Toxicology 314 (2013) 30–38

Contents lists available at ScienceDirect

Toxicology

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

Inflammatory mediators accelerate metabolism of benzo[*a*]pyrene in rat alveolar type II cells: The role of enhanced cytochrome P450 1B1 expression

Lenka Šmerdová[a], Jiří Neča[b], Jana Svobodová[a,c], Jan Topinka[d], Jana Schmuczerová[d], Alois Kozubík[a], Miroslav Machala[b,**], Jan Vondráček[a,*]

[a] *Department of Cytokinetics, Institute of Biophysics AS CR, 61265 Brno, Czech Republic*
[b] *Department of Chemistry and Toxicology, Veterinary Research Institute, 62100 Brno, Czech Republic*
[c] *Institute of Experimental Biology, Faculty of Science, Masaryk University, 61137 Brno, Czech Republic*
[d] *Laboratory of Genetic Ecotoxicology, Institute of Experimental Medicine AS CR, 14220 Prague, Czech Republic*

ARTICLE INFO

Article history:
Received 19 August 2013
Received in revised form 29 August 2013
Accepted 2 September 2013
Available online 8 September 2013

Keywords:
Inflammation
CYP1B1
Polycyclic aromatic hydrocarbons
Metabolism
DNA adducts

ABSTRACT

Long-term deregulated inflammation represents one of the key factors contributing to lung cancer etiology. Previously, we have observed that tumor necrosis factor-α (TNF-α), a major pro-inflammatory cytokine, enhances genotoxicity of benzo[*a*]pyrene (B[*a*]P), a highly carcinogenic polycyclic aromatic hydrocarbon, in rat lung epithelial RLE-6TN cells, a model of alveolar type II cells. Therefore, we analyzed B[*a*]P metabolism in RLE-6TN cells under inflammatory conditions, simulated using either recombinant TNF-α, or a mixture of inflammatory mediators derived from activated alveolar macrophage cell line. Inflammatory conditions significantly accelerated BaP metabolism, as evidenced by decreased levels of both parent B[*a*]P and its metabolites. TNF-α altered production of the metabolites associated with dihydrodiol-epoxide and radical cation pathways of B[*a*]P metabolism, especially B[*a*]P-dihydrodiols, and B[*a*]P-diones. We then evaluated the role of cytochrome P450 1B1 (CYP1B1), which is strongly up-regulated in cells treated with B[*a*]P under inflammatory conditions, in the observed effects. The siRNA-mediated CYP1B1 knock-down increased levels of B[*a*]P and reduced formation of stable DNA adducts, thus confirming the essential role of CYP1B1 in B[*a*]P metabolism under inflammatory conditions. TNF-α also reduced expression of aldo-keto reductase 1C14, which may compete with CYP1B1 for B[*a*]P-7,8-dihydrodiol and divert it from the formation of ultimate B[*a*]P dihydrodiol epoxide. Together, the present data suggests that the CYP1B1-catalyzed metabolism of polycyclic aromatic hydrocarbons might contribute to their enhanced bioactivation and genotoxic effects under inflammatory conditions.

1. Introduction

Polycyclic aromatic hydrocarbons (PAHs) are ubiquitous environmental pollutants produced by incomplete combustion, which are found in tobacco smoke, car exhaust, as well as in smoked and charcoal broiled food. Humans can be exposed to PAHs via inhalation of cigarette smoke or polluted air, consumption of contaminated food or by direct skin absorption. Mixtures containing PAHs, as well as individual PAHs are carcinogenic or co-carcinogenic in multiple organs and species, and a substantial attention has been paid to the mechanisms contributing to their carcinogenicity, in particular to the mechanisms underlying formation of their genotoxic metabolites. Benzo[*a*]pyrene (B[*a*]P) is a potent carcinogen, which has been extensively studied as a model carcinogenic PAH, and which is listed by the International Agency for Research on Cancer (IARC) as a group 1 human carcinogen (IARC, 2010). B[*a*]P is among the carcinogenic compounds found in cigarette smoke that have been implicated as causal factors contributing to the lung cancer development (Hecht, 1999).

B[*a*]P must be metabolically activated to form mutagenic and carcinogenic compounds (reviewed in Xue and Warshawsky, 2005). The three major pathways of B[*a*]P activation include: (i) the formation of radical cation at C6, which may either form depurinating DNA adducts or undergo further oxidations to produce B[*a*]P-1,6-dione and B[*a*]P-3,6-dione (Cavalieri and Rogan, 1995); (ii) diol epoxide pathway, in which cytochromes

* Corresponding author at: Department of Cytokinetics, Institute of Biophysics, Academy of Sciences of the Czech Republic, Královopolská 135, 61265 Brno, Czech Republic.
Tel.: +420 541517168; fax: +420 541211293.
** Corresponding author at: Department of Chemistry and Toxicology, Veterinary Research Institute, Hudcova 70, 621 00 Brno, Czech Republic.
Tel.: +420 533331801; fax: +420 541211229.
E-mail addresses: machala@vri.cz (M. Machala), vondracek@ibp.cz (J. Vondráček).

