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Effect of interleukin (IL)-8 on benzo[a]pyrene metabolism and DNA damage in human lung epithelial cells

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ABSTRACT

It has been well established that inflammation and concurrent mutagenic exposures drive the carcinogenic process in a synergistic way. To elucidate the role of the inflammatory cytokine IL-8 in this process, we studied its effect on the activation and deactivation of the chemical mutagen benzo[a]pyrene B[a]P in the immortalized pulmonary BEAS-2B cell line. After 24 h incubation with B[a]P in the presence or absence of IL-8, the B[a]P induced cytochrome P450 1A1 and 1B1 (*CYP1A1* and *CYP1B1*) gene expression and CYP1A1 enzyme activity was significantly higher in the presence of the cytokine. Consistent with these findings, we observed higher concentration of the metabolite B[a]P-7,8-diol under concurrent IL-8 treatment conditions. Interestingly, we also found higher concentrations of unmetabolized B[a]P. To explain this, we examined the downstream effects of IL-8 on NADPH oxidases (NOXes). IL-8 lowered the intracellular NADPH level, but this effect could not explain the changes in B[a]P metabolism. IL-8 also significantly depleted intracellular glutathione (GSH), which also resulted in enhanced levels of unmetabolized B[a]P, but increased concentrations of the metabolite B[a]P-7,8-diol. No differences in B[a]P-DNA adducts level were found between B[a]P and B[a]P combined with IL-8, and this might be due to a 3-fold increase in nucleotide excision repair (NER) after IL-8 treatment. These findings suggest that IL-8 increased the formation of B[a]P-7,8-diol despite an overall delayed B[a]P metabolism via depletion of GSH, but DNA damage levels were unaffected due to an increase in NER capacity.

1. Introduction

The human lung offers an enormous surface area for exposure to contaminants in air, making this organ particularly vulnerable to a variety of toxic agents including cigarette smoke, automobile exhaust, microorganisms and a wide range of other pollutants (Azad et al., 2008). The inhalation of such toxic agents can induce lung injury by the generation of reactive oxygen/nitrogen species (ROS/RNS), which results in the production of pro-inflammatory cytokines and chemokines (Emmendoerffer et al., 2000). Subsequently, inflammation is triggered and polymorphonuclear leukocytes (PMNs) are recruited to the site of damage to eliminate the pathogenic insult and to restore damaged tissue back to its normal physiological functions (Azad et al., 2008). However, if such an inflammatory condition persists, chronic inflammation develops, which is a causative factor in lung cancer etiology (Knaapen et al., 2006). For example, chronic obstructive pulmonary disease (COPD) patients showed increased number of neutrophils and macrophages in their sputum and bronchoalveolar lavage fluid and this results in the release of proteolytic enzymes and the generation of oxidants which cause tissue damage as well as cytokine and chemokine release (Tetley, 2005). Moreover, large amounts of neutrophils and macrophages in the airways can contribute to alterations of the lung microenvironment, which subsequently increases the risk for developing primary lung cancer (King, 2015; Lee et al., 2009; Raviv et al., 2011).

IL-8, also known as CXCL8, is a pro-inflammatory chemokine, released during inflammation that plays a crucial role in chemoattracting neutrophils (Miyoshi et al., 2010; Waugh and Wilson, 2008). The promoter region of the *IL-8* gene contains binding sites for the redox-sensitive transcription factors nuclear factor-kappa B (NF-κB) and activator protein-1 (AP-1) (Elliott et al., 2001). For example, stimulation of the bronchial epithelial cell line BEAS-2B with lipopolysaccharide (LPS) elevated IL-8 levels in the

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supernatant that were even further enhanced when the LPS-triggerd cells were co-incubated with PMNs (Boots et al., 2012). In addition, upon secretion of IL-8, large amounts of PMN will be attracted to the site of injury/infection. In order to eliminate invading microorganisms, IL-8 induces a respiratory burst and generates ROS, which is initiated by activation of nicotinamide adenine dinucleotide phosphate (NADPH) oxidases (Noxes) (Bréchard et al., 2005; Djeu et al., 1990; Forman and Torres, 2002; Leto et al., 2009).

Moreover, humans are inevitably exposed to benzo[a]pyrene (B[a]P) via cigarette smoke, diesel exhaust emissions and environmental pollutants. B[a]P is the best studied polycyclic aromatic hydrocarbon (PAH) which plays a major role in lung cancer development (Arlt et al., 2015; Baginski et al., 2006; Chiba et al., 2012; Yanagisawa et al., 2016). B[a]P itself is not a direct genotoxin, but gains its carcinogenic and mutagenic effects after metabolic activation (Baird et al., 2005). The most important route of B[a]P metabolic activation is via monooxygenation, catalyzed by the microsomal NADPH-dependent cytochrome P450 isoforms 1A1/1B1 (CYP1A1/CYP1B1), followed by hydration by microsomal epoxide hydrolase (Umannova et al., 2008). Phase I activation of B[a]P results in the generation of an intermediate of (±)-trans-7,8-dihydroxy-7,8-dihydrobenzo[a]pyrene (B[a]P-7,8-diol), that is mostly detoxified via conjugation with glutathione (GSH) by specific glutathione-*S*-transferases (GSTs) in phase II reactions (Kabler et al., 2009; Trush et al., 1985). If not conjugated, B[a]P-7,8-diol is further metabolized by CYPs and yields a highly reactive B[a]P-7,8-dihydrodiol-9,10-epoxide (BPDE) which can covalently bind to DNA. BPDE-DNA adducts may cause mutations and then initiate carcinogenesis (Baird et al., 2005; Xue and Warshawsky, 2005). Apart from the mutagenicity of B[a]P and BPDE, both of them have been found to induce an inflammatory response and elicit the production of inflammatory mediators including IL-8 (Dreij et al., 2010; Podechard et al., 2008a; Vogel et al., 2005). Although the metabolism of B[a]P can be affected by the presence of inflammation by a variety of cytokines, chemokines and enzymes that are released at the inflamed site (Borm et al., 1997; Gungor et al., 2007a,b; Gungor et al., 2010a,b,c; Shachar and Karin, 2013; Shi et al., 2016a; Umannova et al., 2008; Umannova et al., 2011; Van Schooten et al., 2004), there is still little knowledge about the way IL-8 can affect B[a]P induced genotoxicity. Here, we describe the effect of IL-8 on B[a]P metabolism in human bronchial epithelial cells (BEAS-2B) and describe a possible underlying mechanism.

