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Inflammatory Mechanisms of Pulmonary Injury Induced by Mustards

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Abstract

Exposure of humans and animals to vesicants, including sulfur mustard (SM) and nitrogen mustard (NM), causes severe and debilitating damage to the respiratory tract. Both acute and long term pathological consequences are observed in the lung following a single exposure to these vesicants. Evidence from our laboratories and others suggest that macrophages and inflammatory mediators they release play an important role in mustard-induced lung injury. In this paper, the pathogenic effects of SM and NM on the lung are reviewed, along with the potential role of inflammatory macrophages and mediators they release in mustard-induced pulmonary toxicity.

Keywords

vesicant; pulmonary toxicity; lung injury; TNF α ; iNOS

Introduction

Sulfur mustard (SM) and nitrogen mustard (NM), are bifunctional alkylating agents known to cause injury to the respiratory tract (Ekstrand-Hammarstrom et al., 2011; Malaviya et al., 2012; Razavi et al., 2013; Sunil et al., 2011b; Weinberger et al., 2011). They are lipophilic compounds that rapidly penetrate tissues and cells, reacting with sulfhydryl, carboxyl and aliphatic amino groups, as well as heterocyclic nitrogen atoms forming stable adducts (Giuliani et al., 1994; Smith et al., 1998). This causes oxidative and nitrosative stress, impairment of cellular functioning, DNA damage, apoptosis and autophagy (Malaviya et al., 2010; Malaviya et al., 2012; Shakarjian et al., 2010; Weinberger et al., 2011).

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In humans, complications following inhalation exposure to mustards are often lethal in the short term, and a source of ongoing symptoms and morbidity in long-term survivors (Balali-Mood and Hefazi, 2005; Razavi et al., 2013; Wang and Xia, 2007; Weinberger et al., 2011). Chronic obstructive pulmonary disease (COPD), characterized by bronchitis and emphysema, bronchiectasis, bronchiolitis and asthma are common long term effects following mustard inhalation, along with pulmonary fibrosis (Balali-Mood and Hefazi, 2005). In humans and experimental animal models, acute and long term injury induced by mustards is correlated with increases in proinflammatory/profibrotic mediators including reactive oxygen species (ROS) and reactive nitrogen species (RNS), tumor necrosis factor (TNF) α , interleukin (IL)-1, IL-6, IL-8, IL-12 and transforming growth factor (TGF) β (Aghanouri et al., 2004; Weinberger et al., 2011). The role of inflammatory mediators in mustard-induced lung injury is unknown and this represents the focus of our research.

Histopathological alterations in rat lung following mustard exposure

As observed in humans, exposure of rats to SM or NM induces progressive histopathologic changes in the trachea, bronchi and lung (Malaviya et al., 2010; Malaviya et al., 2012; Sunil et al., 2011b; Sunil et al., 2014). Focal attenuation of tracheal epithelium, detachment of epithelium from the mucosa, loss of cilia, and accumulation of fibrin in the lumen are prominent. The bronchus shows evidence of focal ulceration, luminal fibrin plug entrapment of necrotic inflammatory cells and bronchial epithelial cells (Malaviya et al., 2010). SM and NM induce multifocal hyperplasia in the lung parenchyma, as well as patchy mild thickening of alveolar septa, characterized by increased numbers of macrophages, neutrophils and mononuclear cells. In addition, perivascular and peribronchial edema, hyperplasia and hypertrophy of goblet cells, blood vessel hemorrhage, fibrin deposits, inflammatory cell infiltrates and luminal accumulation of cellular debris are evident (Fig. 1). These effects occur early after mustard exposure and persist for at least 28 days (Malaviya et al., 2010; Malaviya et al., 2012; Sunil et al., 2011b; Sunil et al., 2014). Similar persistent histological changes have been described in the lung up to 14 d after exposure of rodents to NM, as well as SM (Calvet et al., 1994; Ucar et al., 2007; Yaren et al., 2007). In lungs of NM treated animals, bronchiolization of alveolar walls, which is an ingrowth of cuboidal cells lining adjacent bronchioles to alveoli which form a tube like alveolar structure, is also observed. Additionally, prominent trichrome staining in the alveolar septal wall and peribronchiolar region is evident; mainly localized within inflammatory lesions with a few areas exhibiting organized fibrin deposits (Malaviya et al., 2012). By 28 d post exposure, multiple areas of fibrosis containing collagen fibers are observed around airways and bronchioles. This is correlated with the appearance of foamy macrophages in the lung which occlude the alveoli. Erythrophagocytosis, fibroplasia, squamous metaplasia of the bronchial wall and emphysema-like changes in the alveolar tissue are also evident. Multifocal hyperplasia and bronchiolization correlate with increased epithelial expression of proliferating cell nuclear antigen (PCNA), indicating cellular proliferation. This is prominent within bronchiolized alveolar walls (Malaviya et al., 2012). Biochemical markers of inflammation and alveolar epithelial damage including bronchoalveolar lavage (BAL) cell and protein content also increase following mustard exposure (Malaviya et al., 2010; Malaviya et al., 2012; Sunil et al., 2011b; Sunil et al., 2014). Evidence of apoptosis,