0300-483X/$ – see front matter © 2013 Elsevier Ireland Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.tox.2013.09.001

P450 (CYP) and epoxide hydrolases contribute to the formation of ultimate genotoxin, (+)-anti-7α,8α-dihydroxy-9α,10α-epoxy-7,8,9,10-tetrahydro-B[*a*]P (BPDE) (Baird et al., 2005; Xue and Warshawsky, 2005); and (iii) the formation of reactive and redox-active B[*a*]P-7,8-dione via activities of several dihydrodiol dehydrogenase members of the aldo-keto reductase (AKR) superfamily (Penning et al., 1999). All three major pathways of B[*a*]P metabolism yield compounds potentially contributing to the process of carcinogenesis. The formation of BPDE, via diol epoxide pathway, received in the past most of the attention, since it is the most efficient mutagenic B[*a*]P metabolite, which readily forms stable DNA adducts, primarily with deoxyguanosine, and plays a prominent role in the B[*a*]P-induced carcinogenesis (Baird et al., 2005; Xue and Warshawsky, 2005). Nevertheless, production of B[*a*]P-7,8-dione via activities of several AKRs, or formation of B[*a*]P-1,6-dione and B[*a*]P-3,6-dione via radical cation pathway, also yields pro-carcinogenic compounds capable of either eliciting both direct and reactive oxygen species-mediated DNA damage, or altering intracellular signaling in a manner contributing to tumor promotion (Burdick et al., 2003; Burchiel et al., 2007; Rodríguez-Fragoso et al., 2009; Park et al., 2008).

PAHs significantly contribute to the adverse effects of airborne particulate matter (PM), which originate from a variety of indoor and outdoor sources, including industry, traffic-related emissions, local heating and others. Exposure to PM has been proposed to lead to a hierarchical response to PM-generated oxidative stress, which includes activation of pro-inflammatory signaling within target cells and production of inflammatory mediators participating in adverse health effects of PM and the associated organic contaminants (Li et al., 2008). Chronic inflammation is also one of the important deleterious effects of smoking in airways and lung tissue (Gonçalves et al., 2011). Among other effects, the inflammatory mediators are known to disrupt expression and/or activity of xenobiotic-metabolizing enzymes (XMEs) in various tissues (Aitken et al., 2006). Importantly, inflammation down-regulates expression and activities of many CYP enzymes, including those participating in B[*a*]P metabolism, such as CYP1A1 (Aitken et al., 2006; Fardel, 2013; Vondráček et al., 2011). The suppression of CYP1A1 expression has been linked to the activation of transcriptional factor NF-κB (nuclear factor kappa-light-chain-enhancer of activated B cells), which is induced by pro-inflammatory cytokines (Tian et al., 2002).

However, in a marked contrast, both we and others have recently observed that expression of CYP1B1 is differentially regulated and that levels of this CYP1 isoform could be increased under infection/inflammatory conditions in various rodent and human cell types within liver, lung or central nervous system (Kurzawski et al., 2012; Malaplate-Armand et al., 2003; Piscaglia et al., 1999; Umannová et al., 2008, 2011). Importantly, the study of Kurzawski et al. (2012) shows that enhanced up-regulation of CYP1B1 can be observed both *in vitro*, upon incubation of hepatocarcinoma cells with pro-inflammatory cytokine, and *in vivo*, in patients suffering from end-stage liver disease. Despite its relatively low homology with other CYP family 1 members, CYP1B1 participates in metabolic activation of PAHs to reactive metabolites at rates similar to or even higher than CYP1A1 (Kim et al., 1998; Shimada et al., 1999). It is expressed both in normal tissues and in tumor cells, where its expression reaches much higher levels as compared with normal tissue (Murray et al., 2001). CYP1B1 has been suggested to play a role in tumor development and progression; it is a potential target for anticancer drugs or a putative tumor biomarker (Sissung et al., 2006). CYP1B1 contributes to bioactivation of numerous chemical carcinogens, as well as genotoxic metabolites of endogenous compounds (Hayes et al., 1996; Shimada et al., 1996). Since chronic inflammation increases risk of cancer and many cancer arises at sites of chronic inflammation (Balkwill et al., 2005), it is important to understand the impact of inflammatory mediators on metabolism of pro-carcinogens, such as B[*a*]P, which may involve enhanced CYP1B1 expression/activity.

