REVIEW

Acrolein – a pulmonary hazard

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Acrolein is a respiratory irritant that can be generated during cooking and is in environmental tobacco smoke. More plentiful in cigarette smoke than polycyclic aromatic hydrocarbons (PAH), acrolein can adduct tumor suppressor p53 (TP53) DNA and may contribute to TP53-mutations in lung cancer. Acrolein is also generated endogenously at sites of injury, and excessive breath levels (sufficient to activate metalloproteinases and increase mucin transcripts) have been detected in asthma and chronic obstructive pulmonary disease (COPD). Because of its reactivity with respiratory-lining fluid or cellular macromolecules, acrolein alters gene regulation, inflammation, mucociliary transport, and alveolar–capillary barrier integrity. In laboratory animals, acute exposures have lead to acute lung injury and pulmonary edema similar to that produced by smoke inhalation whereas lower concentrations have produced bronchial hyperreactivity, excessive mucus production, and alveolar enlargement. Susceptibility to acrolein exposure is associated with differential regulation of cell surface receptor, transcription factor, and ubiquitin-proteasome genes. Consequent to its pathophysiological impact, acrolein contributes to the morbidly and mortality associated with acute lung injury and COPD, and possibly asthma and lung cancer.

Keywords:
ARDS / Asthma / Carbonyl stress / Chronic obstructive pulmonary disease / Countermeasures

1 Introduction

Acrolein (2-propenal) is an α-β-unsaturated aldehyde that is volatile at room temperature and is highly irritating to eyes and respiratory passages. Acrolein can be formed by heating cooking oils and fats above 300°C (e.g. wok cooking [1]), and thus its name refers to pungent ''acrid'' (from Latin stem: acer meaning sharp or sour) smell that is produced from ''oleum'' (Latin meaning ''oil'') [2]. Acrolein also can be formed in domestic cooking with biomass fuels [3], and is present in environmental tobacco smoke [2, 4–6], which remains a significant occupational health hazard in the restaurant workplace [7–10]. Additional human exposure can result from acrolein use as a herbicide and through its use as a chemical feedstock.

As an α,β-unsaturated aldehyde, acrolein contains a reactive carbonyl group and an electrophilic α-carbon and thus is highly reactive with biological macromolecules [11–14]. Irritant stimuli, of which acrolein is one of the most potent, activate respiratory sensory nerve endings, including Ca^{2+}-permeable transient receptor potential cation channel, subfamily A, member 1 (TRPA1) [15]. In addition to nasal irritation, acrolein-activated TRPA1 signaling can lead to cough and inflammation [16, 17]. Although irritant-induced sensory responses can serve as warning signs and promote avoidance behavior or adaptation of protective measures, acrolein exposure remains a global health problem because its release is closely linked to basic human needs (e.g. cooking), emergencies (e.g. fire fighting), or personal habits (e.g. smoking). While acrolein can cause adverse effects in several organ systems, this review focuses on inhaled

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Abbreviations: **BAL,** bronchoalveolar lavage; **COPD,** chronic obstructive pulmonary disease; **CLDN5,** claudin 5; **EGFR,** epidermal growth factor receptor; **GSH,** reduced glutathione; **MMP,** metalloproteinase

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acrolein and its effects in the pathobiology of human pulmonary diseases.

2 Exposure

2.1 Inhalation exposure and endogenous production in the respiratory tract

Acrolein is listed as a hazardous air pollutant (or air toxic) by the US EPA and is often encountered in the ambient atmosphere [18–20]. Acrolein in the atmosphere is relatively unstable and degrades rapidly with a half-life of 0.5–1.2 d. It is removed by reaction with hydroxyl radicals, photolysis, or wet deposition [21]. Nonetheless, acrolein, along with other aldehydes including acetaldehyde, crotonaldehyde, and formaldehyde, is a common ambient air pollutant because it is continuously introduced into the environment from multiple sources. These aldehydes are nasal and tracheobronchial airway irritants, but none more puissant than acrolein [17, 22–24]. Acrolein is emitted from cooking, combustion of wood, grassland, bush, forest, and buildings or from pyrolysis products of biodiesel, petroleum fuel, plastics and cigarette smoke (Supporting Information Table S1). It is also released from facilities manufacturing or using acrolein-containing products (including polyester resin, polyurethane, propylene glycol, acrylic acid, acrylonitrile, and glycerol), from irrigation canals, ditches, and water recirculating systems treated with acrolein as an herbicide and molluscicide due to volatilization, and from fixatives used in electron microscopy [2, 19, 25–30].

Acrolein can constitute up to 8% of total aldehydes generated from vehicles, which can alter with fuel blend and engine type [31–33]. Exhaust gas emission from gasoline and diesel-powered vehicles can contribute to increased acrolein concentration in ambient air [34]. Interestingly, recent diurnal and seasonal analyses of acrolein concentrations in several busy roadways, suggested that vehicles are not the major source of ambient acrolein through primary emissions or secondary oxidation products. Rather, wintertime acrolein concentrations correlated well with 2-furaldehyde, a tracer of biomass burning [3].

The chronic inhalation reference concentration (RfC) for acrolein is 2×10^{-5} mg/m^3 (based on nasal lesions in rats). The RfC is an estimate concentration of lifetime inhalation exposure likely to be without an appreciable risk of deleterious effects in humans (including susceptible subpopulations). Ambient acrolein concentrations ($0.0087–0.41 \times 10^{-3}$ mg/m^3) commonly exceed the RfC throughout the United States. These exposures have been estimated to be associated with additional 2.5 cases per 1000 people (median) adverse pulmonary effects [35, 36].

Indoor acrolein concentrations in homes and commercial establishments are often higher than outdoor levels due to food cooking, wood burning, and smoking (Supporting Information Table S1). For example, the 95 percentile indoor acrolein concentration (64.5×10^{-3} mg/m^3) is ~150 times higher than the national ambient concentration (41×10^{-5} mg/m^3) or ~3000 times the ambient RfC (2×10^{-5} mg/m^3) [36, 37]. Indoor concentration can change about twofold from morning to evening with changes in temperature and cooking activities with indoor acrolein concentrations of $24–64 \times 10^{-3}$ mg/m^3 in homes with vegetable oil cooking compared with 1.8×10^{-3} mg/m^3 in homes with no oil cooking [37, 38]. Indoor concentrations can increase in homes inhabited by at least one smoker [27]. In taverns permitting smoking, indoor acrolein concentration (24×10^{-3} mg/m^3) is equal to 1200 times the ambient RfC [39, 40]. Acrolein levels from 10 cigarettes in a 30 m^3 room can be much higher and have reached 0.23 mg/m^3 [6], which is the Occupational Safety and Health Administration Permissible Exposure Limit for occupational exposure [41].

Acrolein in environmental tobacco smoke also is a major source of human exposure, affecting smokers and more than 30 million nonsmokers in the United States [2, 4–6]. Acrolein concentrations in sidestream smoke are elevated compared with mainstream smoke, because concentrations are increased in sidestream smoke due to the altered combustion chemistry at lower temperatures [2, 19, 42]. For example, acrolein in sidestream smoke is about 17 times higher than in mainstream smoke (i.e. sidestream = 25.2 mg and main-stream = 1.5 mg acrolein per pack of cigarettes) [26]. The amount of acrolein emitted from cigarette smoke depends upon the type of cigarette, smoking conditions, puff volume, puff rate, and other factors [29].