characterized by increased activation of caspase-9 and caspase-3 is noted in lungs of SM treated rats (Malaviya et al., 2010).

A role for nuclear factor (NF)- κ B has previously been suggested in SM-induced apoptosis in human keratinocytes, and it may play a similar role in lung epithelial cells (Ruff and Dillman, 2010). Transmission electron microscopy of lung sections has also revealed the presence of autophagosomes (Malaviya et al., 2010) which correlates with increased expression of LC3B-II, a lipidated form of LC3B-I protein known to associate with autophagosomal membranes (Virgin and Levine, 2009).

Antioxidant expression in rat lung following mustard exposure

Evidence suggests that oxidative stress contributes to mustard induced pulmonary toxicity (Anderson et al., 2009; Gould et al., 2009; Hoesel et al., 2008; Mukhopadhyay et al., 2006b; Shohrati et al., 2010; Weinberger et al., 2011). Thus, following exposure to vesicants, a decrease in glutathione is observed in the respiratory tract, along with increases in markers of oxidative stress, such as malondialdehyde, 8-hydroxyguanosine and 4-hydroxynonenal (Kumar et al., 2001; Mukherjee et al., 2009; O'Neill et al., 2010). These findings, together with reports that antioxidant treatment reduces vesicant-induced lung injury and inflammation provide support for a role of oxidative stress in toxicity (Kumar et al., 2001; McClintock et al., 2002; Ucar et al., 2007; Wigenstam et al., 2009). A characteristic response of cells and tissues to oxidative stress is upregulation of heme oxygenase (HO)-1, a phase II stress response enzyme with antioxidant and antiinflammatory activity (Otterbein et al., 1999; Rahman et al., 2006), and lipocalin-2, a member of the lipocalin superfamily (Roudkenar et al., 2007). After exposure of rodents to mustards, increases in both the intensity of HO-1 expression and the number of macrophages expressing HO-1 are observed (Malaviya et al., 2010; Malaviya et al., 2012; Sunil et al., 2014). Increased levels of HO-1 persist for at least 28 d, suggesting that oxidative stress is an ongoing process after vesicant exposure (Malaviya et al., 2012). Findings that HO-1 expression is predominantly localized in lung macrophages indicate that these cells may be a significant source of cytotoxic oxidants. NM intoxication is also associated with increases in lipocalin-2 in BAL and Mn-superoxide dismutase (SOD) expression in lung macrophages and epithelial cells (Malaviya et al., 2012; Sunil et al., 2014).

Expression of proinflammatory and profibrotic mediators in the lung following mustard exposure

Classically activated proinflammatory/cytotoxic M1 macrophages have been implicated in lung injury induced by pulmonary toxicants [reviewed in (Laskin et al., 2011)]. These cells release ROS and RNS, generated via inducible nitric oxide synthase (iNOS), eicosanoids, produced from arachidonic acid by cyclooxygenase (COX)-2, and matrix metalloproteinases (MMPs), as well as interleukin (IL)s, such as IL-12 and TNF α . We found that exposure of rats to SM or NM results in marked increases in iNOS⁺, COX-2⁺ and TNF α ⁺ macrophages in the lung within 24 h (Malaviya et al., 2010; Malaviya et al., 2012; Sunil et al., 2011b; Sunil et al., 2014) (Fig. 2). Expression of proinflammatory genes including IL-12 and CCR2 is also upregulated in lung macrophages (Venosa et al., 2015). Interestingly, macrophages

expressing these markers persist in the lung for at least 28 d post exposure, indicating a prolonged proinflammatory response. Increases in expression of iNOS and COX-2 have been described in lungs of patients with idiopathic pulmonary fibrosis (Lappi-Blanco et al., 2006; Saleh et al., 1997). These findings, together with reports showing that suppression of iNOS or COX-2 mitigates the development of silica- or bleomycin-induced lung fibrosis in rodents, indicate that products of these enzymes contribute to fibrogenesis following mustard exposure (Arafa et al., 2007; Giri et al., 2002; Kalayarasan et al., 2008). Lung epithelial cells also stain positively for iNOS, COX-2 and TNF α after NM exposure, suggesting that these cells may also be involved in the pathogenesis of tissue injury (Malaviya et al., 2010; Malaviya et al., 2012; Sunil et al., 2011b; Sunil et al., 2014).

