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Single high-dose dexamethasone and sodium salicylate failed to attenuate phosgene-induced acute lung injury in rats

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ABSTRACT

Life-threatening acute lung injury potentially occurs following high-level accidental exposures to phosgene gas. This situation was mirrored in rats exposed nose-only at 900–1000 mg phosgene/m^3 min. At this exposure level, previous studies on rats demonstrated sustained reflexively induced cardiopulmonary dysfunction and evidence of vascular fluid redistribution. These findings challenge the currently applied treatment strategies to mitigate the presumed non-cardiogenic lung edema by steroidal or non-steroidal anti-inflammatory drugs. This study investigates whether high doses of curatively administered dexamethasone (DX; 100 mg/kg bw, ip) and sodium salicylate (SS; 200 mg/kg bw, ip), alone or in combination, show efficacy to mitigate the phosgene-induced lung edema. Exhaled nitric oxide (eNO), animal morbidity and mortality, and increased lung weights one day postexposure served as endpoints of lung injury and drug efficacy. When applying this dosing regimen, SS showed minimal (if any) efficacy while DX, alone or in combination with SS, substantially aggravated the emerging lung edema (lung weights) with 40% mortality. The degree of acute lung injury (ALI) was mirrored by increased eNO. Its direct relationship to ALI-severity was evidenced by decreased eNO following NO-synthetase inhibitor administration (aminoguanidine-aerosol) and associated mitigation of ALI. All non-treated phosgene-exposed as well as treated but non-phosgene-exposed rats survived. This experimental evidence suggests that high-dose corticoid treatments may aggravate the pulmonary toxicity of phosgene. Similarly, this outcome supports the supposition that non-inflammatory, cardiogenic and/or neurogenic factors play a role in this type of acute lung injury.

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1. Introduction

Phosgene (carbonyl chloride) is manufactured from the reaction of carbon monoxide and chlorine gas in the presence of activated charcoal as catalyst. It is an important and indispensable high production volume intermediate in the manufacture of building blocks of various types of plastics and materials. Notably phosgene is produced on demand, *i.e.*, its storage and transportation is discouraged in most countries. Of medical concern are accidental high-level phosgene exposures in production plants (workers) and its usage in terroristic activities causing a mass casualty situation. Evidence of clinically significant edema and onset of mortality has been reported to occur in individuals exposed accidentally at approximately >600 mg/m^3 min and 1200 mg/m^3 min, respectively (Diller, 1985). The phosgene exposure dose on rats used in this study was within this range (1000 mg/m^3 min).

The toxicity of inhaled phosgene gas is confined to the lower respiratory tract as reviewed in detail elsewhere (Pauluhn et al., 2007). Due to its poor water solubility, this gas penetrates the lower respiratory tract without marked retention within the conducting airway and interaction with upper respiratory tract trigeminal sensory nerve endings (Pauluhn, 2006a). Once retained in the lower airways, it may stimulate C-fiber-related nociceptive nerves evidenced by a sustained reflexively induced apnea time, bradycardia, and hemoconcentration early in onset (Pauluhn, 2006a; Li et al., 2013). A similar type of protective vagus reflex-related changes in respiration was also observed in humans (Diller, 1985). Upon inhalation phosgene does not hydrolyze rather than undergoes acylating reactions with nucleophilic moieties. Amines and thiols are amongst the most avidly acylated moieties, often associated

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with changes in redox-sensitive biochemical processes (Pauluhn et al., 2007; Sciuto et al., 1995, 1996; Sciuto and Hurt, 2004). While the post-exposure supplementation with nucleophiles had little or no effect, their prophylactic supplementation abrogates almost entirely the pulmonary toxicity of phosgene (Diller, 1980; Pauluhn and Hai, 2011). Collectively, past (Bruner et al., 1948) and recent (Li et al., 2013) published evidence supports the hypothesis that the pulmonary toxicity of inhaled phosgene seems to elicit a dose-dependently a more cardiogenic pulmonary edema at yet non-lethal exposure doses and a non-cardiogenic edema at lethal exposure intensities. For this type of drug intervention study, the highest level of scientific information as well as human relevance can be gained at an exposure dose of phosgene amenable to study the climax of lung edema approximately 20–24 h post-exposure (Diller, 1985; Pauluhn, 2006b; Pauluhn et al., 2007).