Acrolein is formed endogenously during inflammation – a common characteristic of several respiratory diseases including chronic obstructive pulmonary disease (COPD) and asthma. It is formed from threonine by myeloperoxidase activation [43–46], spermine, or spermidine by amine oxidase-mediated catabolism [47–51], or possibly membrane fatty acids by oxidative degradation [2, 52–54]. Acrolein concentrations in the expired breath condensate and induced sputum [55, 56] were higher in ex-smoking subjects with COPD or subjects with asthma compared with healthy nonsmoking control subjects (Fig. 1). Endogenous acrolein concentrations in COPD or asthma patients exceeded 100–150 nM in induced sputum as compared with ~1.0 nM in control subjects. In contrast, concentrations of a lipid oxidation product, 4-hydroxynonenal, were not increased in COPD or asthma as compared with control and all concentrations measured were approximately 1 nM or less.

2.2 Nasal deposition and penetration to the lower respiratory tract

The extent of penetration and regional deposition of an inhaled gas is controlled by the physical and chemical properties of the compound, physiological and pathological features of the respiratory tract, and other contributing

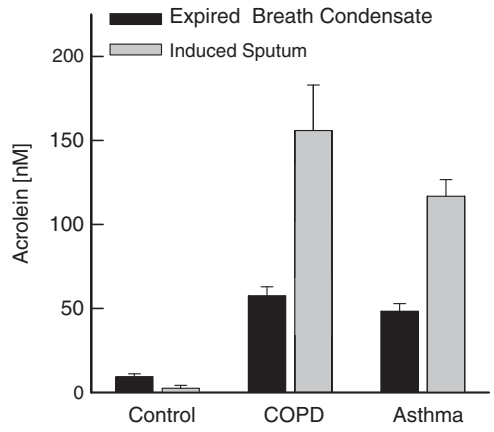

Figure 1. Acrolein concentrations are increased in expired breath condensate and induced sputum from subjects with COPD or asthma as compared with control subjects. Acrolein levels were determined by liquid chromatography/atmospheric chemical ionization tandem mass spectrometry (LC/APCI-MS/MS). (Adapted from [56]).

factors. Acrolein is highly water soluble compared with other common air pollutants (e.g. 210 g acrolein/L versus 0.02 g ozone/L) and water-soluble compounds deposit mainly in aqueous lining fluid of the upper respiratory tract. Acrolein is highly reactive with biological macromolecules that also contribute to nasal retention. In dogs, nasal retention of acrolein is ~80%, and thus ~20% of acrolein can penetrate the nasal passages and be deposited in the lower respiratory tract [11–14].

As determined for acetaldehyde [57], other factors that influence nasal penetration include concentration, altered clearance mechanisms (epithelial metabolism, mucociliary clearance, and regional air and blood flow), vascular congestion, respiratory flow rate, duration of exposure, and airway caliber. The partitioning of inhaled aerosols between the upper respiratory (nose and mouth) and lower respiratory tract (tracheobronchial airways and alveoli) is influenced by the breathing mode, oral, nasal, or oronasal route of inhalation [58]. When acrolein is inhaled by oral breathing (e.g. inhaled in cigarette smoke) lower respiratory tract retention is near complete (i.e. $\geq$98%) [59].

3 Response

3.1 Irritant responses

The nasal tissues appear highly sensitive to acrolein exposure inasmuch as short-term inhalation exposure of rats to $\geq 1.5\,mg/m^3$ acrolein injures respiratory and olfactory epithelium [60–62]. Activation of the sensory nerves in the nasal mucosa of mice, rats, and guinea pigs exposed to $0.7–3.9\,mg/m^3$ acrolein induced vasodilation, increased airflow resistance, and decreased respiratory rate [22, 63–70]. In short term exposures, Swiss–Webster and C57BL/6xC3H/HeJ F1 mice exposed to 3.2 or $2.4\,mg/m^3$, respectively, for 10 min had decreased respiratory rate by 50% [70]. Within 10 min, human volunteers exposed to 0.35 or $0.69\,mg/m^3$ acrolein reported nasal irritation and had decreased respiratory rate, respectively [71]. Decreased respiratory rate may indicate reflex response that could possibly protect the respiratory tract [64].

3.2 Acute lung injury and alteration of host defenses

Because acrolein and other chemicals contained in smoke can cause immediate or delayed acute lung injury or other pulmonary complications [72–74], fire-related injuries involve more than skin burns or asphyxia due to carbon monoxide inhalation. Acrolein inhalation exposure of firefighters can vary greatly but have averaged $0.29–0.58\,mg/m^3$ [75, 76]. Whether or not there are cutaneous burns, smoke inhalation and respiratory injury must be examined in any fire victim [72]. Symptoms resulting from smoke inhalation may include nose, throat and lung irritation, and injury signs include pulmonary edema, lung hemorrhage, and death. Up to 75% of deaths following burn injury may be accounted for by smoke inhalation injury [77]. While every fire and burning condition may present unique chemistry, acrolein appears to be a common toxic component of smoke from most fires [76, 78]. Pulmonary complications following smoke inhalation can lead to increased susceptibility to infection and pneumonia and together with acute lung injury significantly contribute to the morbidity and mortality of fire-related injuries [79].

Death due to smoke inhalation from an overheated fryer has been reported [80]. Histopathology indicated massive cellular desquamation of the bronchial lining. Although the evidence for the diagnosis is not clear, the cause of death was attributed to acrolein inhalation. Studies with laboratory animals indicate that pulmonary edema from smoke inhalation can be produced by acrolein alone in the concentrations found in smoke [81, 82] and that acrolein inhalation can be lethal (Supporting Information Table S2).

A susceptible rat strain succumbs to a high concentration of acrolein exposure for a short duration ($300\,mg/m^3 \times 30$ min) or to a lower concentration for a long duration ($9.2\,mg/m^3 \times 6\,h/d \times 62\,d$) (Supporting Information Table S2) [83–85]. At lower concentrations, acrolein still produced histological features partially consistent with acute lung injury. Studies have been performed with Sprague–Dawley rats, Princeton or Hartley guinea pigs, beagle dogs, and squirrel monkeys continuously exposed to $2.3\,mg/m^3 \times 24\,h/d \times 90\,d$. The lungs were marked by varying degrees of pulmonary inflammation and pulmonary hemorrhage [86].

Breaching of endothelial barriers is a key event in the development of pulmonary edema during acute lung injury. An integral membrane protein, Claudin 5 (CLDN5), is a critical component of endothelial tight junctions that control

pericellular permeability. In mice, acrolein (23 mg/m^3) induced acute lung injury, which was accompanied by alterations in CLDN5 expression. Signs of pulmonary edema, perivascular enlargement, and alveolar membrane thickening were observed earlier in the sensitive (BALB/cByJ) (evident within 10 h) than in the resistant (129X1/SvJ) (not evident at 17 h) mice. Lung CLDN5 increased more in the resistant strain than in the susceptible strain [94]. In human endothelial cells, lower concentration (30 nM) acrolein increased CLDN5 transcripts and increased in phosphorylated forkhead box O1 (p-FOXO1) protein levels. A phosphatidylinositol 3-kinase inhibitor (LY294002) diminished the acrolein-induced increased CLDN5 transcript. Higher acrolein concentrations (300 nM) decreased CLDN5 transcripts, which were accompanied by increased FOXO1 and catenin (cadherin-associated protein), β 1 (CTNNB1). The phosphorylation status of these transcription factors was consistent with the observed CLDN5 alteration. The preservation of endothelial CLDN5 may be a clinical target for smoke inhalation therapy.