MMPs are proteases that degrade extracellular matrix components, an important step in alveolar epithelial injury and detachment of cells from basement membranes following exposure to mustards (Calvet et al., 1999; Guignabert et al., 2005). In rodents, rapid increases in levels of MMP-9 in bronchial epithelium and alveolar macrophages are observed after SM or NM exposure (Malaviya et al., 2010; Sunil et al., 2011b; Sunil et al., 2014). This correlates with an increase in 92 kDa gelatinase activity. Findings that MMP inhibitors such as doxycycline and ilomastat attenuate SM-induced respiratory lesions in rats, demonstrate the importance of proteases in vesicant-induced lung injury (Anderson et al., 2009).

Alternatively activated antiinflammatory/wound repair M2 macrophages play a role in the resolution of lung injury [reviewed in (Laskin et al., 2011)]. Exaggerated responses of M2 macrophages are thought to contribute to the development of fibrosis. In an experimental model of NM toxicity, we observed increases in numbers of lung macrophages expressing markers of M2 macrophages including Ym-1, galectin (Gal)-3, and CD68 and expression of antiinflammatory profibrotic genes (i.e., IL-10, connective tissue growth factor) within 3 d suggesting that the process of fibrosis begins early in the pathogenic response (Malaviya et al., 2012; Sunil et al., 2014; Venosa et al., 2015). This is supported by our findings that the appearance of M2 macrophages in the lung correlates with increases in expression of the profibrotic mitogen, TGF- β and fibroplasia at 3 d post NM, and collagen deposits around bronchioles and alveolar septae after 7 d; by 28 d multiple fibrotic foci with mature collagen are evident around the airways, distorting the normal parenchymal structure (Malaviya et al., 2015; Malaviya et al., 2012). At this time, large and foamy macrophages expressing Ym-1 and Gal-3 occluding the alveoli are prominent (Fig. 2) (Malaviya et al., 2012), which is consistent with a profibrotic phenotype (Wang and Lyster, 2010).

Lung mechanics and function

The effects of mustards on parenchymal mechanical properties of the lung have also been assessed using NM as a model. In these studies pulmonary impedance is measured following deep-inflation as a function of increasing positive end-expiratory pressure (PEEP). NM exposure results in an increase in tissue elastance and resistance, across all levels of PEEP (Sunil et al., 2014). These increases are evident immediately following deep inflation; conversely, the rate and extent of changes in elastance and resistance over the measurement interval are not altered. These studies demonstrate that NM-induced structural and

inflammatory changes are associated with PEEP-dependent increases in tissue damping and elastance. Findings that both elastance and resistance parameters increase, while the kinetics of derecruitment following deep inflation are not significantly altered by NM, indicate that the changes observed are inherent properties of the lung, and not related to the dynamic components of respiration, such as surfactant. Therefore, it is likely that they result as a direct consequence of NM-induced injury, rather than an inflammatory response. NM exposure also causes a reduction in methacholine-induced increases in total lung resistance. Methacholine is a potent bronchoconstrictor used in animal models to examine hyperresponsiveness of the airway, and to assess airway wall stiffness and parenchymal elasticity (Bates and Lauzon, 2007). The observed decrease in lung resistance in response to methacholine could be due to a loss of active airway tone. A significant blunting of methacholine-induced increases in tissue damping and elastance is also noted in NM treated animals. These reduced responses are most likely a consequence of structural alterations in the lung and thus the inability of the airways to respond actively to the bronchoconstrictive agent (Sunil et al., 2011b).

