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Phosgene- and chlorine-induced acute lung injury in rats: Comparison of cardiopulmonary function and biomarkers in exhaled breath

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ABSTRACT

This study compares changes in cardiopulmonary function, selected endpoints in exhaled breath, blood, and bronchoalveolar lavage fluid (BAL) following a single, high-level 30-min nose-only exposure of rats to chlorine and phosgene gas. The time-course of lung injury was systematically examined up to 1-day post-exposure with the objective to identify early diagnostic biomarkers suitable to guide countermeasures to accidental exposures. Chlorine, due to its water solubility, penetrates the lung concentration-dependently whereas the poorly water-soluble phosgene reaches the alveolar region without any appreciable extent of airway injury. Cardiopulmonary endpoints were continually recorded by telemetry and barometric plethysmography for 20 h. At several time points blood was collected to evaluate evidence of hemoconcentration, changes in hemostasis, and osteopontin. One day post-exposure, protein, osteopontin, and cytodifferentials were determined in BAL. Nitric oxide (eNO) and eCO_2 were non-invasively examined in exhaled breath 5 and 24 h post-exposure. Chlorine-exposed rats elaborated a reflexively-induced decreased respiratory rate and bradycardia whereas phosgene-exposed rats developed minimal changes in lung function but a similar magnitude of bradycardia. Despite similar initial changes in cardiac function, the phosgene-exposed rats showed different time-course changes of hemoconcentration and lung weights as compared to chlorine-exposed rats. eNO/eCO_2 ratios were most affected in chlorine-exposed rats in the absence of any marked time-related changes. This outcome appears to demonstrate that nociceptive reflexes with changes in cardiopulmonary function resemble typical patterns of mixed airway-alveolar irritation in chlorine-exposed rats and alveolar irritation in phosgene-exposed rats. The degree and time-course of pulmonary injury was reflected best by eNO/eCO_2 ratios, hemoconcentration, and protein in BAL. Increased fibrin in blood occurred only in chlorine-exposed rats 1-day post-exposure. Hence, the analysis of NO and CO_2 in exhaled breath, including endpoints in blood mirroring changes in the peripheral to pulmonary fluid distribution, seem to be sensitive diagnostic endpoints readily available for early prognostic assessment of severity of injury and efficacy of any chosen countermeasure.

1. Introduction

Phosgene and chlorine gas are high production volume (HPV) chemicals used extensively in numerous industrial processes. At HPV production-scales phosgene is commonly produced 'on demand' by catalyzed gas phase reactions of carbon monoxide and chlorine. Exposure of both humans and laboratory animals to high concentrations of these gases has generally described to result in a spectrum of abnormalities consolidated by the 'hypernyms' acute lung injury (ALI) and acute/adult respiratory distress syndrome (RADS). The prevailing symptomology of both chlorine and phosgene involved in respiratory tract toxicity may be somewhat similar; however, not necessarily their adverse outcome pathways (AOP). Extensive research on these irritant gases has been subject of multiple reviews which reflection is beyond the objective of this paper (for references see below). Current

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treatments for accidental chlorine and phosgene inhalation in humans are largely supportive, much of which are based on reported experience and anecdotal evidence (White and Martin, 2010). Mechanism-based causal countermeasures require an in-depth understanding of the AOPs initiating and amplifying the respective life-threatening condition. Although several of the outcomes of acute inhalation exposures to these gases are predictable, none appear to be specific enough to readily identify the causing agent.

The irritant nature and potential contemporaneous presence of phosgene and chlorine may prompt considerations on 'common modes of action' and equal mitigation strategies suitable for both. Chlorine (Cl_2) is a highly reactive oxidant which dissolves into fluids lining the mucosal surfaces and airways. Its water solubility and direct oxidative injury to the epithelium of first contact is believed to be the primary cause of its respiratory tract toxicity. Cl_2 depletes antioxidants and undergoes hydrolysis to generate hypochlorous (HOCl) and hydrochloric acid (HCl) (Leikauf et al., 2012; Leustik et al., 2008; White and Martin, 2010; Yadav et al., 2010, 2011). All of them may contribute to the initiation and progression of a site- of retention-related localized injury. As referred to above, the sites of injury depend on the inhaled concentration with low-to-intermediate concentrations predominating an enduring airway inflammation and high concentrations causing a life-threatening mix of airway and alveolar damage phenotypically manifested as acute pulmonary edema rapid in onset (White and Martin, 2010,b; Jonasson et al., 2013a,b). Seminal research established a central role of low-molecular-weight antioxidants in the prevention and mitigation of Cl_2-induced injury to both the airway and alveolar epithelium (Leustik et al., 2008; Martin et al., 2003; Yadav et al., 2010, 2011; Zarogiannis et al., 2011, 2014). Industrial accident victims exposed to about 400 ppm Cl_2 and beyond over 30 min were reported to develop potentially fatal pulmonary edema (Weill et al., 1969). Acute rat inhalation studies report a median lethal concentration (LC_{50}) of 700 ppm at 30 min exposure duration (Zwart and Woutersen, 1988). The respective 1-hour LC_{50} was in the range of 293–455 ppm (MacEwen and Vernot, 1972; AEGL, 2004; Back et al., 1972; Withers and Lees, 1985a,b, 1987; Zwart and Woutersen, 1988). The onset of mortality was concentration-dependent, that is 35% of rats exposed for 30 min succumbed within post-exposure days 2–6 whereas 21% of rats that were exposed at lower concentrations for 60 min died between post-exposure days 2 and 11 (Zwart and Woutersen, 1988). These results indicate that the extent of lung injury following chlorine inhalation depends not only on the total dose inhaled (=concentration × exposure duration) but also on the specifics of exposure concentration and time, suggesting that countermeasures against chlorine-induced acute lung injury have to appreciate this concentration-dependent characteristics of mixed airways/alveolar injury (Hoyle et al., 2010). At lethal exposure conditions concentration-dependent effects on ventilation are expected maximal bearing in mind that the RD50 (50% depression of respiratory rate) of chlorine in rats is 1-order of magnitude lower than the 1-hour LC_{50}–range (Barrow and Steinhagen, 1982).