In rodents, acrolein exposure (1.3-4.6 mg/m^3) suppresses intrapulmonary killing of bacteria [87–93]. In Swiss mice, co-exposures to acrolein (5.8 mg/m^3) and carbon black particles (10 mg/m^3) (4 h/d × 4 d) also increased susceptibility to infectious agents and impaired the multiple components of the host defense system [92]. Co-exposures decreased intrapulmonary killing of *Staphylococcus aureus*, indicating suppression of alveolar macrophage surveillance. In contrast, coexposure increased intrapulmonary killing of *Proteus mirabilis*, indicating enhanced killing by accessory phagocytic polymorphonuclear neutrophils, which were also found in bronchoalveolar lavage after exposure. Combined exposure to carbon black and acrolein also resulted in impaired elimination of *Listeria monocytogenes*, indicating suppression of lymphokine-mediated cellular immunity and elimination of influenza A virus, indicating suppression of cytotoxic T-cell-mediated effector mechanisms. Neither exposure to carbon black alone nor exposure to acrolein alone had any effect in these assays. The mechanism for the enhanced biologic effect could have been due to the carbon black particle providing carrier mechanism for greater acrolein penetration into the distal lung.

A major challenge to critical care following smoke exposure is to reliably predict and enhance survival in acute lung injury. Individual susceptibility varies greatly, i.e. patients presenting with the same severity score can have markedly different clinical outcomes. For this reason, studies have begun to investigate the role of genetics in determining survival during acute lung injury. However, because acute lung injury is a sporadic disease produced by heterogeneous precipitating factors, previous genetic analyses are mainly limited to case-control studies that evaluate candidate genes associations. Using a strategy in which single nucleotide polymorphism associations (SNPs) were assessed for functional consequences to survival during acute lung injury in mice, 40 inbred mouse strains were exposed to acrolein and haplotype association mapping and microarray analysis performed [94, 95]. Transcripts for Acvr1, Arhgap15, Cacybp, Rfwd2, and Tgfbr3 differed between the strains with exposure and the genes contained SNPs that could eliminate putative transcriptional factor recognition sites within the regulatory regions or alter the protein-coding sequence. Ccdc148, Fancl, and Tnn had sequence differences that could produce an amino acid substitution. Mycn and Mgat4a had a promoter SNP or 3′UTR SNPs, respectively. An Acvr1 SNP rs6406107 eliminates a putative ELK1 transcription factor binding site and diminished DNA-protein binding. Several genes were related and encoded cell surface receptors (ACVR1, TGFBR3), transcription factors (MYCN, possibly CCDC148), and ubiquitin-proteasome (RFWD2, FANCL, CACYBP) proteins that can modulate transforming growth factor β (TGFB)/ bone morphogenetic protein (BMP) cell signaling (Fig. 2).

Microarray analysis also indicated that lung transcripts in TGFB signaling (e.g. secreted phosphoprotein 1 (SPP1)) increased more in sensitive SM/J as compared to resistant 129X1/SvJ mice (Fig. 3) [94, 95]. Cell death was another pathway enriched with increased transcripts that increased more in sensitive as compared to resistant mice. In contrast, the response of sensitive and resistant strains were similar in the nuclear factor (erythroid-derived 2)-like 2 (NFE2L2 aka NRF2) mediated oxidative stress response, which may be viewed as protective during acute lung injury (31). Decreased transcripts were enriched in pathways of glucocorticoid receptor signaling, lipid metabolism, and retinoic acid receptor (RAR) activation. In general, pathways with decreased transcripts were similar between these strains, whereas individual transcripts among these pathways were decreased more in the sensitive than in resistant 129X1/SvJ mice. Additional pathways that were enriched in sensitive SM/J but were not in 129X1/SvJ mice included (1) increased transcripts: interleukin 10 (IL-10) signaling, Mammalian Target of Rapamycin (mTOR) signaling, and immune cell trafficking, and (2) decreased transcripts: lysine biosynthesis. Pathways that were enriched in 129X1/SvJ but not in SM/J mice included (1) increased transcripts: phospholipid degradation, sphingosine-1-phosphate signaling, protein ubiquitination pathway, cell movement, and anti-apoptosis, and (2) decreased transcripts: ephrin receptor signaling.

3.3 Bronchial hyperreactivity and asthma

Asthma is marked by sporadic airway smooth muscle contraction that induces bronchoconstriction and airflow obstruction. Asthma pathogenesis involves an underlying airway inflammation with altered lymphocyte subtypes and appearance of eosinophils which may or may not be a result of an antigenic stimulus. A hallmark feature of asthma is bronchial hyperreactivity in which low doses of inhaled cholinergic agonists, histamine, antigen, or irritant stimuli can induce bronchoconstriction. Typically, the inhaled dose

necessary for bronchoconstriction is lower in persons with asthma as compared to persons without asthma and can be documented by broncho-provocation challenge [96, 97].

Acrolein has not been reported to produce antigenic-type bronchial hyperreactivity. However, as an irritant it can augment bronchial hyperreactivity in laboratory animals and human tissue in vitro. In guinea pigs, acrolein (>0.7 mg/m^3 × 2 h) increased pulmonary resistance immediately after acrolein exposure, which returned to control values within 30–60 min. Acrolein (2.0 mg/m^3 × 2 h) also increased bronchial hyperreactivity that was evident within 1 h, maximal at 2–4 h, and persisted up to 24 h after exposure [98]. This

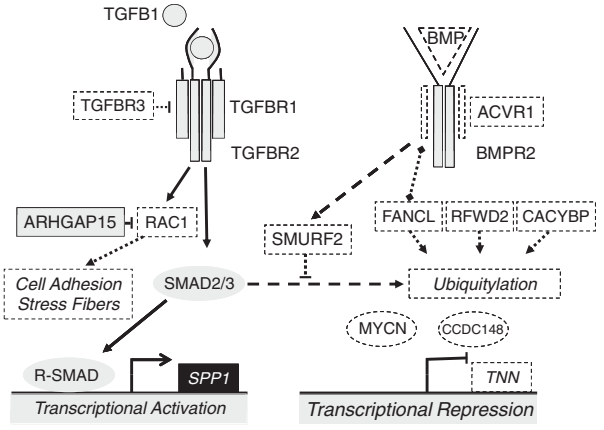

Figure 2. Genetic variants associated with sensitivity to acrolein-induced acute lung injury implicate a transforming growth factor, β (TGFB)/bone morphogenetic protein (BMP) interactome. Haplotype mapping of acrolein-induced acute lung injury in 40 mouse strains identified several related candidate genes. Two of the candidates (TGFBR3 and ACVR1) are receptors for TGFB and BMP ligands. Because TGFBR3 inhibits the heterotetrameric TGFBR1/2 signaling and ACVR1 partners with BMPR2 to interact with FANCL or SMURF proteins that increase SMAD ubiquitylation, TGFB-mediated transcriptional activation or repression is stimulated by decreased TGFBR3 and ACVR1. Additional proteins with ubiquitin-protein ligase activity that include RFWD2 and CACYBP are also decreased by acrolein. Activated TGFBR1/2 also interacts with RAC1, which can be inhibited by acrolein-induced increased ARHGAP15, which in turn, inhibits cell adhesion and stress fiber formation. Gray boxes and solid line = increased members of a pathway, dashed boxes and dashed line: decreased members of a pathway, black box = transcript that increased as a consequence of TGFB signaling. ACVR1: activin A receptor, type 1, ARHGAP15: Rho GTPase activating protein 15, BMP: bone morphogenetic protein (in this case BMP4 and BMP7), BMPR2: bone morphogenetic protein receptor, type II, CACYBP: calcyclin binding protein, CCDC148: coiled-coil domain containing 148, FANCL: Fanconi anemia, complementation group L, MYCN: v-myc myelocytomatosis viral related oncogene, neuroblastoma derived (avian), RAC1: RAS-related C3 botulinum substrate 1, RFWD2: Ring finger and WD repeat domain 2, R-SMAD: regulator SMADs, SMURF2: SMAD specific E3 ubiquitin protein ligases 2, SPP1: secreted phosphoprotein 1, TGFB1: transforming growth factor, beta 1, TGFBR1/2/3: transforming growth factor, beta receptor I/II/III, TNN: tenascin N. (Adapted from [95]).