Approaches to mitigating the pulmonary toxicity of mustards

To explore the role of inflammatory mediators in mustard-induced pulmonary toxicity, mice with targeted deletion of key inflammatory genes were used. Initially, we focused on iNOS, the enzyme mediating the production of RNS by inflammatory macrophages (Laskin et al., 2010). Mice deficient in iNOS were found to be less sensitive to the cytotoxic effects of the half mustard, 2-chloroethyl ethyl sulfide (CEES) than wild-type controls (Sunil et al., 2012). CEES-mediated alterations in total lung resistance and compliance in response to methacholine are also abrogated in iNOS^{-/-} mice, suggesting that RNS generated via iNOS, play a role in the pathogenic responses to mustards. To explore this further, the effects of blocking iNOS using aminoguanidine (AG), a specific inhibitor of this enzyme (Southan and Szabo, 1996) was analyzed in a rat model of NM-induced lung toxicity. We observed that administration of AG (50 mg/kg, 2×/day, 1 d – 3 d) beginning 30 min after NM, reduces histopathological changes in the lung induced by NM at 1 d and 3 d post exposure and blunts NM-induced increases in BAL cell and protein content (Malaviya et al., 2012). AG treatment also suppresses NM-induced increases in oxidative stress, and numbers of COX-2⁺ and iNOS⁺ proinflammatory macrophages in the lung. Early increases in hyperplasia, as measured by PCNA expression and fibrogenesis, are also blunted by AG. This is correlated with reduced numbers of Ym-1⁺ and Gal-3⁺ profibrotic M2 macrophages in the lung (Malaviya et al., 2012). Although AG is effective in inhibiting NM-induced injury, oxidative stress and inflammation at 1 d and 3 d post exposure, no effects are observed at 7 d or 28 d. Similar effects were observed using 1400W, another selective iNOS inhibitor (Malaviya et al., 2014). These findings suggest that nitric oxide generated via iNOS contributes mainly to acute NM-induced lung toxicity.

In further studies we focused on TNF α , a macrophage-derived proinflammatory cytokine known to promote oxidative stress, inflammatory cell influx, cytotoxicity and apoptosis, and pulmonary fibrosis (Aggarwal, 2003; Bradley, 2008; Mukhopadhyay et al., 2006a; Thrall et al., 1997). In our first series of studies we used mice lacking TNF receptor (TNFR) 1, the major receptor mediating the proinflammatory actions of TNF α (Aggarwal, 2003; Bradley,

2008). Binding of TNF α to TNFR1 activates mitogen activated protein kinase (MAPK) signaling pathways in mustard-exposed lung which potentially contributes to injury and inflammation (Mukhopadhyay et al., 2009). In mice lacking TNFR1, significant protection from CEES-induced injury, oxidative stress, and inflammation is observed. CEES-induced expression of iNOS, COX-2 and monocyte chemotactic protein (MCP)-1 mRNA is also attenuated in TNFR1^{-/-} mice relative to wild type mice, while CEES-mediated upregulation of CuZn-SOD and Mn-SOD are delayed or absent in TNFR1^{-/-} mice and functional alterations are blunted (Sunil et al., 2011a). These findings prompted us to assess the effects of pharmacologic inhibition of TNF α on mustard-induced toxicity in the lung using pentoxifylline, a methyl xanthine phosphodiesterase inhibitor reported to down regulate TNF α production (Fernandes et al., 2008), or a specific antibody to TNF α . In these experiments, rats were treated with pentoxifylline (46.7 mg/kg, *i.p.*) daily for 3 d beginning 15 min after NM, or with anti-TNF α antibody (15 mg/kg, *i.v.*) once every 9 d, beginning 30 min after NM. Inhibition of TNF α with pentoxifylline or anti-TNF α antibody reduces progressive histopathologic alterations in the lung beginning at 3 d including perivascular and peribronchial edema, macrophage/monocyte infiltration, interstitial thickening, bronchiolization of alveolar walls, fibrin deposition, emphysema and fibrosis (Malaviya et al., 2015; Sunil et al., 2014). Inhibition of TNF α also reduces NM-induced damage to the alveolar-epithelial barrier, measured by BAL protein and cell content, along with expression of the oxidative stress markers, HO-1 and lipocalin-2. TNF α inhibitors also effectively suppress the accumulation of proinflammatory/cytotoxic M1 macrophages in the lung in response to NM, whereas antiinflammatory/wound repair M2 macrophages are increased or unchanged. Treatment of rats with anti-TNF α antibody also reduces NM-induced increases in expression of the profibrotic mediator, TGF- β (Malaviya et al., 2015). This is associated with marked inhibition of NM-induced fibrosis and collagen deposition in the lung. Etanercept, a recombinant soluble TNF receptor that binds to and neutralizes soluble TNF α , has been reported to reduce the progression of idiopathic pulmonary fibrosis in humans (Raghu et al., 2008). These findings support the notion that TNF α is an important mediator of vesicant-induced pulmonary injury.

