Toxicology and Applied Pharmacology 278 (2014) 53–64

Contents lists available at ScienceDirect

Toxicology and Applied Pharmacology

journal homepage: www.elsevier.com/locate/ytaap

Acute chlorine gas exposure produces transient inflammation and a progressive alteration in surfactant composition with accompanying mechanical dysfunction

Christopher B. Massa, Pamela Scott, Elena Abramova, Carol Gardner, Debra L. Laskin, Andrew J. Gow *

Department of Pharmacology & Toxicology, Ernest Mario School of Pharmacy, Rutgers University, USA

ARTICLE INFO

Article history:
Received 30 October 2013
Revised 29 January 2014
Accepted 4 February 2014
Available online 25 February 2014

Keywords:
Respiratory impedance
Pulmonary mechanics
Nitric oxide
Alternative activation
Bronchoalveolar lavage

ABSTRACT

Acute Cl_2 exposure following industrial accidents or military/terrorist activity causes pulmonary injury and severe acute respiratory distress. Prior studies suggest that antioxidant depletion is important in producing dysfunction, however a pathophysiologic mechanism has not been elucidated. We propose that acute Cl_2 inhalation leads to oxidative modification of lung lining fluid, producing surfactant inactivation, inflammation and mechanical respiratory dysfunction at the organ level. C57BL/6J mice underwent whole-body exposure to an effective 60 ppm-hour Cl_2 dose, and were euthanized 3, 24 and 48 h later. Whereas pulmonary architecture and endothelial barrier function were preserved, transient neutrophilia, peaking at 24 h, was noted. Increased expression of ARG1, CCL2, RETLNA, IL-1b, and PTGS2 genes was observed in bronchoalveolar lavage (BAL) cells with peak change in all genes at 24 h. Cl_2 exposure had no effect on NOS2 mRNA or iNOS protein expression, nor on BAL NO_3^- or NO_2^-. Expression of the alternative macrophage activation markers, Relm-α and mannose receptor was increased in alveolar macrophages and pulmonary epithelium. Capillary surfactometry demonstrated impaired surfactant function, and altered BAL phospholipid and surfactant protein content following exposure. Organ level respiratory function was assessed by forced oscillation technique at 5 end expiratory pressures. Cl_2 exposure had no significant effect on either airway or tissue resistance. Pulmonary elastance was elevated with time following exposure and demonstrated PEEP refractory derecruitment at 48 h, despite waning inflammation. These data support a role for surfactant inactivation as a physiologic mechanism underlying respiratory dysfunction following Cl_2 inhalation.

Introduction

Acute inhalation of chlorine gas (Cl_2) is known to produce respiratory dysfunction, although the underlying pathophysiological mechanism remains undefined (Malo et al., 2009). Such exposures – though presently rare – have occurred in the setting of both industrial and transportation accidents as well as in chemical warfare/terrorism scenarios (Jones et al., 2010). Clinically, exposures are characterized by hyperacute respiratory irritation, followed by respiratory distress and increased work of breathing (Leroyer et al., 1998). In the setting of human exposure incidents individuals are subjected to a wide range of doses. Severe exposures may produce acute lung injury, necessitating mechanical ventilation and increasing the likelihood of significant morbidity or death (Hedges and Morrissey, 1979). Among those exposed are also persons receiving lower-doses that produce minimal tissue injury, yet cause respiratory distress that may require mechanical ventilator support (White and Martin, 2010). In these patients the mechanism by which Cl_2 inhalation results in increased work of breathing and organ level dysfunction is unclear (Malo et al., 2009).