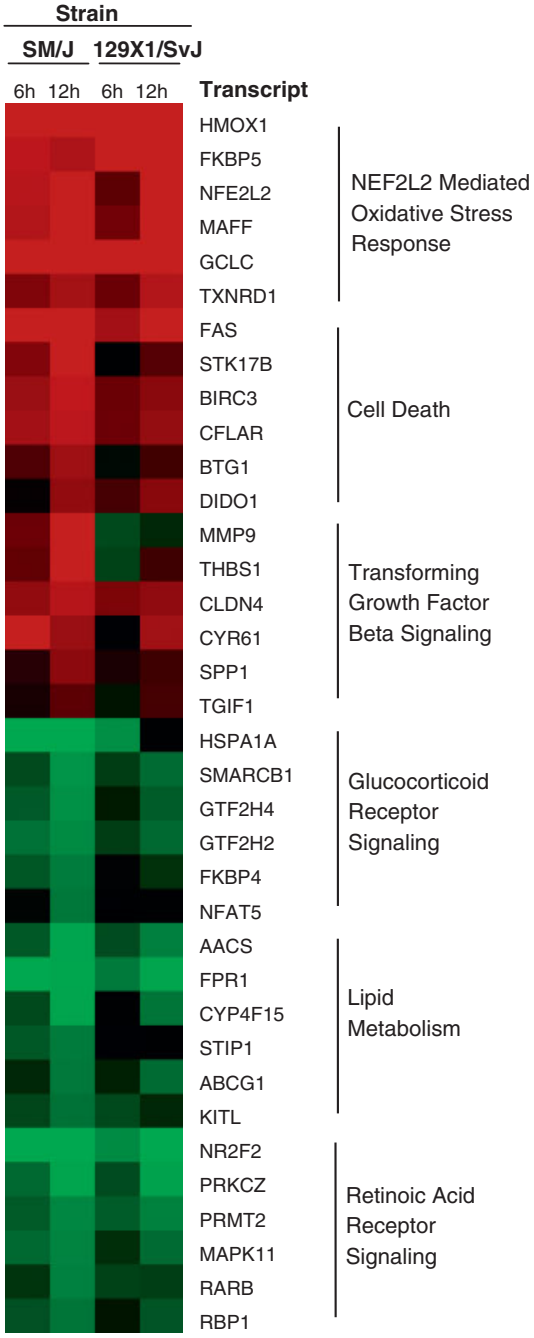

Figure 3. Pathways enriched in transcripts in sensitive (SM/J) and resistant (129X1/SvJ) mouse lung during acrolein exposure (23 mg/m^3) for 0 (control), 6, or 12 h. The top three enriched pathways/lists for ''Canonical Pathway'', ''Biological Function'', or ''Toxicology List'' categories were selected based on the combined 6 and 12 h -log (P). Increased transcripts were enriched in (A) nuclear factor, erythroid derived 2, like 2 (NFE2L2) mediated oxidative stress, (B) cell death, and (C) transforming growth factor, β (TGFB) signaling, Decreased transcripts were enriched in (A) glucocorticoid receptor signaling, (B) lipid metabolism, and (C) retinoic acid receptor signaling (Adapted from [95]).

exposure was accompanied by increases in bronchoconstrictive lipid mediators (thromboxane B2, prostaglandin F2 α, and leukotrienes) immediately after exposure, and delayed influx of neutrophils (24 h) [98, 99]. These temporal relationships suggest that neutrophil infiltration may be a sufficient but not a necessary condition for the onset of bronchial hyperreactivity and that injury to cells normally present in the lung, e.g. airway epithelial cells [100], are responsible for the mediators thought to influence bronchial responsiveness.

Interaction between acrolein exposure and passive sensitization can alter contractile responses in isolated human airways [101]. In tissues sensitized by incubation in sera from asthmatic patients, pre-exposure to 0.3 μM acrolein (10 min) increased the maximal contractile response to the antigen *Dermatophagoides pteronyssinus* (house dust mite). Acrolein (0.3 μM × 10 min) also increased the contractile response of bronchial rings to carbachol (a chlolinergic agonist) or histamine following passive sensitization. Thus acrolein exposure and passive sensitization interact (possibly in synergy) on human bronchial smooth muscle reactivity in response to both specific antigen and nonspecific agonists.

3.4 Chronic obstructive pulmonary disease – bronchitis

Characterized by progressive airflow obstruction due to varying degrees of bronchitis and emphysema, COPD is a leading cause of morbidity and mortality in the United States [102, 103] and worldwide [104–106]. COPD pathogenesis results from gene–environmental interactions evoked by cigarette smoking and several nonsmoking risk factors including exposure to second-hand smoke, biomass fuel smoke, and air pollution [107–109].

Bronchitis in COPD involves airway inflammation with excessive mucus production from surface epithelial (goblet) cell and submucosal glands [110, 111]. The associated decreased mucociliary clearance and mucus retention may obstruct the airways and contribute to COPD exacerbations and possibly mortality [112, 113]. Mucus consists mainly of water (95%) combined with salts, lipids, proteins, and mucin glycoprotein [114]. Mucin glycoproteins provide the gel-like viscoelastic properties of mucus. Of the 20 identified membrane-associated or secretory mucin gene products, 16 have been identified in the airways [115]. Airway mucin 5, subtypes A and C (MUC5AC) and MUC5B are the predominant mucin proteins expressed in the lung [116–120].

Acrolein can induce excessive mucus production either directly by acting on lung epithelial cells or indirectly via inflammation. In rats, acrolein exposure (6.9 mg/m^3 × 6 h/d × 5 d/wk × 2 wk) increased tracheal MUC5AC mRNA and protein within 2 days and increased lung MUC5AC mRNA and protein within 5–12 days. In FVB/NJ mice, acrolein exposure (4.6 mg/m^3 × 6 h/d × 5 d/wk × 4 wk) increased MUC5AC transcripts and protein and increased lung metalloproteinase 9 (MMP9) transcripts, protein, and activity (Fig. 4). In contrast, mMUC5AC transcript and mucin protein levels were reduced in MMP9 knockout mice (*Mmp9*$^{(-/-)}$, FVB.Cg-*Mmp9*^{tm1Tvu}/J), or with an epidermal growth factor receptor (EGFR) inhibitor (erlotinib) in mice [56], or (gefitinib) in rats [121]. Low levels of acrolein