Summary

Vesicant exposure causes both acute and long term pathologic consequences in the lung. Acute cytotoxicity, oxidative stress and disruption of tissue architecture lead to alterations in lung function, and induce myofibroblast activation, fibroblast proliferation and collagen deposition resulting in fibrosis. Our studies demonstrate that while the acute pulmonary effects of NM intoxication can be mitigated by inhibition of iNOS, blocking TNF α suppresses both acute and long term pathologic effects of mustards on the respiratory system. Taken together, these findings support the notion that inflammatory molecules including RNS and TNF α are important mediators of vesicant-induced pulmonary injury and that targeting these molecules may be an efficacious approach to mitigating the toxic effects of mustards in humans.

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References

- Aggarwal BB. Signalling pathways of the TNF superfamily: a double-edged sword. *Nat. Rev. Immunol.* 2003; 3:745–756. [PubMed: 12949498]
- Aghanouri R, Ghanei M, Aslani J, Keivani-Amine H, Rastegar F, Karkhane A. Fibrogenic cytokine levels in bronchoalveolar lavage aspirates 15 years after exposure to sulfur mustard. *Am. J. Physiol. Lung Cell. Mol. Physiol.* 2004; 287:L1160–L1164. [PubMed: 15286001]
- Anderson DR, Taylor SL, Fetterer DP, Holmes WW. Evaluation of protease inhibitors and an antioxidant for treatment of sulfur mustard-induced toxic lung injury. *Toxicology.* 2009; 263:41–46. [PubMed: 18852015]
- Arafa HM, Abdel-Wahab MH, El-Shafeey MF, Badary OA, Hamada FM. Anti-fibrotic effect of meloxicam in a murine lung fibrosis model. *Eur. J. Pharmacol.* 2007; 564:181–189. [PubMed: 17462625]
- Balali-Mood M, Hefazi M. The pharmacology, toxicology, and medical treatment of sulphur mustard poisoning. *Fundam. Clin. Pharmacol.* 2005; 19:297–315. [PubMed: 15910653]
- Bates JH, Lauzon AM. Parenchymal tethering, airway wall stiffness, and the dynamics of bronchoconstriction. *J. Appl. Physiol.* 2007; 102:1912–1920. [PubMed: 17204575]
- Bradley JR. TNF-mediated inflammatory disease. *J. Pathol.* 2008; 214:149–160. [PubMed: 18161752]
- Calvet JH, Jarreau PH, Levame M, D'Ortho MP, Lorino H, Harf A, Macquin-Mavier I. Acute and chronic respiratory effects of sulfur mustard intoxication in guinea pig. *J. Appl. Physiol.* 1994; 76:681–688. [PubMed: 8175578]
- Calvet JH, Planus E, Rouet P, Pezet S, Levame M, Lafuma C, Harf A, D'Ortho MP. Matrix metalloproteinase gelatinases in sulfur mustard-induced acute airway injury in guinea pigs. *Am. J. Physiol.* 1999; 276:L754–L762. [PubMed: 10330031]
- Ekstrand-Hammarstrom B, Wigenstam E, Bucht A. Inhalation of alkylating mustard causes long-term T cell-dependent inflammation in airways and growth of connective tissue. *Toxicology.* 2011; 280:88–97. [PubMed: 21129433]
- Fernandes JL, de Oliveira RT, Mamoni RL, Coelho OR, Nicolau JC, Blotta MH, Serrano CV Jr. Pentoxifylline reduces pro-inflammatory and increases anti-inflammatory activity in patients with coronary artery disease--a randomized placebo-controlled study. *Atherosclerosis.* 2008; 196:434–442. [PubMed: 17196208]
- Giri SN, Biring I, Nguyen T, Wang Q, Hyde DM. Abrogation of bleomycin-induced lung fibrosis by nitric oxide synthase inhibitor, aminoguanidine in mice. *Nitric Oxide.* 2002; 7:109–118. [PubMed: 12223180]
- Giuliani I, Boivieux-Ulrich E, Houcine O, Guennou C, Marano F. Toxic effects of mechlorethamine on mammalian respiratory mucociliary epithelium in primary culture. *Cell Biol. Toxicol.* 1994; 10:231–246. [PubMed: 7895152]
- Gould NS, White CW, Day BJ. A role for mitochondrial oxidative stress in sulfur mustard analog 2-chloroethyl ethyl sulfide-induced lung cell injury and antioxidant protection. *J. Pharmacol. Exp. Ther.* 2009; 328:732–739. [PubMed: 19064720]
- Guignabert C, Taysse L, Calvet JH, Planus E, Delamanche S, Galiacy S, d'Ortho MP. Effect of doxycycline on sulfur mustard-induced respiratory lesions in guinea pigs. *Am. J. Physiol. Lung Cell. Mol. Physiol.* 2005; 289:L67–L74. [PubMed: 15778244]
- Hoesel LM, Flierl MA, Niederbichler AD, Rittirsch D, McClintock SD, Reuben JS, Pianko MJ, Stone W, Yang H, Smith M, Sarma JV, Ward PA. Ability of antioxidant liposomes to prevent acute and progressive pulmonary injury. *Antioxid. Redox Signal.* 2008; 10:973–981. [PubMed: 18257742]
- Kalayarasan S, Sriram N, Sudhandiran G. Diallyl sulfide attenuates bleomycin-induced pulmonary fibrosis: critical role of iNOS, NF- κ B, TNF- α and IL-1 β . *Life Sci.* 2008; 82:1142–1153. [PubMed: 18462759]