2. Materials and methods

2.1. Chemicals

All chemicals were purchased from Sigma-Aldrich Chemie B.V. (Zwijndrecht, The Netherlands) unless stated otherwise.

2.2. Cell culture

BEAS–2 B cells (SV40-immortalized normal bronchial epithelium cells) from the American Tissue Culture Collection (ATCC, Manassas, USA), were cultured in Dulbecco's Modified Eagle's Medium (DMEM/F-12; Gibco, Bleiswijk, The Netherlands) supplemented with 1% penicillin/streptomycin, 15 μg/ml bovine pituitary extract, 0.5 ng/ml bovine serum albumin (Invitrogen, Breda,The Netherlands), 10 ng/ml epidermal growth factor (Merck Millipore, Darmstadt, Germany), 10 ng/ml cholera-toxin (list Biological Laboratories, Inc., Campbell, California), 5 μg/ml insulin, 5 μg/ml transferrin and 0.1 μM dexamethasone (Sigma-Aldrich, St. Louis, MO, USA) and maintained in a 5% CO_2 environment at 37 °C. Cell passages between 10 and 20 were used for experiments and cells were routinely screened for mycoplasma contamination by a Mycoplasma Detection Kit (InvivoGen, The Netherlands).

2.3. Cell treatments

To investigate the effect of IL-8 on B[a]P metabolism, B[a]P (1 μM) in the present or absence of IL-8 (10 ng/ml) was used for exposure of BEAS–2 B cells (80% confluency) for 6, 24 and 48 h. Previous studies have revealed that simulating these lung cells with LPS or particles resulted in supernatant IL-8 levels ranging from 1 ng/ml to 100 ng/ml (Boots et al., 2012; Schins et al., 2002). To mimic such a pro-inflammatory condition, 10 ng/ml IL-8 was added to BEAS–2 B cells and coincubated with 1 μM B[a]P. B[a]P was dissolved in dimethyl sulfoxide (DMSO) and added into the medium with a final DMSO concentration of 0.5% (v/v). IL-8 was dissolved in phosphate-buffered saline (PBS, pH 7.4). To study the possible influence of GSH and ROS on the interaction studied, pre-treatments with a glutathione synthesis inhibitor (buthionine sulphoximine, BSO) and a general NOXs pathway inhibitor (diphenyleneiodonium chloride, (DPI)) were included. To deplete intracellular GSH levels, BEAS–2 B cells were preincubated with a non-toxic dose of 400 μM BSO for 24 h, following by washing the cells with cell media and then 24 h treatment with 1 μM B[a]P. For DPI treatments, cells were directly coincubated with different concentrations of this inhibitor (100, 10, 1, 0.1, and 0.01 μM). In concordance with literature, DPI doses above 1 μM was toxic to the cells (Gatley and Martin, 1979; Jaquet et al., 2011; Riganti et al., 2004). Consequently, 0.1 and 1 μM DPI were used in the current study to inhibit NOXes activity. All cell media and pellets were collected and stored at −20 °C for further analysis upon these incubations. Experiments were performed at least in duplicate in two independent cultures (n = 4).

2.4. Detection of cellular viability

The 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-tetrazolium bromide (MTT) assay was used to assess cell cytotoxicity and cell viability. As previous described by Shi et al. (Shi et al., 2016b), BEAS–2 B cells were cultured in 96-well plates (Costar, Cambridge, MA) at 1×10^4 cells/100 μl medium and exposed to B[a]P, IL-8, DPI or a combination of these three triggers. After all treatments, wells were washed with warm (37 °C) Hank's Balanced Salt Solution (HBSS) and incubated with MTT (0.5 mg/ml) at 37 °C in the dark for 1 h. Then, wells were emptied and cells were incubated with 200 μl DMSO for 30 min at room temperature. Finally, absorption was measured by using a microplate reader at 540 nm (Biorad, Veenendaal, The Netherlands) and the cell viability was expressed as percentage of DMSO control.

2.5. Measurement of IL-8 protein levels

In order to determine the IL-8 protein levels in cell medium after 6, 24 and 48 h incubation, an enzyme-linked immunosorbent assay (ELISA) (cat. no. M950050192; CLB/Sanquin, The Netherlands) was carried out according to the manufacturer's instructions.

2.6. NADPH quantification

To assess the concentration of intracellular NADPH, a NADPH quantification kit was applied (cat. no. MAK038; Sigma-Aldrich). After incubating BEAS–2 B cells with different treatments for 6, 24 and 48 h, cells were washed with cold PBS, trypsinized and harvested as pellets by centrifuging at 1200 RPM at 4 °C for 5 min. Next, cells were extracted by adding 800 μl NADPH extraction buffer vortexing for 10 s and centrifuging at 14,000 RPM for 5 min.