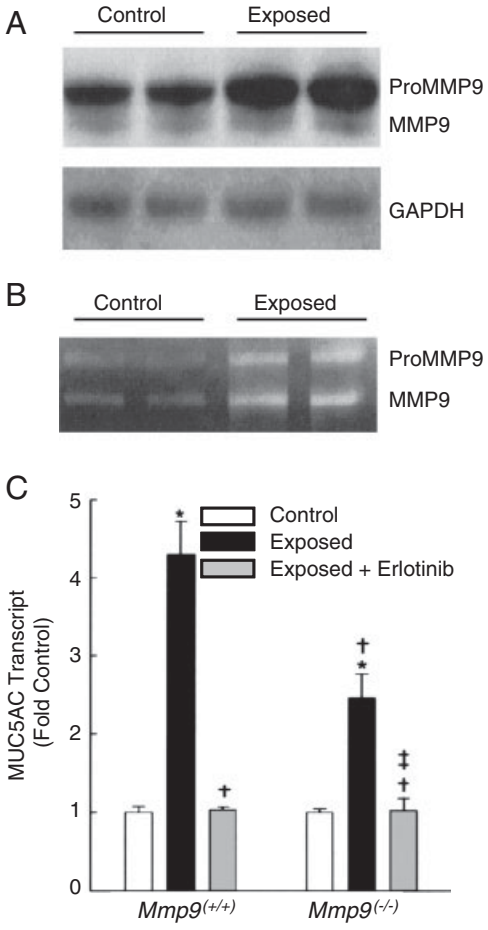

Figure 4. Acrolein activates MMP9 and contributes to persistent mucin production in mice. Acrolein (4.6 mg/m^3 × 6 h/d × 5 d/wk × 4 wk) increased mucin 5AC (MUC5AC) and MMP9 protein and activity in the mouse lung after acrolein exposure. Acrolein increased (A) MMP9 protein whereas GAPDH protein levels remained unchanged (loading control) as determined by Western blot and (B) gelatinase activity in mouse lung. Acrolein increased (C) MUC5AC transcripts in the lung of *Mmp9*$^{(+/+)}$ control mouse as compared to *Mmp9*$^{(-/-)}$ gene-targeted mouse. This effect was diminished in mice treated with an EGFR antagonist, erlotinib (100 mg/kg). Anti-MMP9 immunostaining also increased in the respiratory epithelium in acrolein exposed as compared with control FVB/NJ mice (filtered air). *Significantly different from the control, †significantly different from the acrolein-exposed *Mmp9*$^{(+/+)}$, or ‡significantly different from the acrolein-exposed *Mmp9*$^{(-/-)}$ as determined by ANOVA. (Adapted from [56]).

(100–300 nM) also increased MMP9 transcripts in human airway cells and pro-MMP9 cleavage and activity in a cell-free system [121]. This has clinical relevance because nearly equivalent acrolein concentrations were measured in induced sputum in persons with COPD (Fig. 1), supportive of endogenous acrolein generation, MMP activation and persistent mucin overproduction.

Rat strain differences in sensitivity to acrolein exposure have also been extended to gene regulation studies. Eight rat strains (ACI, Brown-Norway, Dahl salt-resistant (Rapp), Dahl salt-sensitive (Rapp), Fisher 344, Lewis, Sprague–Dawley (outbred), and Wistar–Kyoto) were exposed to acrolein for 2 wk. MUC5AC mRNA levels were induced in all but the Wistar–Kyoto and Dahl salt-resistant rats [114].

In a human lung epithelial cell line (NCI-H292), acrolein, inflammatory mediators (prostaglandin E2, 15-hydroxyeicosatetraenoic acid, or tumor necrosis factor (TNF)), or a protein kinase C agonist (phorbol 12-myristate 13-acetate) increased MUC5AC mRNA. The increased MUC5AC mRNA was due, in part, to transcript stabilization [114, 122, 123]. In differentiated human bronchial epithelial cell culture, acrolein, and inflammatory mediators (IL-6, IL-13, or IL-17) increased MUC5AC [124].

In airway epithelial cells, EGFR mediates MUC5AC expression either directly via stimulation by EGFR ligands (EGF or transforming growth factor, α (TGFA)), or indirectly via cytokines (TNF, IL-4, or IL-13), cigarette smoke extract, or neutrophil elastase. While more work is needed to fully delineate the mechanisms of action, matrix metalloproteinases (MMP9, MMP12, and MMP14) activation and EGFR signaling regulate acrolein-induced mucin production [56, 125–129]. Held inactive in a proprotein state, pro-MMP9 can be activated by MMP14 (MT1-MMP) [130]. Once activated, MMPs cleave and release EGFR ligand from the cell membrane and enable EGFR binding and thereby initiate EGFR activation. In cell culture, acrolein also increased MMP14 expression and activity [131] (Fig. 5). In cells, acrolein-induced MMP14 transcripts were inhibited by an EGFR neutralizing antibody, an EGFR kinase inhibitor, an MMP inhibitor, or a proprotein convertase inhibitor (furin), hexa-D-arginine inhibitor. MMP14 expression knock-down using siRNA reduced acrolein-induced MUC5AC transcripts. In cell-free system, acrolein directly activates MMP14. Acrolein was found to adduct to cysteine 319 in the MMP14 hemopexin-like domain. Mucin induction can be inhibited by EGFR kinase inhibitors (BIBX1522 and AG-1478) or an EGFR neutralizing antibody.

Acrolein-induced MUC5AC also can be inhibited by other cell signaling modulators including mitogen-activated protein kinase (MAPK) 3/2 (aka ERK1/2) [131], MAPK8 (aka JNK) [131], or MAPK14 (aka p38) inhibitors [132], statin (simvastatin) [133], or the peroxisome proliferator receptor-γ agonist (rosiglitazone) [134]. The findings that acrolein-induced MUC5AC production can be blocked by EGFR neutralizing antibody and a variety of chemical inhibitors suggest that treatment for mucus hypersecretion may be developed by targeting select EGFR signaling cascades or proteinases.

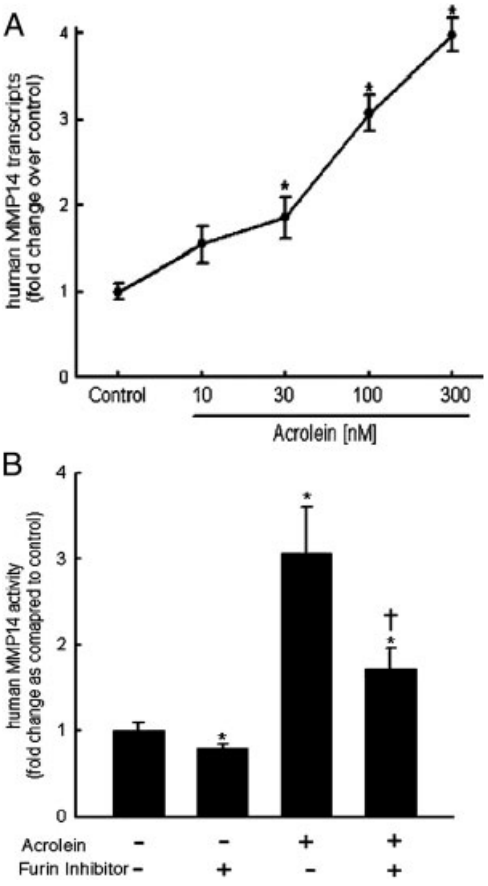

Figure 5. Acrolein increases MMP14 transcripts, protein, and activity. (A) MMP14 transcripts increased in human airway epithelial cells (NCI-H292) in a concentration-dependent manner after acrolein treatment. This response was similar in normal human bronchial epithelial cells (NHBE). (B) Acrolein (300 nM, 4 h, 37°C) increased MMP14 activity is inhibited by a proprotein convertase inhibitor (furin), hexa-D-arginine. (Adapted from [131]).

3.5 COPD – emphysema

Emphysema in COPD is characterized by alveolar enlargement, reduced lung elastic recoil (increased compliance), airflow obstruction in the distal airways, labored breathing, and progressive shortness of breath [135, 136]. Proteinase–anti-proteinase imbalance has been proposed to lead to the remodeling of the supportive connective tissue in the parenchyma and separate lesions coalesce to destroy large volumes of lung tissue [137]. This hypothesis was based on observations that linked severe emphysema to serpin peptidase inhibitors, clade A (α-1 antiproteinase, antitrypsin), member 1 (SERPINA1) deficiency [138] and the

ability to induce emphysema in rodents instilled with proteinase, e.g. papain [139]. SERPINA1 inhibits neutrophil elastase and neutrophil elastase-deficient mice are partially protected against emphysema [140]. Similarly, MMP12- (aka macrophage elastase) deficient mice do not develop cigarette smoke-induced increased alveolar enlargement [141]. In cigarette-exposed mice, exogenous SERPINA1 treatment protected against alveolar enlargement and increased serum TNF [142] possibly by suppression of TNF and MMP12 production by cigarette smoke-stimulated macrophages [143].