- Kumar O, Sugendran K, Vijayaraghavan R. Protective effect of various antioxidants on the toxicity of sulphur mustard administered to mice by inhalation or percutaneous routes. *Chem. Biol. Interact.* 2001; 134:1–12. [PubMed: 11248218]
- Lappi-Blanco E, Kaarteenaho-Wiik R, Maasilta PK, Anttila S, Paakko P, Wolff HJ. COX-2 is widely expressed in metaplastic epithelium in pulmonary fibrous disorders. *Am. J. Clin. Pathol.* 2006; 126:717–724. [PubMed: 17050069]
- Laskin DL, Sunil VR, Gardner CR, Laskin JD. Macrophages and tissue injury: agents of defense or destruction? *Annu. Rev. Pharmacol. Toxicol.* 2011; 51:267–288. [PubMed: 20887196]
- Laskin, JD.; Heck, DE.; Laskin, DL. Nitric oxide pathways in toxic responses. In: Ballantine, B.; Marrs, T.; Syversen, T., editors. *General and Applied Toxicology*. Wiley-Blackwell, UK: 2010. p. 425–438.
- Malaviya R, Sunil V, Venosa A, Vayas K, Sinko PJ, Shen J, Hall L, Shealy D, Heindel ND, Lacey CJ, Laskin JD, Laskin DL. Attenuation of nitrogen mustard (NM)-induced pulmonary injury and inflammation by antitumor necrosis factor (TNF) α antibody and the inducible nitric oxide synthase (iNOS) inhibitor, N-(3-(aminomethyl)benzyl) acetamidine (1400W). *The Toxicologist*. 2014; 138:A561–A146.
- Malaviya R, Sunil VR, Cervelli J, Anderson DR, Holmes WW, Conti ML, Gordon RE, Laskin JD, Laskin DL. Inflammatory effects of inhaled sulfur mustard in rat lung. *Toxicol. Appl. Pharmacol.* 2010; 248:89–99. [PubMed: 20659490]
- Malaviya R, Sunil VR, Venosa A, Verissimo VL, Cervelli JA, Vayas KN, Hall L, Laskin JD, Laskin DL. Attenuation of nitrogen mustard-induced pulmonary injury and fibrosis by anti-tumor necrosis factor- α antibody. *Toxicol. Sci.* 2015
- Malaviya R, Venosa A, Hall L, Gow AJ, Sinko PJ, Laskin JD, Laskin DL. Attenuation of acute nitrogen mustard-induced lung injury, inflammation and fibrogenesis by a nitric oxide synthase inhibitor. *Toxicol. Appl. Pharmacol.* 2012; 265:279–291. [PubMed: 22981630]
- McClintock SD, Till GO, Smith MG, Ward PA. Protection from half-mustard-gas-induced acute lung injury in the rat. *J. Appl. Toxicol.* 2002; 22:257–262. [PubMed: 12210543]
- Mukherjee S, Stone WL, Yang H, Smith MG, Das SK. Protection of half sulfur mustard gas-induced lung injury in guinea pigs by antioxidant liposomes. *J. Biochem. Mol. Toxicol.* 2009; 23:143–153. [PubMed: 19367648]
- Mukhopadhyay S, Hoidal JR, Mukherjee TK. Role of TNF α in pulmonary pathophysiology. *Respir. Res.* 2006a; 7:125. [PubMed: 17034639]
- Mukhopadhyay S, Mukherjee S, Stone WL, Smith M, Das SK. Role of MAPK/AP-1 signaling pathway in the protection of CEES-induced lung injury by antioxidant liposome. *Toxicology*. 2009; 261:143–151. [PubMed: 19464336]
- Mukhopadhyay S, Rajaratnam V, Mukherjee S, Smith M, Das SK. Modulation of the expression of superoxide dismutase gene in lung injury by 2-chloroethyl ethyl sulfide, a mustard analog. *J. Biochem. Mol. Toxicol.* 2006b; 20:142–149. [PubMed: 16788954]
- O'Neill HC, White CW, Veress LA, Hendry-Hofer TB, Loader JE, Min E, Huang J, Rancourt RC, Day BJ. Treatment with the catalytic metalloporphyrin AEOL 10150 reduces inflammation and oxidative stress due to inhalation of the sulfur mustard analog 2-chloroethyl ethyl sulfide. *Free Radic. Biol. Med.* 2010; 48:1188–1196. [PubMed: 20138141]
- Otterbein LE, Lee PJ, Chin BY, Petrache I, Camhi SL, Alam J, Choi AM. Protective effects of heme oxygenase-1 in acute lung injury. *Chest*. 1999; 116:61S–63S. [PubMed: 10424595]
- Raghu G, Brown KK, Costabel U, Cottin V, du Bois RM, Lasky JA, Thomeer M, Utz JP, Khandker RK, McDermott L, Fatenejad S. Treatment of idiopathic pulmonary fibrosis with etanercept: an exploratory, placebo-controlled trial. *Am. J. Respir. Crit. Care Med.* 2008; 178:948–955. [PubMed: 18669816]
- Rahman I, Biswas SK, Kode A. Oxidant and antioxidant balance in the airways and airway diseases. *Eur. J. Pharmacol.* 2006; 533:222–239. [PubMed: 16500642]
- Razavi SM, Ghanei M, Salamati P, Safiabadi M. Long-term effects of mustard gas on respiratory system of Iranian veterans after Iraq-Iran war: a review. *Chin. J. Traumatol.* 2013; 16:163–168. [PubMed: 23735551]

