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Short communication

Differential chemokine induction by 1-nitropyrene and 1-aminopyrene in bronchial epithelial cells: Importance of the TACE/TGF-α/EGFR-pathway

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ABSTRACT

1-nitropyrene (1-NP), a common PAH in diesel exhaust, and its amine metabolite 1-aminopyrene (1-AP) induce distinctly different chemokine-responses in bronchial epithelial cells (BEAS-2B) characterized by increases in CXCL8 and CCL5, respectively. Tumor necrosis factor-α converting enzyme (TACE), which cleaves membrane-bound transforming growth factor (TGF)-α, activating the epidermal growth factor receptor (EGFR), may regulate pro-inflammatory responses induced by a variety of endogenous and exogenous agents. The present results suggest that CXCL8, but not CCL5 responses in 1-NP- or 1-AP-exposed cells required TACE/TGF-α/EGFR-signaling. The findings strengthen the notion that TACE/TGF-α/EGFR-signaling is central in epithelial CXCL8-regulation upon exposure to multiple airborne pollutants.

1. Introduction

Nitro-polycyclic aromatic hydrocarbons (nitro-PAHs) are a subgroup of PAHs formed during combustion processes through interactions between PAHs and nitrogen oxides. Nitro-PAHs are ubiquitous air pollutants found in ambient air particles, in particular those derived from diesel emissions (Zwirner-Baier and Neumann, 1999). Many nitro-PAHs are mutagenic and carcinogenic. 1-nitropyrene (1-NP), a suggested marker of diesel exhaust particles (DEP), appears to be among the main contributors to the mutagenicity of DEP (Scheepers et al., 1995).

Inflammation is considered a central step in the development and exacerbation of adverse health effects of DEP and other airborne particulates. However, compared to the

Abbreviations: 3-ABA, 3-aminobenzanthrone; 3-AF, 3-aminofluoranthene; 1-AP, 1-aminopyrene; COPD, chronic obstructive pulmonary disease; DEP, diesel exhaust particles; EGFR, epidermal growth factor receptor; ERK, extracellular regulated kinase; IL-8, interleukin-8; JNK, c-Jun n-terminal kinase; MAPK, mitogen activated kinase; 3-NBA, 3-nitrobenzanthrone; 3-NF, 3-nitrofluoranthene; 1-NP, 1-nitropyrene; PAH, polycyclic aromatic hydrocarbon; RANTES, regulated upon transcription normal T-cell expressed and secreted; SFK, Src-family kinases; TACE, tumor necrosis factor-α converting enzyme; TGF-α, transforming growth factor-α.

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knowledge on genotoxicity, considerably less is known about the pro-inflammatory potential of nitro-PAHs. Previously, we have investigated the ability of 1-NP, 3-nitrofluoranthene (3-NF) and 3-nitrobenzanthrone as well as their corresponding amine metabolites, 1-aminopyrene (1-AP), 3-aminofluoranthene (3-AF) and 3-aminobenzanthrone, to induce expression of pro-inflammatory cytokines and chemokines in human bronchial epithelial cells, BEAS-2B (Øvrevik et al., 2010). 1-NP, 1-AP, 3-NF and 3-AF induced marked increases in several cytokine and chemokine genes. However, 1-NP and 3-NF induced a distinct gene-expression pattern characterized by high levels of CXCL8 (IL-8), while 1-AP and 3-AF induced a completely different pattern characterized by high levels of CCL5 (RANTES) (Øvrevik et al., 2010). CXCL8 is a potent neutrophil recruiting chemokine (Fox et al., 2005), while CCL5 preferentially activates and attracts eosinophils (Bisset and Schmid-Grendelmeier, 2005). Thus, the substitution of the nitro-group with an amino-group seems to completely alter the pro-inflammatory potential of PAH-derivatives. The reason for this difference in effect is unclear, but the responses were not directly related to typical PAH-induced endpoints, such as aryl hydrocarbon receptor-activation, DNA-damage, cell-cycle alterations or general cytotoxicity (Øvrevik et al., 2010).