However, $<1\%$ of COPD patients have SERPINA1 deficiency, and many genetic variants of *SERPINA1* that are associated with lower-than-normal serum levels of this proteinase inhibitor have not been clearly associated with an increased COPD risk [144]. In addition to MMP12 and other MMPs [145–148], elastases, [149–152], proteinase 3 [153], and plasminogen activator, tissue [154] have been demonstrated to mediate alveolar enlargement in laboratory animals.

In addition to increased MMP9 and MMP14 transcript, protein, and activity, acrolein decreased the anti-proteinase, tissue inhibitor of metalloproteinase 3 (TIMP3) levels, in mouse lungs [56, 131]. Consistent with the proteinase–anti-proteinase hypothesis, this would tip the balance in favor of proteolytic activity contributing to tissue degradation [130]. It remains to be determined whether increased MMP14 expression and activity exacerbates or protects against emphysema. MMP14 is critical to lung development as evidenced by a defect in formation of alveolar septae in $MMP14^{-/-}$ mice [155, 156].

Histopathologic examination of tissues and bronchoalveolar lavage (BAL) from COPD patients contained increased macrophages, neutrophils, and CD(8^+) T cells [157–160]. Macrophages have been implicated in COPD pathogenesis as noted by: (i) from 5- to 10-fold increased macrophage numbers in BAL and at sites of tissue damage in persons with COPD, (ii) the long life-span of macrophages, and (iii) the ability of macrophages to produce high levels of cytokines and proteases that can mediate tissue injury and remodeling in COPD pathogenesis [161–163].

In vivo animal models illustrate the significance of macrophages in acrolein-induced pulmonary disorders that mimic COPD. In addition to protection from alveolar enlargement, MMP12-deficient mice are resistant to cigarette smoke-induced macrophage accumulation [141]. Similarly, acrolein exposure ($6.9\,mg/m^3 \times 6\,h/d,\ 5\,d/wk \times 3\,wk$) of FVB/NJ mice increased macrophages in BAL at day 5 and peaked at day 19 [164]. In contrast, neutrophils increased transiently on day 1 had decreased by day 5 (Fig. 6). Repeated acrolein exposure also increased BAL macrophages in C57BL/6J mice. In addition, acrolein-induced excessive macrophage accumulation (as with cigarette smoke) and MUC5AC mRNA was observed in the lung of $MMP12^{+/+}$ strain-matched control mice as compared with the lung of $MMP12^{-/-}$ gene-targeted mice [56].

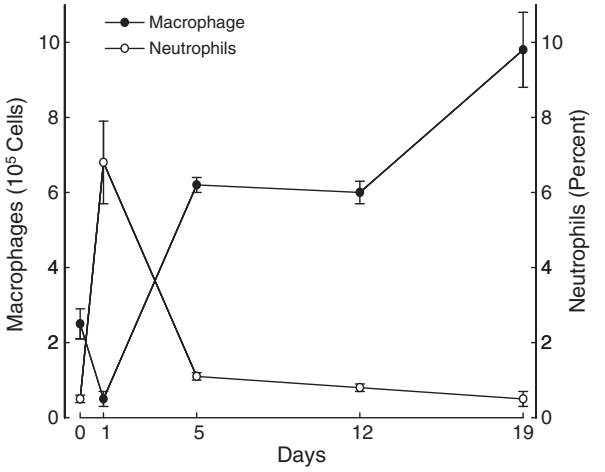

Figure 6. Acrolein ($6.9\,mg/m^3 \times 6\,h/d,\ 5\,d/wk$ for $3\,wk$) significantly increased macrophages in BAL from FVB/NJ mice at days 5, 12, and 19 ($p<0.05$). In contrast, neutrophils increased transiently on day 1 had decreased by day 5 (Adapted from [164]).

Differences in the prevalence of COPD in cigarette smokers, different ethnic groups, and α1-antitrypsin-deficient patients strongly suggest that genetic factors [165] and/or other environmental factors contribute to individual susceptibility to acrolein. This interpretation is supported by animal studies. Two Dahl rat strains that vary in their response to salt-induced hypertension, one being sensitive (DS) and the other resistant (DR), were tested for the pulmonary effects of acrolein exposure (filtered air, 0.9, 3.2, or $9.2\,mg/m^3$ acrolein $6\,h/d \times 5\,d/wk \times 62\,d$). Strain-associated acrolein sensitivity was evident because all of the DS rats exposed to $9.2\,mg/m^3$ acrolein died within the first 11 days, while 60% of the DR rats survived the duration of the study. Severe airway epithelial necrosis with edema and hemorrhage marked the lungs of DS rats whereas cell proliferation was the primary pathologic change in DR rats [84].

The effects of acrolein and cigarette smoke may be modulated by $CD8^+$ T cells, inasmuch as these cells are required for MMP12 induction. $CD8^+$ T cells secrete chemokine (C-X-C motif) ligand 10 (CXCL10 aka IFN-γ-inducible protein-10), which induces production of MMP12 leading to elastin degradation [166]. To examine the role of T lymphocytes on macrophage accumulation, repeated acrolein exposure ($4.6\,mg/m^3 \times 6\,h/d \times 5\,d/wk$ for up to $12\,wk$) was investigated in control (C57BL/6J) and CD8-deficient mice ($B6.129S2Cd8a^{tm1Mak}$). Acrolein exposure increased $CD8^+$ cells in the lungs of C57BL/6J mice and increased macrophages that remained elevated for the duration of the exposure. In contrast, increased macrophage accumulation did not occur in CD8-deficient mice exposed to acrolein suggesting that $CD8^+$ T cells actively contribute to macrophage accumulation in the lungs of acrolein-exposed mice (Fig. 7). Neutrophil accumulation did not differ between

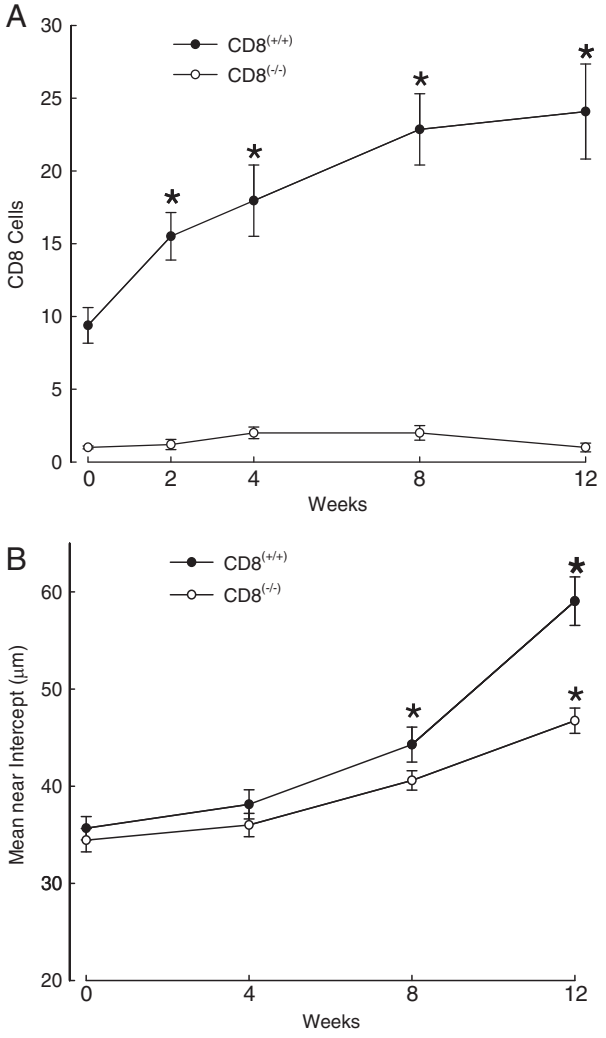

Figure 7. Acrolein increased $CD8^+$ T cells and airspace enlargement in C57BL/6J ($CD8^{(+/+)}$) mice is attenuated in CD8-deficient ($CD8^{(-/-)}$: B6.129S2Cd8a^{tm1Mak}) mice. (A) Lung $CD8^+$ cells were quantified as the number of positive cells per high-power field in mice exposed to acrolein (4.6 mg/m^3 × 6 h/d × 5 d/wk) for up to 12 wk. (B) Acrolein-induced airspace enlargement (measured as mean linear intercept) is attenuated in CD8-deficeint mice. *Significantly different from strain-matched control ($p<0.05$). (Adapted from [167]).