In humans, chlorinated oxidant inhalation has principally been examined in the context of recurring, low-dose exposure either in occupational settings or as experienced through common use as swimming pool disinfectants. Such exposures produce asthma-like obstructive lung disease, characterized by chronic cough and airway hyperresponsiveness following methacholine challenge (Bherer et al., 1994). Pathology is thought to involve recurrent disruption of redox homeostasis, with antioxidant depletion and consequent inflammation (Squadrito et al., 2010; Yadav et al., 2010). Recent studies (Hoyle, 2010; Hoyle et al., 2010; Leustik et al., 2008; Martin et al., 2003; Song et al., 2011; Tian et al., 2008; Zarogiannis et al., 2011) have examined the effect of acute high-dose Cl_2 inhalation on pulmonary inflammation and injury in rodents. These studies have employed exposure regimens that produce profound tissue injury, epithelial barrier disruption and

* Corresponding author at: Department of Pharmacology & Toxicology, Ernest Mario School of Pharmacy, Rutgers University, Piscataway, NJ 08854, USA. Fax: +1 732 445 1191.
E-mail address: Gow@rci.rutgers.edu (A.J. Gow).

http://dx.doi.org/10.1016/j.taap.2014.02.006
0041-008X/

alveolar flooding, carrying a high risk of mortality. Treatment with antioxidants (Fanucchi et al., 2012; Zarogiannis et al., 2011), sodium nitrite (Samal et al., 2012; Yadav et al., 2011) and restoration of transepithelial fluid balance through pharmacologic manipulation of cAMP dependent signaling (Chang et al., 2012; Song et al., 2011) have been demonstrated to be effective in reducing lung pathology and mortality in these exposure conditions. Little is known regarding the acute effects of Cl_2 exposure on inflammation and mechanobiology below the threshold for overt injury with epithelial barrier damage.

Due to its high water solubility Cl_2 readily partitions into the lung lining fluid and may exert prolonged or delayed toxicologic effects (Nodelman and Ultman, 1999; Squadrito et al., 2010). The chemistry of Cl_2 in aqueous environments favors the production of both hydrochloric acid and hypochlorous acid/hypochlorite anion (Wang and Margerum, 1994). The pH change that this reaction produces can likely be mitigated by the buffering capacity of the lung lining fluid. Oxidative reactions between hypochlorite and nucleophilic lung lining constituents may also occur, with thiols being particularly sensitive to these oxidations (Squadrito et al., 2010). Disruption of local redox homeostasis may promote the inflammatory response through induction of redox sensitive stress signaling, or as a secondary response to protein oxidation with consequent alteration in function. Through its proposed oxidative reactions with lung lining fluid (Yadav et al., 2010) Cl_2 gas exposure may result in impaired surfactant activity with consequent mechanical organ dysfunction either from direct oxidative modification of lung lining components or potentially through signaling which alters type II pneumocyte function as a secondary effect.

To examine the proposal that pulmonary dysfunction results from altered lung lining fluid we have employed a murine whole-body Cl_2 gas exposure model that produces inflammation and mechanical dysfunction in the absence of overt injury. As outcomes we have measured the inflammatory response within the tissue and the BAL cells, surfactant composition and function, as well as organ-level lung mechanical function via forced oscillation technique and inverse modeling. Using broadband respiratory impedance measurements with increasing positive end expiratory pressure to recruit collapsed lung units allowed the measurement of pathophysiologic changes arising from heterogeneous responses to inhalation. Empiric modeling of respiratory impedance allowed direct comparison of resistance and elastance spectra between exposure conditions, with curve parameterization separating the contributions of proximal airways from distal/tissue components. Comparing measured surfactant function with computational analysis of respiratory mechanics supports the notion that small airway collapse contributes to the physiological mechanism underlying the mechanical dysfunction arising from low-dose Cl_2 inhalation.

Methods

Exposure and ventilation protocol. For a target chlorine exposure of 300 ppm for 1 h, 1% Cl_2 gas in N_2 (Scott Specialty gasses) was blended with compressed room air (Praxair) at 0.16 L/min and 5 L/min respectively using bubble flow meter. The two gas flow streams were mixed in a round-bottom flask with stir agitation prior to entry into the whole-body exposure chamber and allowed to equilibrate for 10 min before mice entered the chamber. All experiments using Cl_2 gas were conducted in a designated fume hood for toxic gas exposures with safety protocols developed in consultation with Rutgers Environmental Health Services. Protocol was developed for proper tank storage, transport, maintenance, regulator use and gas stream purging. As Cl_2 gas is a potent oxidizing agent, traffic near exposures was reduced; personnel in proximity to exposures were informed of risks of exposure as per the MSDS, proper emergency procedure in event of gas leak, required PPE (laboratory coat, gloves and protective eye wear) and monitoring of ambient gas concentration within the hood. All exposures were performed with chamber top closed and 4 outlet ports open to atmospheric air. In-chamber concentrations were monitored via one outlet port both with and without mice for 1 h, by drawing 0.6 L/min gas directly from the chamber into an ITX-4 multiple gas monitoring system (Industrial Scientific Corporation, Pittsburgh, PA). The Cl_2 concentration–time profile was fit to a logarithmic regression line and integrated in order to determine the effective exposure within the chamber. Pathogen free, male 10-week-old C57BL/6J mice (Jackson Labs, Bar Harbor, ME) received whole body exposure to Cl_2 gas in room air or room air alone for 1 h. Five mice were exposed in the chamber at a given time, with animals in each exposure randomized to measurement time points. All animal procedures were performed in accordance with institutional IACUC approved protocols as outlined in the *NIH Guide for the Care and Use of Laboratory Animals*.

