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Chlorine gas exposure disrupts nitric oxide homeostasis in the pulmonary vasculature☆

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ABSTRACT

Exposure to chlorine (Cl_2) gas during industrial accidents or chemical warfare leads to significant airway and distal lung epithelial injury that continues post exposure. While lung epithelial injury is prevalent, relatively little is known about whether Cl_2 gas also promotes injury to the pulmonary vasculature. To determine this, rats were subjected to a sub-lethal Cl_2 gas exposure (400 ppm, 30 min) and then brought back to room air. Pulmonary arteries (PA) were isolated from rats at various times post-exposure and contractile (phenylephrine) and nitric oxide (NO)-dependent vasodilation (acetylcholine and mahmanonoate) responses measured ex vivo. PA contractility did not change, however significant inhibition of NO-dependent vasodilation was observed that was maximal at 24–48 h post exposure. Superoxide dismutase restored NO-dependent vasodilation suggesting a role for increased superoxide formation. This was supported by ~2-fold increase in superoxide formation (measured using 2-hydroethidine oxidation to 2-OH-E^+) from PA isolated from Cl_2 exposed rats. We next measured PA pressures in anesthetized rats. Surprisingly, PA pressures were significantly (~4 mmHg) lower in rats that had been exposed to Cl_2 gas 24 h earlier suggesting that deficit in NO-signaling observed in isolated PA experiments did not manifest as increased PA pressures in vivo. Administration of the iNOS selective inhibitor 1400W, restored PA pressures to normal in Cl_2 exposed, but not control rats suggesting that any deficit in NO-signaling due to increased superoxide formation in the PA, is offset by increased NO-formation from iNOS. These data indicate that disruption of endogenous NO-signaling mechanisms that maintain PA tone is an important aspect of post-Cl_2 gas exposure toxicity.

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1. Introduction

Chlorine gas (Cl_2) is utilized in various manufacturing processes and is a chemical that is transported by rail in close proximity to significant population centers worldwide. High levels of Cl_2 gas exposure have been documented in numerous case reports of accidental release during transport or industrial use, or after intentional release in the military arena (Cevik et al., 2009; Evans, 2005; Jones et al., 2010; Matalon and Maull, 2010; Van Sickle et al., 2009; Wenck et al., 2007; White and Martin, 2010) which has led to recent interest into a more detailed understanding of the mechanisms of Cl_2 gas induced toxicity (Hoyle, 2010; Matalon and Maull, 2010; Samal et al., 2010; White and Martin, 2010).

Cl_2 gas toxicity is dependent on the dose and length of exposure with several studies showing that it comprises an initial 'during exposure' injury that is likely mediated by direct reactions of Cl_2 (or its hydrolysis product, hypochlorous acid) with biomolecules and a robust and chronic (days-weeks) 'post exposure' injury period (Donnelly and FitzGerald, 1990; Gunnarsson et al., 1998; Leustik

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et al., 2008; Martin et al., 2003; Tian et al., 2008; Tuck et al., 2008). The latter culminates in acute lung injury (ALI), adult respiratory distress syndrome (ARDS) and reactive airway syndrome (RAS) and reflects increased permeability and inflammatory tissue injury. Several mechanisms have been identified including loss of endogenous antioxidants and surfactant, stimulation of inflammation, epithelial and alveolar ion transport dysfunction and activation of sensory neurons to name but a few (Bessac and Jordt, 2010; Fanucchi et al., 2012; Lazrak et al., 2012; Leikauf et al., 2012; Song et al., 2010; Tian et al., 2008; Tuck et al., 2008).

Notably, these insights have centered on Cl_2-dependent injury to airway (bronchial), and distal lung (alveolar type II) epithelial, cell function (Lazrak et al., 2012; Song et al., 2010). It is important to recognize however, that injury is not limited to the epithelial cells. Our previous studies documented significant dysfunction in systemic vascular function, illustrated by a loss of control over nitric oxide-dependent vasodilation (Honavar et al., 2011). Specifically, post-Cl_2 gas toxicity led to an inhibition of endothelial nitric oxide synthase (eNOS) activity and an increase in inducible nitric oxide synthase (iNOS) activity in the systemic circulation. eNOS derived NO is a critical modulator of vascular homeostasis mechanisms including regulating vessel tone, cellular respiration, inhibiting smooth muscle proliferation, maintaining an anti-thrombotic and anti-inflammatory luminal surface (Moncada, 1999; Napoli and Ignarro, 2009) and critical in maintaining low pulmonary arterial pressures. Moreover, a deficit in NO-bioavailability, is one mechanism for pulmonary arterial hypertension. If and how Cl_2 gas exposure affects regulation of pulmonary arterial tone is unknown and was tested in this study.

2. Materials and methods

2.1. Materials

Unless stated otherwise all reagents and antibodies were purchased from Sigma (St. Louis, MO, USA) and AbCam (Cambridge, MA, USA) respectively except Mahma/NONOate (MNO) which was purchased from Axxora Platform (San Diego, CA, USA). Male Sprague Dawley rats (200–300 g) were purchased from Harlan (Indianapolis, IN, USA) and kept on 12 h light dark cycles with access to standard chow and water ad libitum prior to and post chlorine exposure. 1400W was purchased from Enzo Life Sciences International, Inc. (Plymouth Meeting, PA, USA). Hydroethidine was purchased from Invitrogen and 2-OH-E^+ standards were synthesized as previously described (Zielonka et al., 2008).