control and CD8-deficient mice exposed to acrolein for up to 12 wk [167].

In addition to macrophage accumulation, $CD8^+$ T cells have been associated with airspace enlargement and epithelial cell apoptosis [167]. $CD8^+$ T cells can produce several soluble mediators, including chemokines [168] and cytokines (e.g. interferon-γ (IFNG)) [169] that have been implicated in the recruitment and activation of macrophages. Inflammatory cytokines (CXCL10, IFNG, IL12, chemokine (C-C motif) ligand2 (CCL2 aka MCP-1) and CCL5 (aka RANTES)), MMP2 and MMP9 activity, and caspase 3 in pulmonary epithelial cells were attenuated in acrolein-exposed CD8-deficient mice compared with the control mice [167].

To further investigate T-cell subpopulations, mice genetically deficient in either αβ T cells (B6.129P2-Tcrb^{tm1Mom}) or γδ T cells (B6.129P2-Tcrd^{tm1Mom}) were used [170, 171]. Acrolein (4.6 mg/m^3 × 6 h/d × 5 d/wk for up to 12 wk) resulted in a persistent increase in macrophage accumulation in the lungs of control and γδ-T-cell-deficient mice whereas αβ T–cell-deficient mice lack macrophage accumulation following any of the exposure periods. The γδ T-cell-deficient mice had increased epithelial cell sloughing compared with the control indicating that γδ-T cells are critical in protecting the pulmonary epithelium against acrolein-induced necrotic and apoptotic cell death.

3.6 Lung cancer

Acrolein is likely to contribute to health problems especially among smokers because of its role in COPD, and emerging evidence suggests a possible role in cancer. Acrolein can interact with DNA forming two major adduct isomers of 1,N^2-propano-deoxyguanoside: α-hydroxy-acrolein-deoxyguanosine and γ-hydroxy-acrolein-deoxyguanosine [172–174]. Acrolein reacted preferentially at methylated CpG sites in the commonly mutated tumor suppressor protein 53 (*TP53*) gene isolated from lung tumors in smokers and induced predominantly G to T transversions. These findings raised the possibility that acrolein contributes to *TP53* mutations in cigarette smoke-induced lung cancer [173].

However, mutagenicity of acrolein-induced DNA adducts has been questioned [175] and the total acrolein-deoxyguanosine levels in smokers do not differ from nonsmokers [176]. Moreover, International Agency for Research on Cancer concluded that there was inadequate evidence for its carcinogenicity in humans and experimental animals [177]. Yet, as a component of cigarette smoke, which contains >4000 compounds and >60 established carcinogens [178], and given its ability to form DNA adducts, a carcinogenic role of acrolein in combination with other carcinogens cannot be ruled out. In addition, acrolein can bind to TP53 protein and inhibit TP53 protein–DNA binding and activity. Such interaction may contribute to the initiation of lung cancer by altering the tumor suppressor activity of TP53 [179]. Finally, acrolein is metabolized in vitro by liver and lung microsomes to glycidaldehyde, which produces tumors in mice and rats when administered subcutaneously [180].

3.7 Respiratory cell apoptosis and necrosis

Acrolein inhalation can produce nasal and pulmonary epithelial cell lesions [181]. To investigate the molecular basis of acrolein toxicity, in vitro models of tissue culture cell lines and primary cells have been used. Acrolein (≥10 μM)

is toxic to a variety of cultured respiratory cells (Supporting Information Table S3) including airway epithelial cells, alveolar macrophages, and fibroblasts [182–186]. Acrolein causes cell apoptosis and necrosis as demonstrated by phosphatidylserine (PS) externalization, DNA fragmentation, decreased cell viability and growth and increased cell membrane permeability. Interestingly, acrolein inhibits neutrophil apoptosis [187, 188]. Acrolein toxicity is dependent on the cell type, acrolein concentration, duration of exposure, and possibly genetic background. Acrolein (25 μM)-induced apoptosis of alveolar macrophages isolated from different volunteers ranged from 10 to 60%, possibly due to susceptibility differences within the sampled population [183].

Gas-phase cigarette smoke and 3–30 μM acrolein can deplete reduced glutathione (GSH) or protein thiols, and antioxidants [24, 182, 185, 186]), and increase formation of carbonylated structural and/or functional biomolecular cell constituents [189–191]. Depletion of GSH may mediate acrolein-induced cellular toxicity [192]. Thiol depletion in human fetal lung fibroblasts by culturing in cysteine-free medium or in the presence of thiol-depeleting agents induced oxidant accumulation, leukotriene production, and MAPK14 phosphorylation and its nuclear substrate activating transcription factor 2 (ATF2) leading to apoptotic cell death [193].

Lung carcinoma A-427 cells, which have low GSH, are more sensitive to acrolein-induced growth inhibition and GSH depletion than SK-LU-1 cells [194]. However, other reports indicate that GSH depletion by itself may not cause cytotoxicity (apoptosis and/or necrosis). The acrolein-induced GSH depletion is followed by a recovery phase, likely due to a feedback de novo synthesis [192] through enhanced expression of glutamate-cysteine ligase, catalytic subunit (GCLC), a rate-limiting enzyme of GSH biosynthesis [195]. Besides, despite buthionine sulfoximines (BSO) effect on GSH depletion, BSO-treated macrophages maintain normal viability [196, 197]. Rather, the redox status of the thioredoxin (TXN) system could be more critical to cell survival than is glutathione [198].

Increased oxidant activity is associated with mitochondrial membrane hyperpolarization and cytochrome c release triggering lung cell apoptosis through the mitochondrial pathway [183, 186, 193, 199, 200]. In support of oxidants being mediators of cytotoxicity, supplementation of cells with antioxidants (α-tocopherol, ascorbic acid, catalase, superoxide dismutase, sodium pyruvate, MnTBAP) inhibited acrolein-induced apoptosis and prevented the increase in the generation of intracellular oxidants [185, 199]. However, the oxidative response can be induced by lower acrolein concentrations without imparting significant cell apoptosis. Acrolein (5 μM) increased heme oxygenase 1 (HOMX1) expression in A549 cells at a low dose, whereas at higher cytotoxic doses (25 and 50 μM) decreased HOMX1 protein levels [183]. In addition, the activity of several proteins critical in maintaining redox homeostasis, including thioredoxin reductase, thioredoxin 1, and thioredoxin 2 are irreversibly inhibited by 5 μM acrolein [201]. In addition, acrolein ($<15\,\mu M \times 30$ min) induced activation of the survival factor AKT, which paralleled initiation of apoptotic signaling pathways [199].