At 3, 24 and 48 h following exposure mice were anesthetized with 300 μg ketamine/15 μg xylazine per gram of body weight via i.p. injection. Mice were tracheostomized and maintained on mechanical ventilation using the Flexivent-small animal ventilator (SCIREQ, Montreal, QC). Mechanical ventilation was performed using a quasi-sinusoidal inspiratory flow waveform to deliver tidal volume of 10 mL/kg of body weight at a rate of 120 breaths/min and 2:3 ratio of inspiratory to expiratory time. The protocol for measurement of pulmonary mechanical function was created to assess the effect of Cl_2 inhalation on two principal end-points: A) basal airway constriction as determined by increases in high-frequency resistance in the absence of methacholine challenge, and, B) small airway collapse assessed by an increase in effective pulmonary elastance that may be overcome with airway pressure support. To examine the pressure-responsiveness of these endpoints, measurements of respiratory mechanical function were made in triplicate at 5 levels of positive end expiratory pressure (PEEP = 0, 1, 3, 6, 9 cm H_2O). Prior experiments demonstrated that 1 min of ventilation is sufficient to allow for mechanical equilibration following step increases in PEEP. At each level of PEEP respiratory mechanical function was assessed using two approaches: broad-band respiratory impedance measurement for the partitioning of mechanical function into tissue and airway components and quasi-static pressure–volume loops for the evaluation of PEEP-dependent changes in effective elastance. Measurement of respiratory impedance and pressure–volume curves are detailed below.

Following ventilation, mice were euthanized by exsanguination and bronchoalveolar lavage (BAL) was performed with 1 mL of isotonic HEPES buffered saline × 4 washes. BAL cells were isolated by centrifugation (300 ×*g* for 10 min) and resuspended in 1 mL of sterile, phosphate buffered saline at pH 7.4 and counted using a Multisizer particle counter (Beckman Coulter, Brea, CA) with particles between 4 and 20 μm diameter considered as cells. The BAL cells were spun onto glass slides (30,000 cells/slide, 800 ×*g* for 3 min), air dried for 24 h, methanol fixed than stained with Diff-Quik buffered modified Wright–Giemsa stain for manual analysis of cell differentials. Remaining cells were lysed in Trizol for RNA collection. BAL supernatant was stored at −80 °C for protein, nitric oxide (NO) metabolite, phospholipid and surface tension analysis. Right lung lobes were isolated and tied off at the mainstem bronchus, then frozen at −80 °C for Western Blot and RT-PCR, while the left lung was inflation fixed with 3% paraformaldehyde in 2% sucrose and paraffin embedded for hematoxylin and eosin (H + E) stain and immunohistochemistry. All reagents were purchased from Sigma-Aldrich unless stated otherwise.

Histology and tissue inflammation scoring. Tissue sections were obtained from paraffin embedded lung tissue following inflation–fixation. Sections were H + E stained, examined by light microscopy and scored on a five point scale [0–4] for airway epithelial thickening/membrane blebbing, peribronchial/perivascular infiltration, septal thickening and interstitial destruction/airspace enlargement (Rudmann et al., 1998). The extent of inflammation and injury was independently scored by two blinded observers. Comparison of injury severity scores from Cl_2 exposed lungs to control was performed using the Wilcoxon