2.2. Rat exposure to chlorine gas

Whole body exposure of rats (Harlan Laboratories, USA) to 400 ppm Cl_2 gas was performed as previously described (Leustik et al., 2008) and according to IACUC approved protocols. This is a sub-lethal exposure protocol that results in significant acute lung injury and chronic development of reactive airways and systemic endothelial dysfunction. Two rats were exposed in the same chamber at any one time and all exposures were performed between 8 and 9 am, and were 30 min in length followed by return to room air. Two mass flow controllers (MFCs) with Kalrez seals (Scott Specialty Gases, Los Angeles, CA; part no. 05236A1V5K) and a microprocessor control unit (Scott Specialty Gases; part no. 05236E4) were used to control the compressed air and Cl_2 (1000 ppm Cl_2 in air; Airgas, Birmingham, AL) flow rates to achieve the chamber Cl_2 target concentrations. A bubble flow meter was used to validate MFC performance on a weekly basis. Air and Cl_2 were initially mixed at a three-way junction, and they were further mixed by passing through a diffuser located inside the top lid of the exposure chamber. Gases exited the chamber via two large-bore diameter ports in its bottom half. The exposure chamber was placed inside a chemical fume hood located in a negative-pressure room. At the end of each exposure, the Cl_2 gas was turned off, the chamber was vented with compressed air for 2–3 min, the two halves were separated, and the rats were removed and returned to their cages, where they breathed room air. Food and water were provided ad libitum.

2.3. Pulmonary artery pressure measurements

Sprague-Dawley rats weighing 200–300 g were divided into 4 groups, 3 of which were exposed to 400 ppm Cl_2 gas for 30 min. These were then subdivided into chlorine only, chlorine + 1400W dihydrochloride and chlorine + L-NMMA groups. One group of rats was not exposed to chlorine and used as air controls. 24 h after Cl_2 exposure rats were anesthetized and PA pressures measured as described below. Then animals in the Cl_2 only group received 200 μl IP injection of PBS while rats in chlorine + 1400W dihydrochloride and chlorine + L-NMMA group received intravenous injection of 10 mg/kg dose of 1400W dihydrochloride or 10 mg/kg dose of L-NMMA respectively and PA pressure measured again 60 min post injection. For PA pressure measurements, rats were anesthetized using isoflurane and the jugular vein exposed above the clavicle. An incision was made in the external jugular vein and a polyethylene tube (introducer) with internal diameter of 0.76 mm and outer diameter of 1.2 mm (PE-60, Clay Adams) was inserted into the external jugular vein and advanced until it reached the right ventricle (RV). Relative position of the introducer was monitored via pressure transducers using AcqKnowledge software with Biopac system. Once the introducer reached the RV it was detached from the pressure transducer and the pressure catheter was attached. This catheter was a silastic tubing of Micro Renathane (MRE025, Braintree Scientific, MA). The catheter was inserted into the introducer and extended past the tip of the introducer till it reached the RV. Once in the RV, the catheter was further advanced until the pressure traces resembled those of PA pressures measured by previous investigators (Rabinovitch et al., 1979). After every measurement, rats were sacrificed by exsanguination and the position of the catheter in the PA verified by opening of the chest cavity and dissection. Data were excluded if it was determined post-measurement that the catheter was pressed against the PA wall.

2.4. Isolated pulmonary artery studies

At the indicated times post Cl_2 exposure, pulmonary arteries were collected, cleansed of adherent fat and responses to the indicated vasoconstrictive and vasoactive stimuli determined in vessel bioassay chambers (Radnoti, Monrovia, CA), as previously described (Honavar et al., 2011). All vessel bioassay studies were performed in indomethacin (5 μM) pre-treated vessels (2–3 mm segments) and in bicarbonate buffered Krebs Henseleit buffer of the following composition (mM): NaCl 118; KCl 4.6; $NaHCO_3$ 27.2; KH_2PO_4 1.2; $MgSO_4$ 1.2; $CaCl_2$ 1.75; Na_2 EDTA (ethylenediaminetetraacetic acid) 0.03, and glucose 11.1 and perfused with 21% O_2, 5% CO_2 balanced with N_2. A passive load of 1.5 g was applied to all ring segments and maintained at this level throughout the experiments. At the beginning of each experiment ring segments were depolarized with KCl (70 mM) to determine the maximal contractile capacity of the vessel. Rings were then washed extensively and allowed to equilibrate and again depolarized with KCl (70 mM). The rings were then washed and allowed to re-equilibrate. Vasoconstrictor responses were tested by cumulative addition of phenylephrine (PE) doses ranging from 1 nM to 3000 nM. Endothelium-dependent vasodilator responses were tested by administering cumulative doses of acetylcholine (Ach), ranging from 1 nM to 3000 nM after tension development at maximal PE dose. In subsequent experiments, vessels were sub-maximally contracted (50% of KCl response) with PE (300 nM–1000 nM). When tension development reached a plateau, endothelium-independent vasodilator responses were induced by administering the NO

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