Exposure of plasma, as a model for respiratory tract lining fluids, to gas-phase cigarette smoke can cause anti-oxidant depletion, lipid oxidation, and protein carbonyl formation. GSH inhibited protein carbonyl formation, whereas other plasma antioxidants, including ascorbate, which prevented lipid peroxidation, were ineffective. In addition to acrolein, gas-phase cigarette smoke contains several other chemical components including free radicals and reactive oxygen species. A focus of great interest in the study of the toxicity of cigarette smoke free radicals ($\sim 1 \times 10^{15}$ per puff) have been implicated in the oxidative damage of biological macromolecules [189, 202–204]. The presence of free radicals and reactive oxygen species in cigarette smoke have been proposed to contribute to the depletion of antioxidants and initiate unsaturated lipid peroxidation and protein and amino acid modifications [205–207]. However, gas-phase cigarette exposure produces little free radical lipid peroxidation products, e.g. <1 μmol/L lipid hydroperoxide. In contrast, this exposure yielded mainly aldehyde adduction products, e.g. ~450 μmol/L protein carbonyls. In addition, α,β-unsaturated aldehydes, including acrolein, added to plasma in quantities present in cigarette smoke increased protein carbonyl formation and reduced free sulfhydryls in glutathione, but not other plasma antioxidants. Cigarette smoke-induced sulfhydryl depletion therefore is likely to be due to carbonyl-mediated protein modification rather than due to lipid oxidation. Thus, α,β-unsaturated aldehydes, like acrolein, are the major contributors to the oxidative damage induced by gas-phase cigarette smoke [206–208]. These observations also support that protein carbonyl formation occurs to a larger extent than does lipid peroxidation [190, 206, 208].

It appears that carbonyl formation [190] is a critical determinant of acrolein-induced cytotoxicity in respiratory cells in culture. At lower acrolein doses, GSH is sufficient to inhibit excessive carbonyl formation and play a protective role [190, 209] in addition to other tissue repair processes that are activated at sites of injury. Still, acrolein may counteract, at sub-lethal concentrations, the repair processes by inhibiting epithelial cell chemotaxis, matrix substrate contraction, and matrix protein production [210, 211]. The lower level of carbonyl formation in combination with the induced protective changes may cause gene expression and cell activity alterations that influence indirectly the pathophysiological response by activating inflammatory processes [123].

Lower dose repetitive exposure to acrolein may lead to adaptive chronic responses [212]. As acrolein concentration increases, protein damage and functional interference would increase proportionally [191] and cause apoptosis followed by necrosis. Reactive oxygen species and radicals may also contribute to the apoptotic process. Thus, the induced cytotoxicity may account for acrolein-induced

sloughing of epithelial cells [83, 170, 213–215] impaired mucociliary transport [216–218], and suppression of pulmonary host defense [89, 90, 183, 219–222].

3.8 Comparative in vivo and in vitro acrolein exposure

It is difficult to compare human acrolein environmental exposures with experimental exposures used in cell culture experiments. Assuming 40% mouth breathing (with 98% acrolein deposition), 60% nasal breathing (with 20% acrolein deposition) and 7.5 L/min volume, the resulting hourly acrolein exposure rate would be $\sim 100 \times 10^{-3}$ μg/h from US average ambient air exposure (0.4×10^{-3} μg/m^3) [36] or ~140 μg/h during fires (0.58 mg/m^3). Indoor levels have ranged from 5 to 50 μg/h in smoke-filled rooms to ~200 μg/h by cigarette smoking (2 packs/day). The surface area of the lung ($\sim 7 \times 10^5$ cm^2), and thus the highest dose rate per unit area would be ~0.1 ng/h per cm^2 for environmental tobacco smoke-exposed nonsmokers, ~0.2 ng/h per cm^2 for fire fighters, and ~0.3 ng/h per cm^2 for smokers.

In cell culture, 10 μM acrolein (4 h exposure in a 10 mL volume in 100 mm cell-culture plate) will yield ~20 ng/h per cm^2 dose rate. This estimated dose could be achieved by inhalation concentration >50 mg/m^3. Such doses could be considered extremely high in that ~23 mg/m^3 acrolein is lethal to laboratory animals [95]. In contrast, doses of 0.1 μM acrolein will yield 0.2 ng/h per cm^2. This dose is somewhat more reasonable in that similar concentration (0.05–0.1 μM) have been measured in expired breath from persons with COPD and asthma [55, 56]. Such exposures could be similar to those experienced by high-level environmental tobacco smoke, fires, or by cigarette smokers.

3.9 Molecular basis of acrolein action

As a water-soluble α-β-unsaturated aldehyde, acrolein possesses a C-3 carbon that can react with the sulfhydryl group of cysteine, the imidazole moiety of histidine, the ε-amino group of lysine, the guanidine group of arginine, and the deoxyguanosine moiety of DNA forming biomolecular adducts [2]. The carbonylated adduct can undergo nucleophilic attack by adjacent proteins to form cross-links [223].

In vitro studies and analyses of lung cells and human lung samples have demonstrated that acrolein can interact with cell membrane, cytoskeletal, cytosolic, and nuclear proteins as well as nucleic acids. For example, acrolein exposure forms nonreducible glutathione-aldehyde derivatives [224], vimentin as well as keratins-7, -8, -9, -18, and heat shock protein 90 (Hsp90) adducts and cross-links [225] or modified nuclear factor of kappa light polypeptide gene enhancer in B-cells (NF-κB) due to Cys-61 and Arg-307 alkylation [226, 227], modified Jun due to lysine alkylation [228], modified TP53 due to alkylation [179], and acrolein–DNA adducts [172, 173, 176, 229].

The reactive property of acrolein and its ability to modify cellular components underlie acroleins multifaceted effect on lung pathogenesis, cell survival, and cell signaling. It remains to be determined whether acrolein adducts contribute to perpetuating mechanisms that maintain chronic pulmonary disorders. Proteomic profiling identified 34 differentially expressed proteins between control and acrolein-treated lung epithelial cells [230] whereas genome-wide transcriptional analyses revealed alteration of several more transcripts belonging to a broad range of cellular pathways including those involved in apoptosis, cell cycle control, transcription, cell signaling, and protein biosynthesis [231].

Conceivably, acrolein-cell membrane receptor adducts and cross-links form oligomeric or multimeric complexes, which initiate apoptotic, inflammatory, as well as many other signaling events independent of the "lock-and-key" mode of activation. However, acrolein-induced responses are not limited to cell membrane protein activation. Because of its reactivity with numerous intracellular targets, acrolein can alter upstream and/or downstream components of a signaling cascade. For example, alkylation of the p50 subunit of the NF-κB transcription factor by acrolein inhibited binding to an NF-κB consensus DNA sequence [226]. Direct modification of NF-κB may thus account for acrolein-mediated inhibition of endotoxin-, TNF-, or 12-*O*-tetradecanoylphorbol-13-acetate (TPA)-induced NF-κB activation [221, 227, 232, 233]. Furthermore, acrolein can regulate gene expression indirectly via altered levels of cytokine release.

4 Concluding remarks

The relevance of acrolein as a significant inhalation hazard is borne by its wide distribution and the fact that the pathological changes due to smoke-related lung diseases can be replicated using acrolein alone in animal models. Higher dose of acrolein exposure causes acute lung injury whereas lower dose exposure represents a risk factor for development of chronic pulmonary diseases, such as COPD. Currently, treatment of acute lung injury is limited to ventilator control strategies and no effective treatment for COPD currently exists. Additional research is needed to advance our understanding of the biological effects of acrolein and develop novel treatment strategies. Investigation of the cellular and molecular mechanisms that mediate acrolein dose-dependent pathologic alternations is needed.

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