Based on this evidence, as well as on our own previous results, documenting an increased formation of stable DNA adducts in epithelial cells co-treated with B[*a*]P and pro-inflammatory cytokine (Umannová et al., 2008, 2011), the present study aimed to characterize the impact of inflammatory mediators on B[*a*]P metabolism within lung alveolar epithelial type II (AEII) cells. As a model, we used rat lung epithelial RLE-6TN cells, derived from AEII cells, in which CYP1B1 can be highly induced upon co-treatment with B[*a*]P and tumor necrosis factor-α (TNF-α), a key pro-inflammatory cytokine. We have also evaluated the impact of a mixture of inflammatory mediators derived from lipopolysaccharide (LPS)-activated alveolar macrophages. We then assessed the role of CYP1B1 in B[*a*]P metabolism in AEII cells using short interfering (si) RNA-mediated knock-down of CYP1B1. Finally, we investigated possible impact of pro-inflammatory cytokine on expression of further enzymes and/or transporters potentially contributing to the B[*a*]P activation and/or detoxification.

2. Materials and methods

2.1. Chemicals

B[*a*]P (CAS No. 50-32-8, purity 99.9%) was provided by Ehrenstorfer (Augsburg, Germany). Stock solutions were prepared in dimethyl sulfoxide (DMSO) (Merck, Darmstadt, Germany) and stored in the dark. The unlabeled B[*a*]P metabolite standards B[*a*]P-r-7,t-8,t-9,c-10-tetrahydrotetrol(±) (B[*a*]P-tetrol I-1), B[*a*]P-trans-7,8-dihydrodiol(±) (B[*a*]P-7,8-DHD), B[*a*]P-trans-9,10-dihydrodiol (B[*a*]P-9,10-DHD), B[*a*]P-trans-4,5-dihydrodiol(±) (B[*a*]P-4,5-DHD), B[*a*]P-3,6-dione, B[*a*]P-7,8-dione, B[*a*]P-1,6-dione, B[*a*]P-6,12-dione, 1-OH-B[*a*]P, 3-OH-B[*a*]P, 7-OH-B[*a*]P, 8-OH-B[*a*]P and 9-OH-B[*a*]P were purchased from the National Cancer Institute's Chemical Carcinogen Standard Reference Repository (Midwest Research Institute, Kansas City, MO). The identities and purity of all the standards were established by LC–MS/MS. Ethyl acetate (p.a. ACS), methanol (p.a. ACS) and methanol (HPLC gradient grade) were purchased from Merck. Ultrapure water was obtained from a Milli-Q UF Plus water system (Millipore, Molsheim, France). Liquid cell culture media were obtained from Life Technologies (Carlsbad, CA). Recombinant rat TNF-α (Sigma–Aldrich, Prague, Czech Republic) was dissolved in phosphate-buffered saline (PBS). Goat polyclonal antibody against CYP1A1 and rabbit polyclonal antibodies against CYP1B1 were obtained from BD Biosciences (San Jose, CA). Rabbit polyclonal antibodies against Ser15-phosphorylated p53 and cyclooxygenase-2 (COX-2) were purchased from Santa Cruz Biotechnology (Santa Cruz, CA). Mouse monoclonal antibody against β-actin was obtained from Sigma–Aldrich. Spleen phosphodiesterase was purchased from ICN Biomedicals (Aurora, OH); micrococcal nuclease, nuclease P1 was from Sigma–Aldrich; polyethylene-imine cellulose TLC plates (0.1 mm) were from Macherey-Nagel (Düren, Germany); T4 polynucleotide kinase was from Affymetrix (Santa Clara, CA); γ-^{32}P-ATP (3000 Ci/mmol, 10 μCi/μl) was from Perkin Elmer (Waltham, MA). All other specific reagents and media supplements are indicated below.

2.2. Cell culture and treatments

The RLE-6TN rat lung epithelial cell line (ATCC® CRL-2300™) (Driscoll et al., 1995) was obtained from American Type Culture Collection (Manassas, VA). This cell line has been derived from alveolar type II cells, which were isolated from male F344 rats. RLE-6TN cells are thought to be derived from a spontaneous transformant, are T-antigen negative and have been previously successfully used to analyze genotoxicity of PAHs (Borm et al., 1997; Umannová et al., 2011). Cells were maintained in Ham's F12 medium supplemented with 2 mM L-glutamine, bovine pituitary extract (10 μg/ml), insulin (5 μg/ml), insulin-like growth factor (2.5 ng/ml), transferrin (1.25 μg/ml), epidermal growth factor (2.5 ng/ml) and 5% heat-inactivated fetal bovine serum (GE Healthcare). All tissue culture supplements were obtained from Sigma–Aldrich. Cells were cultivated at 37 °C in a humidified incubator maintained at 5% CO_2. For the treatments, cells were seeded at the density of 30,000 cells per cm^2 in 60-mm diameter cell culture dishes. For the individual treatments, see legends to figures.

2.3. siRNA transfection

Cells were plated at a density of 20,000 cells per cm^2 in 6-well plates in the cultivation medium without antibiotics. After 24-h cultivation, transfections were performed, using rat CYP1B1 mRNA siGENOME smart pool provided by Thermo Scientific Dharmacon (Lafayette, CO). The transfection was performed in a total volume of 2 ml containing 200 pmol siRNA (100 nM) and 5 μl of Lipofectamine 2000