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The tobacco smoke component acrolein induces glucocorticoid resistant gene expression via inhibition of histone deacetylase

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HIGHLIGHTS

- Acrolein-mediated pro-inflammatory gene expression is resistant to hydrocortisone.
- Acrolein treatment of macrophage-like cells decreases nuclear HDAC acitivity.
- Cysteine 274 on HDAC2 is a target for acrolein adduction.

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ABSTRACT

Chronic obstructive pulmonary disease (COPD) is the leading cause of cigarette smoke-related death worldwide. Acrolein, a crucial reactive electrophile found in cigarette smoke mimics many of the toxic effects of cigarette smoke-exposure in the lung. In macrophages, cigarette smoke is known to hinder histone deacetylases (HDACs), glucocorticoid-regulated enzymes that play an important role in the pathogenesis of glucocorticoid resistant inflammation, a common feature of COPD. Thus, we hypothesize that acrolein plays a role in COPD-associated glucocorticoid resistance. To examine the role of acrolein on glucocorticoid resistance, U937 monocytes, differentiated with PMA to macrophage-like cells were treated with acrolein for 0.5 h followed by stimulation with hydrocortisone for 8 h, or treated simultaneously with LPS and hydrocortisone for 8 h without acrolein. GSH and nuclear HDAC activity were measured, or gene expression was analyzed by qPCR. Acrolein-mediated TNFα gene expression was not suppressed by hydrocortisone whereas LPS-induced TNFα expression was suppressed. Acrolein also significantly inhibited nuclear HDAC activity in macrophage-like cells. Incubation of recombinant HDAC2 with acrolein led to the formation of an HDAC2-acrolein adduct identified by mass spectrometry. Therefore, these results suggest that acrolein-induced inflammatory gene expression is resistant to suppression by the endogenous glucocorticoid, hydrocortisone.

1. Introduction

Chronic obstructive pulmonary disease (COPD), characterized by increased mucus production, chronic inflammation, and destruction of the lung, continues to be one of the leading causes of cigarette smoke-related death worldwide (Adhikari et al., 2008). In a study by Corradi et al. (2004) exhaled breath condensates and sputum from both asthmatic and COPD patients were shown to contain higher levels reactive aldehydes, and lung tissue from COPD patients contain elevated levels of the reactive aldehyde 4-hydroxy-2-nonenal (4-HNE) as compared to patients without COPD (Rahman et al., 2002). Whether these reactive aldehydes

Abbreviations: COPD, chronic obstructive pulmonary disease; HDAC, histone deacetylase; PMA, phorbol 12-myristate 13-acetate; LPS, lipopolysaccharide; GSH, glutathione; qPCR, quantitative polymerase chain reaction; TNFα, tumor necrosis factor α; 4-HNE, 4-hydroxynonenal; IL-8, interleukin 8; Cys, cysteine; FBS, fetal bovine serum; RPMI, Roswell Park Memorial Institute media; HBSS, Hanks balanced salt solution; SSA, sulfosalicylic acid; DTNB, Ellman's reagent; MALDI-TOF, Matrix-assisted laser desorption/ionization-time of flight.

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have a causal role in COPD development is rather poorly understood.

Cigarette smoke contains many volatile compounds which include the reactive aldehydes, crotonaldehyde and acrolein (Counts et al., 2004). Cigarette smoke contains approximately 45 μg of acrolein per cigarette (Reznick et al., 1992) and can be found as high as 90 ppm in mainstream cigarette smoke (Costa, 2001). Acrolein is an α,β-unsaturated aldehyde. Acrolein is categorized as a soft electrophile due to its electrochemical polarity. The oxygen of the aldehyde moiety is electron dense thus making the α-β-unsaturated bond electron poor at the β-carbon (LoPachin et al., 2009) (Fig. 1). As a highly reactive electrophile acrolein has high affinity for adduction with cysteine (Cys) amino acid residues (LoPachin et al., 2009; Esterbauer et al., 1991) and upon adduction the biological activity of proteins is often drastically altered (Randall et al., 2013a). Similarly to cigarette smoke, acrolein has been shown to produce several characteristics of COPD including mucus hypersecretion (Borchers et al., 1998), and secretion of pro-inflammatory cytokines such as interleukin 8 (IL-8) and tumor necrosis factor α (TNFα) (Facchinetti et al., 2007).

Glucocorticoids, a class of steroid hormones, are frequently utilized for the treatment of chronic inflammation associated with COPD. Although glucocorticoids are the main therapeutic used for COPD, many patients are resistant to the effects of or completely unaffected by inhaled synthetic glucocorticoids (Barnes, 2000). Furthermore, innate suppression of inflammation requires the endogenously produced glucocorticoid hydrocortisone (Oakley and Cidlowski, 2013). Both the endogenous glucocorticoid hydrocortisone, and the synthetic glucocorticoids that are administered to patients with chronic inflammatory diseases including COPD, function via histone deacetylase 2 (HDAC2)-regulated epigenetic modifications to suppress pro-inflammatory gene transcription (Adcock et al., 2004). HDACs along with histone acetyltransferases are the major regulators of histone deacetylation and acetylation respectively and thereby directly regulate gene transcription (de Ruijter et al., 2003). Moreover, it is known that HDAC2 has a significant role in COPD pathophysiology (Barnes, 2009). HDAC enzymes have been demonstrated as being sensitive to oxidative modification (Yao and Rahman, 2012). Likewise, *S*-nitrosylation of HDAC on Cys^{274} facilitated the release of HDAC from chromatin (Nott et al., 2008) and alkylation of HDACs by 4-HNE and prostaglandin 15d-PGJ_2 inhibited deacetylase activity (Doyle and Fitzpatrick, 2010).

