Toxicology Letters 289 (2018) 54–62

Contents lists available at ScienceDirect

Toxicology Letters

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

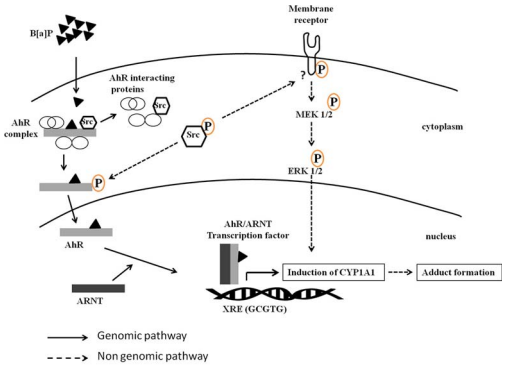
# Benzo[a]pyrene activates an AhR/Src/ERK axis that contributes to CYP1A1 induction and stable DNA adducts formation in lung cells

G. Vázquez-Gómez[a,*], L. Rocha-Zavaleta[b], M. Rodríguez-Sosa[c], P. Petrosyan[a], J. Rubio-Lightbourn[a]

[a] Universidad Nacional Autónoma de México, Instituto de Investigaciones Biomédicas, Departamento de Medicina Genómica y Toxicología Ambiental, A.P. 70228, Ciudad Universitaria, 04510, Ciudad de México, Mexico
[b] Universidad Nacional Autónoma de México, Instituto de Investigaciones Biomédicas, Departamento de Biología Molecular y Biotecnología, A.P. 70228, Ciudad Universitaria, 04510, Ciudad de México, Mexico
[c] Universidad Nacional Autónoma de México, Facultad de Estudios Superiores (FES) Iztacala, C.P. 54090, Tlalnepantla, México, Mexico

## GRAPHICAL ABSTRACT

Benzo[a] pyrene induces a classical genomic pathway by activating the Aryl hydrocarbon Receptor (AhR), along with a non-genomic pathway through the activation of SRC and ERK 1/2 kinases.Interaction between the genomic and non-genomic pathways is required for an adequate AhR signaling pathway, B[a]P metabolic process and its related genotoxic damage production.




Benzo[a]pyrene induces a classical genomic pathway by activating the Aryl hydrocarbon Receptor (AhR), along with a non-genomic pathway through the activation of SRC and ERK 1/2 kinases. Interaction between the genomic and non-genomic pathways is required for an adequate AhR signaling pathway, B[a]P metabolic process and its related genotoxic damage production.

## ARTICLE INFO

*Keywords:*
Benzo[a]pyrene
CYP1A1
AhR
MAP kinases

## ABSTRACT

Benzo[a]pyrene (B[a]P), the most extensively studied carcinogen in cigarette smoke, has been regarded as a critical mediator of lung cancer. It is known that B[a]P-mediated Aryl hydrocarbon Receptor (AhR) activation stimulates the mitogen activated protein kinases (MAPK) signaling cascade in different cell models. MAPK pathway disturbances drive alterations in cellular processes, such as differentiation, proliferation, and apoptosis, and the disturbances may also modify the AhR pathway itself. However, MAPK involvement in B[a]P metabolic activation and toxicity in lung tissues is not well understood. Here, we used a non-transformed human bronchial epithelial lung cell line, BEAS-2B, to study the participation of ERK 1/2 kinases in the metabolic activation of B[a]P and in its related genotoxic effects. Our results indicate that B[a]P is not cytotoxic to BEAS–2B cells at relatively low concentrations, but it enhances *CYP1A1* gene transcription and protein induction. Additionally, B

* Corresponding author.
*E-mail address:* gvazquezg@iibiomedicas.unam.mx (G. Vázquez-Gómez).

https://doi.org/10.1016/j.toxlet.2018.03.012
Received 27 July 2017; Received in revised form 24 February 2018; Accepted 10 March 2018
Available online 12 March 2018
0378-4274/ 

[a]P promotes Src and ERK 1/2 phosphorylation. Accordingly, inhibition of both Src and ERK 1/2 phosphorylation decreases CYP1A1 protein induction, AhR nuclear translocation and production of B[a]P adducts. Together, these data suggest a crosstalk between AhR and the members of the MAPK pathway, ERK 1/2 mediated by Src kinase. This interaction is important for the adequate AhR pathway signaling that in turn induces transcription and protein induction of CYP1A1 and B[a]P-induced DNA damage in BEAS–2B cells.