- Roudkenar MH, Kuwahara Y, Baba T, Roushandeh AM, Ebishima S, Abe S, Ohkubo Y, Fukumoto M. Oxidative stress induced lipocalin 2 gene expression: addressing its expression under the harmful conditions. *J. Radiat. Res.* 2007; 48:39–44. [PubMed: 17229997]
- Ruff AL, Dillman JF 3rd. Sulfur mustard induced cytokine production and cell death: investigating the potential roles of the p38, p53, and NF- κ B signaling pathways with RNA interference. *J. Biochem. Mol. Toxicol.* 2010; 24:155–164. [PubMed: 20143454]
- Saleh D, Barnes PJ, Giaid A. Increased production of the potent oxidant peroxynitrite in the lungs of patients with idiopathic pulmonary fibrosis. *Am. J. Respir. Crit. Care Med.* 1997; 155:1763–1769. [PubMed: 9154889]
- Shakarjian MP, Heck DE, Gray JP, Sinko PJ, Gordon MK, Casillas RP, Heindel ND, Gerecke DR, Laskin DL, Laskin JD. Mechanisms mediating the vesicant actions of sulfur mustard after cutaneous exposure. *Toxicol. Sci.* 2010; 114:5–19. [PubMed: 19833738]
- Shohrati M, Ghanei M, Shamspour N, Babaei F, Abadi MN, Jafari M, Harandi AA. Glutathione and malondialdehyde levels in late pulmonary complications of sulfur mustard intoxication. *Lung.* 2010; 188:77–83. [PubMed: 19862574]
- Smith KJ, Smith WJ, Hamilton T, Skelton HG, Graham JS, Okerberg C, Moeller R, Hackley BE Jr. Histopathologic and immunohistochemical features in human skin after exposure to nitrogen and sulfur mustard. *Am. J. Dermatopathol.* 1998; 20:22–28. [PubMed: 9504665]
- Southan GJ, Szabo C. Selective pharmacological inhibition of distinct nitric oxide synthase isoforms. *Biochem. Pharmacol.* 1996; 51:383–394. [PubMed: 8619882]
- Sunil VR, Patel-Vayas K, Shen J, Gow AJ, Laskin JD, Laskin DL. Role of TNFR1 in lung injury and altered lung function induced by the model sulfur mustard vesicant, 2-chloroethyl ethyl sulfide. *Toxicol. Appl. Pharmacol.* 2011a; 250:245–255. [PubMed: 21070800]
- Sunil VR, Patel KJ, Shen J, Reimer D, Gow AJ, Laskin JD, Laskin DL. Functional and inflammatory alterations in the lung following exposure of rats to nitrogen mustard. *Toxicol. Appl. Pharmacol.* 2011b; 250:10–18. [PubMed: 20883710]
- Sunil VR, Shen J, Patel-Vayas K, Gow AJ, Laskin JD, Laskin DL. Role of reactive nitrogen species generated via inducible nitric oxide synthase in vesicant-induced lung injury, inflammation and altered lung functioning. *Toxicol. Appl. Pharmacol.* 2012; 261:22–30. [PubMed: 22446026]
- Sunil VR, Vayas KN, Cervelli JA, Malaviya R, Hall L, Massa CB, Gow AJ, Laskin JD, Laskin DL. Pentoxifylline attenuates nitrogen mustard-induced acute lung injury, oxidative stress and inflammation. *Exp. Mol. Pathol.* 2014; 97:89–98. [PubMed: 24886962]
- Thrall RS, Vogel SN, Evans R, Shultz LD. Role of tumor necrosis factor- α in the spontaneous development of pulmonary fibrosis in viable motheaten mutant mice. *Am. J. Pathol.* 1997; 151:1303–1310. [PubMed: 9358756]
- Ucar M, Korkmaz A, Reiter RJ, Yaren H, Oter S, Kurt B, Topal T. Melatonin alleviates lung damage induced by the chemical warfare agent nitrogen mustard. *Toxicol. Lett.* 2007; 173:124–131. [PubMed: 17765411]
- Venosa A, Malaviya R, Choi H, Gow AJ, Laskin JD, Laskin DL. Characterization of distinct macrophage subpopulations during nitrogen mustard-induced injury and fibrosis. *Am. J. Respir. Cell Mol. Biol.* 2015
- Virgin HW, Levine B. Autophagy genes in immunity. *Nat. Immunol.* 2009; 10:461–470. [PubMed: 19381141]
- Wang GQ, Xia ZF. Tissue injury by hot fluid containing nitrogen mustard. *Burns.* 2007; 33:923–926. [PubMed: 17532574]
- Wang L, Lyerla T. Histochemical and cellular changes accompanying the appearance of lung fibrosis in an experimental mouse model for Hermansky Pudlak syndrome. *Histochem. Cell Biol.* 2010; 134:205–213. [PubMed: 20603711]
- Weinberger B, Laskin JD, Sunil VR, Sinko PJ, Heck DE, Laskin DL. Sulfur mustard-induced pulmonary injury: therapeutic approaches to mitigating toxicity. *Pulm. Pharmacol. Ther.* 2011; 24:92–99. [PubMed: 20851203]
- Wigenstam E, Rocksén D, Ekstrand-Hammarström B, Bucht A. Treatment with dexamethasone or liposome-encapsulated vitamin E provides beneficial effects after chemical-induced lung injury. *Inhal. Toxicol.* 2009; 21:958–964. [PubMed: 19572781]