Asthmatics who smoke cigarettes were observed to have an impaired glucocorticoid response (Chalmers et al., 2002). Although cigarette smoke is known to decrease protein levels and activity of HDACs within macrophages (Adenuga et al., 2009; Winkler et al., 2012), the possibility that acrolein can alkylate HDAC and thereby suppress glucocorticoid responses, has not been addressed to date and may be crucial for the cigarette-smoke mediated pathogenesis of COPD. Therefore, the current study was designed to further evaluate the causal role of acrolein in glucocorticoid resistance and COPD development. Our long-term goal is to develop a more efficacious therapeutic for COPD patients.

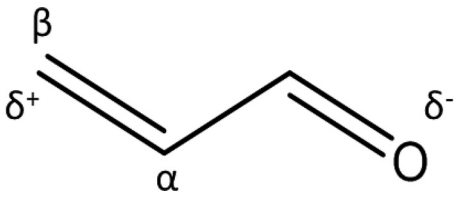

Fig. 1. Acrolein skeletal structure, and electrochemical polarity. Acrolein is an α,β-unsaturated aldehyde which has a partial negative charge on the oxygen of the aldehyde moiety and a partial positive charge on the β-carbon.

2. Methods and materials

2.1. Cell culture and treatments

U-937 monocytes (CRL-1593.2™; ATCC®) were cultured at 37 °C in 95% humidified air containing 5% CO_2 using RPMI medium supplemented with 10% fetal bovine serum (FBS) and 5% penicillin/streptomycin. For experimentation, cells were differentiated to macrophage-like cells by stimulation with 50 ng/ml phorbol 12-myristate 13-acetate (PMA) for 4 h at a density of 5×10^5 cells/ml according to previous literature (Ruijters et al., 2014). Cells were seeded in 24-well plates at 1×10^6 cells/well and allowed to differentiate for 48 h. Prior to treatment, cells were serum-starved overnight in RPMI media. Acrolein was administered in 1 ml Hank's Balanced Salt Solution (HBSS) (to avoid unwanted reactions of acrolein with other constituents present in the culture media), and cells were collected after 0.5 h for GSH and deacetylase activity measurements. When indicated, cells were treated with 1 ng/ml lipopolysaccharide (LPS) or 50 nM hydrocortisone for 8 h (Fig. S1).

Supplementary material related to this article found, in the online version, at http://dx.doi.org/10.1016/j.toxlet.2015.10.009.

2.2. mRNA gene expression

Eight hour after LPS/hydrocortisone treatment, cells were lysed using 200 μl *QIAzol lysis Reagent* (Qiagen, Venlo, NL). RNA was isolated with chloroform by phase separation and precipitated with isopropanol. RNA was re-suspended in RNase/DNase free water and incubated at 60 °C for 10 min. Synthesis of cDNA was performed with 100–500 ng RNA using *iScript cDNA Synthesis Kit* (Bio-Rad, Hercules, CA, USA) according to manufacturer's protocol. Reactions for qPCR were made with primers for IL-8 and TNFα (Eurofins MWG Operon, Ebersberg, Germany) with SYBR green mix (Bio-Rad). PCR was performed using a (iCycler system, BioRad) with 25 μl total volume. Data are expressed as relative quantitative value as normalized to β-actin gene expression.

2.3. Analysis of cellular GSH levels

Cells were lysed 0.5 h post acrolein treatment with 200 μl 100 mM potassium phosphate buffer, pH 7.5, containing 10 mM EDTA and 0.1% Triton X-100. Protein was quantified via the bicinchoninic acid assay (BCA protein assay kit, Pierce, Rockford, IL, USA). Sulfosalicylic acid (SSA) was added to a concentration of 0.6% and samples were diluted 5×. GSH analysis was determined by 5,5′-dithiobis-(2-nitrobenzoic acid) (DTNB) reduction as described previously (Rahman et al., 2006). Briefly, cell lysates, with and without a 1 h pre-incubation with vinyl pyridine, were incubated with glutathione reductase and DTNB for 30 s after which NADPH was added and immediately, absorbance was measured kinetically over 3 min at 412 nm. The absorbance values measured from the lysates without vinyl pyridine were subtracted from their counterparts with vinyl pyridine to obtain the total amount of free GSH. Glutathione concentrations were calculated based on a standard curve and results are expressed as a percent of control values.

2.4. Nuclear deacetylase activity

Nuclear extracts were made 0.5 h after acrolein treatment. Cells were washed with PBS and incubated on ice for 15 min in hypotonic buffer. Cells were scraped into Eppendorf tubes, and nuclei were isolated and lysed according to Life Technologies™ Protocols. HDAC activity in nuclear extracts was measured by Fluor de Lys® Fluorescent Assay System according to manufacturer's protocol (HDAC Fluorimetric Cellular Activity Assay, ENZO Life Sciences, Farmingdale, NY, USA).