## 1. Introduction

Polycyclic aromatic hydrocarbons (PAHs) are abundant environmental contaminants that are produced by the incomplete combustion of organic matter, combustion engines, residential heating, biomass burning, and industrial activities (Gelboin, 1980; Phillips, 1999, 2002). Some PAHs have been recognized as mutagenic, carcinogenic and teratogenic to humans (Ellard et al., 1991; Nebert, 1989). Benzo[a]pyrene (B[a]P) is the major carcinogenic PAH; its ability to induce lung tumor formation has been well documented, and it is currently listed by the International Agency for Research on Cancer (IARC) as a carcinogen to humans (IARC, 2012). The B[a]P mutagenic effects are mediated by the metabolites produced during the biotransformation of B[a]P by the cytochrome P450 1 family enzymes through the Aryl hydrocarbon Receptor (AhR) pathway (Schmidt and Bradfield, 1996; Fujii-Kuriyama and Mimura, 2005). AhR is a ligand-activated member of the Per-Arnt-Sim family of basic helix–loop–helix transcription factors. Inactivated AhR forms a cytoplasmic complex in association with a dimer of heat shock protein 90 and other scaffold proteins, such as p23 protein and immunophilin-like AhR interacting protein (AIP). It has also been reported that the AhR complex can interact with other cytosolic proteins, including kinome chaperone, Cdc37, and the non-receptor tyrosine kinase, Src (Park et al., 2007; Enan and Matsumura, 1996; Perdew, 1988; Ma and Whitlock, 1997; Sogawa and Fujii-Kuriyama, 1997; Kazlauskas et al., 1999). The binding of ligands to AhR induces the release of chaperone proteins and the translocation of the receptor into the nucleus, where it forms a heterodimer with the aryl hydrocarbon receptor nuclear translocator (ARNT). Following AhR/ARNT heterodimer formation, it binds to a cognate sequence (TNGCGTG) known as the xenobiotic response element (XRE) in the promoters of responsive genes and regulates its transcription. The regulated genes include cytochrome P450 1A1 (*CYP1A1*), 1A2 (*CYP1A2*) and 1B1 (*CYP1B1*) (Nebert et al., 2004; Hao and Whitelaw, 2013). At the end of the metabolic processing, B[a]P is activated to B[a]P-7,8-diol-9,10 epoxide (BPDE), which can form stable $N^2$-B[a]PDE-deoxyguanosine (B[a]P-N2-dG) DNA adducts (Osborn and Crosby, 1987); these adducts are well known risk factors for lung cancer (Hecht, 2003; Rojas et al., 2004; Shimada and Fujii-Kuriyama, 2004; Alexandrov et al., 2010). Crosstalk between classical AhR and other cell signaling pathways may also occur as a consequence of AhR activation. There is evidence of AhR-dependent activation of mitogen activated protein kinases (MAPK). This activation of MAPK may produce changes in cellular processes, such as differentiation, proliferation, and apoptosis (Perez et al., 2008; Chramostová et al., 2004; Andrysík et al., 2006; Hoffer et al., 1996; Tan et al., 2002, 2004; Ding et al., 2009; Occhi et al., 2015). However, the potential participation of MAPK in the B[a]P biotransformation, the production of its metabolites, and the formation of B[a]P-N2-dG adducts have not been explored yet. It is known that toxic metabolites of B[a]P concentrate exclusively in bronchial epithelial cells (Rojas et al., 2004; Alexandrov et al., 2010), making the lung the major target of carcinogenic PAH. In spite of this, most studies on PAH have used hepatocytes or cancer-derived cell lines, which already have an impaired cell cycle regulation. For these reasons, in this work, we have investigated the participation of the ERK 1/2 kinases on AhR-dependant B[a]P bioactivation and the genotoxic damage caused by its metabolic product, BPDE, in a non-tumorigenic bronchial epithelial human cell line, BEAS-2B. We found that exposure of cells to B[a]P induced the activation of both Src and ERK 1/2 kinases and that the activity of these kinases promoted both the expression of CYP1A1 and the production of B[a]P-N2-dG adducts.