Phosgene (carbonyl chloride, $COCl_2$) is highly reactive, acylating gas reacting with nucleophilic moieties at its site of first contact. Due to its low water solubility phosgene gas is not retained at airways level to any appreciable extent and injury is generally confined to the alveolar level. Nucleophiles administered prophylactically prior to phosgene exposure were highly protective. However, when administered therapeutically post-exposure they were ineffective (Diller, 1980; Pauluhn and Hai, 2011). Previous inhalation studies with phosgene in rats yielded a non-lethal time-adjusted threshold concentration (LCt_{01}) of 250 ppm × min. Opposite to chlorine, phosgene-induced pulmonary injury was clearly Cxt-related with minimal, if any, modifying concentration-dependent effects (apart from transient changes in ventilation; Pauluhn, 2006a). The ensuing pulmonary edema reaches its climax about 1 day post-exposure (Pauluhn, 2006a,b,b). An involvement of different types of nociceptive reflexes leading to bradycardia and dysregulated hemodynamics was postulated elsewhere (Li et al., 2013). Interestingly, somewhat similar reduction in heart rate were demonstrated after acute chlorine inhalation exposure of rats at 500 ppm for 30 min) which was associated with decreased total ATP content and loss of sarco-endoplasmic reticulum calcium ATPase, SERCA, activity (Ahmad et al., 2014). It is beyond the scope of this paper to reiterate the wealth of information available on mechanistic research of phosgene (Diller, 1985a,b,b; Duniho et al., 2002; IPCS, 1998; Li et al., 2013; Liu et al., 2013; Luo et al., 2013; Pauluhn et al., 2007).

The focus of this paper is to compare time-course changes in cardiopulmonary function and endpoints considered to integrate pulmonary inflammation and edema occurring within the first 24 h post-exposure of rats acutely exposed to chlorine and phosgene at exposure intensities producing a somewhat similar degree of pulmonary edema within this time period. This condition seemed to be fulfilled at exposure intensities of about 240 and 12,000 ppm × min of phosgene and chlorine, respectively. With regard to translational toxicology and clinical needs, it appears timely to identify early, non-invasive diagnostic and prognostic biomarkers of chlorine- and phosgene gas-induced acute lung injury suitable for triage and to rationalize treatment.

2. Materials and methods

2.1. Test materials

Phosgene (carbonyl chloride) and chlorine gases were from Linde, Germany (certified gases contained in pressurized cylinders; the certified concentrations of phosgene and chlorine in synthetic air were 150 and 413 ppm, respectively). The conversion factors applied for phosgene and chlorine were 1 ppm = 4.1 mg/m^3 and 2.9 mg/m^3, respectively. Nitric oxide in synthetic air ($NO_x \leq 0.1$ ppm specified, NO ~11 ppb measured) was also from Linde.

2.2. Animals, diet, and housing conditions

Healthy SPF-bred young adult male Wistar rats (8–9 weeks of age, 200–250 g), strain Hsd Cpb:WU, from the experimental animal breeder Harlan-Nederland (NL) were acclimatized to the housing and nose-only exposure conditions for at least 5 days and then sequentially assigned at random to the respective exposure group. Animals were housed single in polycarbonate cages, containing low dust wood bedding. Feed and water were accessible ad libitum except during inhalation exposures. Animal holding rooms were maintained at approximately 22 °C with relative humidity of 40%–60% and a 12-hour light cycle beginning at 0600 h. This study was conducted in an animal care-approved laboratory in accordance with the current German Animal Welfare Act and European Council Directive 2010/63/EU (Directive 2010/63/EEC, 2010) on the 'Care and Protection of Animals Used for Experimentation and other Scientific Purposes' and observed those called for by OECD (2009).

2.3. Experimental protocol

Three groups of rats were sequentially nose-only exposed for 30 min to conditioned air, ~8 ppm phosgene, and 413 ppm chlorine gas. Each group consisted of 32 rats; out of these 8 were intraperitoneally implanted with telemetry transmitters and were also used for lung function measurements.