be emitted by natural sources, such as forest fires or volcanic eruptions, most are produced by anthropogenic activities (municipal waste, industrial effluents, petroleum spills and automobile exhaust). The most important sources of this class of contaminants include gasoline emissions and particulate material produced by the fossil fuels in vehicles and industrial processes (Harvey, 1997; Ziad, 2008; Visciano and Perugini, 2009).

Humans are exposed to PAH by various pathways, but mainly occur through inhalation, ingestion and dermal contact; the great concern of these clases of pollutants is due to the fact that they can cause deleterious effects in the exposed organisms, because some of them are well known mutagenic and carcinogenic compounds (WHO/IPCS, 1998; IARC, 2010). Furthermore many of them are also potential endocrine disruptors causing adverse effects on the hormone homeostasis in at least three possible ways: by mimicking the action of a naturally produced hormone, bindind to their hormone receptors; by blocking the receptors in target cells for these hormones an therefore preventing the action of natural hormones; by altering the synthesis and function of hormone receptors and modify the synthesis, transport, metabolism and excretion of hormones (Santodonato, 1997; Gozgit et al., 2004; Fertuck et al., 2001; Ropero et al., 2006). Moreover the

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metabolism of PAHs can increase the toxicity of the compounds by the formation of reactive metabolites which can bind, covalently, with DNA, RNA and proteins, which can result in several alterations in the cells (Shimada and Fujii-Kuriyama, 2004).

In Mexico City, the atmospheric contaminants are distributed according to the characteristics and activities of each city zone. The research by Amador-Muñoz et al. (2011), confirms that concentrations of pollutants has seasonal and meteorological variation, showing a tendency towards increased concentrations of $PM_{2.5}$, heavy-PAH (containing more than four fused rings) and nitro-PAH in the dry season (November-April) compared to the rainy season (May–October), with the highest concentrations occurring in the central zone of the city (Amador-Muñoz et al., 2011).

In the study carried by Amador-Muñoz et al. (2013) it was demonstrated that B*ghi*P was the most abundant PAH in the PM_{10} in the Mexico City atmosphere since 1998–2002 with a mean of 1457.25 pg/m^3 during the 4 years. These same results were obtained by Guzmán-Torres et al. (2009), who measured levels of the 16 US-EPA priority pollutant PAH from March 17–31 in 2003 at Merced (source site) and at Pedregal (receptor area). B*ghi*P levels were, in general, the highest among the target PAH, both at the source (7.2 ng/m^3) and the receptor site (2.8 ng/m^3). Amador-Muñoz et al. (2011) also report that in 2006 B*ghi*P was the most abundant HAP in $PM_{2.5}$ with annual means of 1.19 ng/m^3 and 1.84 ng/m^3 in the southwestern and central zones of the city, respectively. (Guzmán-Torres et al., 2009; Amador-Muñoz et al., 2011, 2013).

B*ghi*P is a *peri*-condensed, 6-ring hydrocarbon with a molecular weight of 276, whose structure has neither "bay" nor "gulf" regions. This compound can be used as a marker of gasoline powered vehicle activity, as it has the highest particle-phase emission factor of the 16 priority PAH in light-duty vehicle exhaust but is not detected in heavy-duty diesel exhaust. The high concentrations of B*ghi*P in the atmosphere confirms that gasoline-powered vehicles are an especially significant source of PAH in Mexico City, which correlates with the large number of vehicles that circulate there: above 3 million (Marr et al., 1999; Amador-Muñoz et al., 2011; Arenas-Huertero et al., 2011).

The importance of studying this compound lies on the fact that the results of the first studies performed in 1983 by the IARC, led to its classification as non carcinogenic in animals and humans. Since then, it has kept such status as no further information has been obtained. Its most recent evaluation and report conduced by the IARC dates to 2010, and states that B*ghi*P did not produce cancer in experiments when it is applied to the skin of female mice (Lijinsky and Saffiotti, 1965; Hoffmann and Wynder, 1966; IARC, 1983), nor showed initiator or promoter effects in a tumor induction model in mouse skin (Van Duuren et al., 1970, 1973; IARC, 1983). Two other studies with mice similarly failed to prove tumor induction after subcutaneous injection (Müller, 1968; IARC, 1983). Meanwhile, intrapulmonary application in rats generated inconclusive results; however some pulmonary tumors did appear (Deutsch-Wenzel et al., 1983; IARC, 1983).