## 2. Materials and methods

### 2.1. Cell lines and culture conditions

Human bronchial epithelial BEAS-2B, human hepatocarcinoma HepG2 and human neuroepithelioma SK-N-MC cell lines were purchased from the American Type Culture Collection (ATCC). BEAS–2B and HepG2 cells were cultured in Dulbecco's Modified Eagle Medium (DMEM) (Sigma-Aldrich Co. N.Y. USA). SK-N-MC cells were cultured in Minimum Essential Medium (MEM) (Sigma-Aldrich Co. N.Y. USA). All cell lines were cultured in medium supplemented with 10% fetal bovine serum (FBS), (Gibco, N.Y. USA.), 1% non-essential amino acids (Sigma-Aldrich Co. N.Y. USA.), and 1% antibiotics (penicillin/streptomycin) (Gibco, N.Y. USA.), and they were maintained in a humidified atmosphere at 37 °C and 5% $CO_2$. The cells were subcultured once per week using trypsin.

### 2.2. Chemicals and reagents

B[a]P (CAS No. 50-32-8, purity 99.9%); ERK inhibitor, U0126; AhR inhibitor, CH223191; Src inhibitor, dasatinib; CYP1 inhibitor, α-naphthoflavone; and dimethyl sulfoxide (DMSO) were purchased from Sigma-Aldrich Co. (N.Y. USA). Stock solutions were diluted in DMSO and stored at −20 °C, in the dark.

### 2.3. Cell viability and inhibition assays

To evaluate the effect of B[a]P on cell viability, cells were seeded in duplicate in 96-well plates at a density of 35,000 cell/$cm^2$, in a volume of 200 µL of medium per well. After 24 h, the cells were treated with different concentrations of B[a]P in the range of 1–50 µM for 12, 24, and 36 h. Then, the culture medium containing B[a]P was discarded and rinsed with phosphate-buffered saline (PBS). Cell viability was evaluated by incubating the cells for 2 h at 37 °C with 1 mg/mL 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) in fresh medium. Precipitated formazan was solubilized by adding 100 µL of isopropanol. The absorbance was measured at 570 nm using a plate reader, Biotek EL × 800. The relative cell viability (%) related to control was calculated by [OD]test/[OD]control × 100%. For the inhibition assay, cells were cultured in 20 mm dishes until they reached 70% confluence. Culture medium was replaced by FBS-free DMEM medium. After 24 h, the cells were exposed to 20 µM of U0126, 100 nM of dasatinib, 25 µM of α-naphthoflavone, 10 µM of CH223191 and/or 1 µM of B[a]P for the appropriate times according to the assays as indicated in the figure legends. DMSO was used as a vehicle control. Then, cellular extracts were obtained and stored at −80 until use.

### 2.4. Evaluation of CYP1A1 expression by RT-PCR

BEAS–2B and SK-N-MC cells were cultured in 20 mm dishes until they reached 70–80% confluence. Culture medium was replaced by FBS-free medium. After 24 h, the cells were exposed to 1, 5, 10, 20, 30, 40 or 50 µM B[a]P for 24 h, using DMSO vehicle as a control. Total mRNA was isolated using RNeasy Plus Mini Kit (QIAGEN, Germany), according to the instructions of the manufacturer. cDNA synthesis and