We have recently observed that CXCL8-responses in BEAS-2B cells triggered by a variety of air pollutants including 1-NP, seemed to be regulated at least partly through a signaling-pathway involving tumor necrosis factor-α converting enzyme (TACE), transforming growth factor (TGF)-α and the epidermal growth factor receptor (EGFR) (Øvrevik et al., 2011). TACE (or ADAM-17) is a metalloprotease that cleaves membrane-bound TGF-α, leading to release of TGF-α ectodomains and transactivation of EGFR. The TACE/TGF-α/EGFR-pathway has also been implicated in CXCL8-induction by endogenous and microbial agents in airway epithelial cells (Koff et al., 2008; Kuwahara et al., 2006). Furthermore, other signaling molecules central in chemokine-regulation, such as the non-receptor tyrosine kinase c-Src and other Src family kinases (SFKs) and the mitogen activated protein kinases (MAPKs), have also been linked to TACE/TGF-α/EGFR-signaling (Kuwahara et al., 2006; Murillo et al., 2005; Øvrevik et al., 2004). In the present study we set out to explore to what extent the TACE/TGF-α/EGFR-pathway could explain the difference in effect between 1-NP- and 1-AP-induced chemokine-responses induced in BEAS-2B cells.

2. Materials and methods

2.1. Reagents

1-NP, 1-AP (Fig. 1) and dimethyl sulphoxide (DMSO) and were purchased from Sigma–Aldrich (St. Louis, MO, USA). SB202190, PD98059, SP600125, PP2, AG1478, TAPI-1 and TGF-α-neutralizing antibody were all from Calbiochem (La Jolla, CA, USA). LHC-9 cell culture medium was from Invitrogen (Carlsbad, CA, USA). Cytokine ELISA assays for CCL5 and CXCL8 were purchased from Biosource International (Camarillo, CA, USA). All other chemicals used were purchased from commercial sources at the highest purity available.

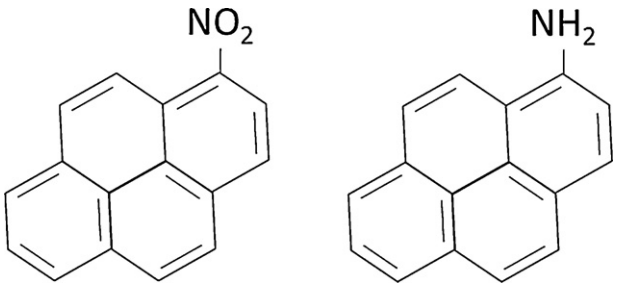

Fig. 1 – Chemical structures of the test compounds.

2.2. Cell cultures

BEAS-2B cells, a SV40 hybrid (Ad12SV40) transformed human bronchial epithelial cell line, were from European Collection of Cell Cultures (ECACC, Salisbury, UK). Cells were maintained in LHC-9 medium in collagen-coated (PureColTM, Inamed Biomaterials, Fremont, CA, USA) flasks in a humidified atmosphere at 37 °C with 5% CO_2, and passaged twice per week. Prior to exposure, cells were plated in 12-well culture dishes, grown to near confluence in serum-free LHC-9 medium and exposed as described elsewhere. DMSO-concentrations in all samples were below 0.5%.

2.3. Chemokine release

CXCL8- and CCL5-protein levels in cell-supernatants were determined by ELISA (Biosource International, Camarillo, CA, USA) as described elsewhere (Øvrevik et al., 2010). Absorbance was measured using a plate reader (TECAN Sunrise, Phoenix Research Products, Hayward, CA, USA) complete with software (Magellan V 1.10).

2.4. Statistical analysis

Statistical significance was evaluated by GraphPad Prism software (GraphPad Software Inc., San Diego, CA, USA), using repeated measures-ANOVA with Tukey's post-test for multiple comparisons. In experiments with large variations statistical analysis was performed on log-transformed data.

3. Results

To assess the role of TACE/TGF-α/EGFR-signaling in 1-NP- and 1-AP-induced chemokine-responses, BEAS-2B cells were pre-incubated with the TACE-inhibitor TAPI-1, a TGF-α-neutralizing antibody or the EGFR-inhibitor AG1478, prior to exposure with 1-NP or 1-AP. In line with previous observations (Øvrevik et al., 2010), 1-NP induced a strong increase in CXCL8 without affecting CCL5 levels, whereas 1-AP induced a moderate increase in both CXCL8 and CCL5 from BEAS-2B cells. Both TAPI-1 and anti-TGF-α caused around 50% reduction in 1-NP-induced CXCL8 release without affecting basal CXCL8 levels in the BEAS-2B cells (Fig. 2A and B). TACE-inhibition also attenuated 1-AP-induced CXCL8 release (Fig. 2C), but did not affect the CCL5 response (Fig. 2D). In comparison, inhibiting EGFR activity by AG1478 almost completely blocked CXCL8