The data on human only suggests that the exposure to B*ghi*P occurs primarily through the inhalation of polluted air, smoking of tobacco and by ingestion of food and water contaminated by combustion effluents (IARC, 1983; Seto et al., 1993; Tokiwa et al., 1998). B*ghi*P is mutagenic to *Salmonella typhimurium* only in the presence of an exogenous metabolic system (rat liver S9 fraction) (Platt and Grupe, 2005). And Cho et al. (2005) have reported that B*ghi*P is highly correlated with dithiothreitol activity, a quantitative measure of *in vitro* reactive oxygen species formation. Thus, evaluations of biological systems must consider the formation of these molecules (Cho et al., 2005; Amador-Muñoz et al., 2011).

The exposure to xenobiotic compounds in skin, gut, lung, and eyes can trigger adaptive physiologic responses. The cells in these organs have various protective mechanisms against toxic compounds, including molecular ones that induce or reduce gene activity in response to xenobiotic compounds. PAH can generate their own biotransformation by increasing the expression of diverse genes upon binding to an aryl-hydrocarbon receptor (AhR) (Esser and Rannug, 2015). AhR is a ligand-dependent transcription factor that belongs to the basic helix-loop-helix (bHLH) Per/Arnt/Sim (PAS) family, which binds to a wide variety of endogenous and exogenous compounds, including xenobiotics as benzo(*a*)pyrene and 2,3,7,8- tetrachlorodibenzo-*p*-dioxin (Ma, 2012; Murray et al., 2014). It is localized in the cytoplasm in a multi-protein complex containing two molecules of the chaperone protein hsp90 (90 kDa heat shock proteins) that help maintain the conformation of the receptor, an AIP protein (auxiliary interacting protein, also known as XAP2 or Ara9) that stabilizes interaction between the hsp90 and the receptor, and a co-chaperone protein p23 that stabilizes the intermediary complex AhR-ligand. Interaction between AhR and ligands leads to the release of the chaperone proteins, and a rapid receptor nuclear translocation, where it forms a heterodimer with Arnt (nuclear translocator of the aryl-hydrocarbon receptor) (Arenas-Huertero et al., 2011; Ma, 2012; Esser and Rannug, 2015). The AhR-Arnt complex binds to the XRE (xenobiotic response elements) with the consensus core recognition sequence 5′-TNGCGTG-3′ to induce transcription of target genes that codify for enzymes involved in the xenobiotic activation and detoxification: *i.e.*, Phase I and Phase II enzymes like CYPs and AKRs, respectively. This mechanism corresponds to the canonical AhR pathway (Sorg, 2014; Esser and Rannug, 2015).

Several types of cross-talks have been reported as a result of molecular interaction between AhR and other proteins, in at least 3 ways: by competition for transcriptional coactivators/repressors; by coactivator-like interactions; by direct binding (Sorg, 2014; Esser and Rannug, 2015). This activation of genes can induce diverse biological effects implicated in processes such as the cell cycle (Puga et al., 2002; Levine-Fridman et al., 2004), apoptosis (Tian et al., 2002; Kimura et al., 2009), inhibition of differentiation (Apetoh et al., 2010; Hughes et al., 2014), perturbation of the endocrine balance (Kim et al., 2000; Vogel et al., 2004, 2007), and tumor promotion (Opitz et al., 2011; Feng et al., 2013). Once AhR is released from its ligand, the proteolytic pathway that degrades the nuclear receptor involves ubiquitination and the proteasome pathway (Roberts and Whitelaw, 1999). The AhR pathway has its own negative regulation performed by AhRR (aryl-hydrocarbon receptor repressor) that is trans-activated by the AhR/Arnt heterodimer to suppress the transcriptional activity of AhR by competing for dimerization with Arnt and the binding to XRE (Arenas-Huertero et al., 2011; Fujii-Kuriyama and Kawajiri, 2012).

The canonical AhR pathway activates processes in order to detoxificate the cells, but in some cases exactly the opposite occurs, the metabolites of these reactions may turn out to be just as toxic, or even more so, than the original compound. For example "knock out" mice of the AhR receptor (AhR −/−) show no TCDD-induced toxicity, but the "wild type" does, indicating that TCDD toxicity is mediated by AhR. The same phenomenon occurs with BaP, which only generates tumors in mice with AhR and not in "knock out" mice (Fernandez-Salguero et al., 1996; Mimura and Fujii-Kuriyama, 2003).

Using an *in vitro* reporter gene assay in the rat hepatoma H4IIE cell line, Machala et al. (2001) studied the AhR-inducing potency of B*ghi*P, demonstrating only a partial agonist AhR-mediated activity. They also showed that B*ghi*P has a relatively high mutagenic equivalent value (MEQs) based on mutagenic potency of B*a*P in river sediments samples.

Till et al. (1999) studied the induction of CYP1A1 as an AhR-mediated cellular response, by quantifying CYP1A1-catalyzed 7-