Yaren H, Mollaoglu H, Kurt B, Korkmaz A, Oter S, Topal T, Karayilanoglu T. Lung toxicity of nitrogen mustard may be mediated by nitric oxide and peroxynitrite in rats. *Res. Vet. Sci.* 2007; 83:116–122. [PubMed: 17196628]

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Highlights

- Pulmonary exposure to mustards causes acute injury which progresses to fibrosis
- Macrophages and mediators they release play role in acute toxicity and fibrosis induced by mustards.
- Targeting reactive nitrogen species and TNF α may be useful in mitigating the toxic effects of mustards.

Fig. 1. Effects of NM on histopathology of the lung

Lung sections, prepared 3 d after exposure of rats to NM or control (PBS) were stained with H & E. NM-induced acute structural changes include multifocal inflammatory lesions (*), characterized by accumulation of inflammatory cells in alveolar spaces (arrow), thickened bronchial epithelium (arrow head), perivascular edema (e), hyperproliferation and hypertrophy of goblet cells (g) and fibrin deposits (f). Original magnification, 4× (top panels), 200× (bottom panels). Representative section from 3 – 7 rats/treatment group are shown.

Fig. 2. Effects of NM exposure on expression of proinflammatory and profibrotic proteins
Lung sections, prepared 3 d and 28 d after exposure of rats to NM or control (PBS), were stained with antibody to COX-2, iNOS, Ym-1 or Gal-3. Binding was visualized using a Vectastain kit. Original magnification, 600 $\times$. Representative section from 3 – 7 rats/ treatment group are shown. Control, 3 d post PBS exposure.