Steroids were reported to be neither beneficial nor particularly detrimental in the treatment of phosgene lung edema and their usage is limited by the side effects which could diminish the chances for recovery (Grainge and Rice, 2010a; de Lange and Meulenbelt, 2011). Assessment of the benefit of corticoids in treating phosgene-induced acute lung injury seems to be complicated by the variability in treatment regimen used. In regard to route, dose, and dosing frequency relative to the exposure to phosgene optimal treatment regimen for this indication could not be identified. One characteristic of phosgene-induced lung injury is the increase in peripheral white blood cell counts (pigs exposed at 2500 mg/m^3 min; Grainge et al., 2010b) which has also been observed in dogs subjected to a similar exposure dose (unpublished findings from this laboratory). Also in rats an increased trafficking of neutrophils from blood to the alveoli is amongst the most sensitive endpoints following exposure to phosgene (Pauluhn, 2006b; Pauluhn et al., 2007). Glucocorticoids control the interaction of leukocytes (PMN) with the vessel wall by affecting both the leukocyte responsiveness and endothelial-cell reactivity (Igarashi et al., 2013; Perretti and D'Acquisto, 2009). The hypercellularity caused by PMNs may attribute to the stagnant blood blow and anoxia described by Bruner et al. (1948). Dexamethasone (DX) has been successfully used in the clinical treatment of paraquat poisoning, its positive effects being attributed to the down-regulation of neutrophil recruitment, collagenase activity, and proliferation of Type II pneumocytes (Dinis-Oliveira et al., 2006a,b 2007a,b). All these factors are conducive to mitigate the phosgene-induced lung edema as well. Sodium salicylate (SS) has remarkable hydroxyl scavenging properties, which leads to neuro- and cytoprotection in models of ischemia/reperfusion injury. Transcription factors, such as NF-κB and AP-1, are responsible for the expression of a myriad of pro-inflammatory factors, pro-oxidant enzymes and cytokines and inhibits cyclooxygenase (Baltazar et al., 2011; de Lange and Meulenbelt, 2011; Dinis-Oliveira et al., 2007a,b). The antioxidant, anti-inflammatory, anti-apoptotic and anti-thrombogenic properties of SS proved to protect lungs from paraquat poisoning (Dinis-Oliveira et al., 2007b). In fact, the accumulated properties of both DX and SS should also be applicable to phosgene poisoning as long the etiology follows a non-cardiogenic, inflammatory pathway.

The main focus of study was to challenge current treatment paradigms focusing, *inter alia*, on anti-inflammatory mitigation strategies typical for non-cardiogenic types of lung edema. This included an analysis whether phosgene-induced acute lung injury is unequivocally linked to increased levels of exhaled nitric oxide (eNO), a non-invasive tool to follow-up phosgene-induced acute lung injury and drug efficacy. An ancillary proof of concept study on rats investigated the therapeutic impact of inhalation treatments with the NO-synthetase inhibitor (aminoguianidine aerosol). This served the purpose of studying whether eNO and the lung water content change in concert.

2. Materials and methods

2.1. Test materials

Phosgene (carbonyl chloride), certified gas of 150 ppm in synthetic air contained in 10 L cylinders @150 bar, was from Linde, Germany. The conversion 1 ppm in 4.1 mg/m^3 phosgene is based on 25 °C and 1 atm. Synthetic air (20% oxygen in 80% nitrogen ($NO_x \leq 0.1$ ppm specified, NO ~ 11 ppb measured) was also from Linde. Aminoguanidine (aminoguanidine hemisulfate salt), dexamethasone, and sodium salicylate were from Sigma, Germany.

2.2. Animals, diet, and housing conditions

Healthy male SPF-bred Wistar rats of the strain Hsd Cpb:WU from the experimental animal breeder Harlan-Nederland (NL), AD Horst were used. Animals were placed in polycarbonate cages containing bedding material. Both feed and water were given *ad libitum* except during inhalation exposures. Animal rooms were maintained at approximately 22 °C with relative humidity of 40–60% and a 12-h light cycle beginning at 0600 h. The studies described were in accordance with contemporary, internationally harmonized testing standards/guidelines (OECD, 2009, Available at: http://oberon.sourceoecd.org). The experiments were performed in an animal care-approved laboratory in accordance with the German Animal Welfare Act and European Council Directive 86/609/EEC (Directive 86/609/EEC, 1986) as well as the updated Directive 2010/63/EU as of 22 September 2010.

2.3. Experimental protocol

Male Wistar rats were randomly allocated into 8 groups as shown in Table 1. The exposure dose of phosgene (~1000 mg/m^3 min; ~35 mg/m^3 for 30 min) was in the range of the maximum non-lethal concentration, and similar exposure intensities to phosgene have not produced any consistent evidence of mortality beyond the postexposure period used in this study (Pauluhn, 2006a,b; Pauluhn et al., 2007). Signs and survival rate were recorded systematically once per day as called for by OECD (2009). Body weights were collected before treatment and at the day of necropsy. Measurements started approximately 20 h postexposure to phosgene, the time point where lung edema has been shown to be maximal (Pauluhn, 2006b). Shortly after eNO analysis the rats were anesthetized using sodium pentobarbital (Narcoren®; 120 mg/kg body weight, intraperitoneal injection). After exsanguination (by severing of the abdominal aorta), the weight of the excised lungs was determined. Body weight gains represent the weight change between phosgene exposure and sacrifice. This study examined DX and SS alone as well as in combination in control and phosgene-exposed rats, and compared the outcome based on the endpoints detailed above. The dosages used were those shown to be efficacious following paraquat poisoning of rats (Dinis-Oliveira et al., 2006a, 2007a,b).

2.4. Exposure to phosgene

Details were published elsewhere (Pauluhn, 2006a). In brief, rats were exposed under highly controlled conditions to phosgene gas by directed-flow nose-only inhalation during an exposure period for 30 min. Temperature and humidity measurements in the inhalation chamber were performed by a computerized Data Acquisition and Control System using HC-S3 sensors (Rotronic, http://www.rotronic-usa.com/prod_oem/hc2%20probes/hc2_main.htm). All airflow controllers were calibrated using a Bios DryCal Defender 510 (http://www.smglink.com/bios/drycaldefender.html). The exposure conditions were adjusted to maintain an airflow rate of 0.75 L/min per exposure port. Actual concentrations were determined real-time using a calibrated Gasmet Dx-4000 FT-IR (Fourier transform infrared spectroscopy) gas analysis system.

2.5. Measurement of NO and CO_2 in exhaled breath and breathing rate

The measurement of eNO in rats was published in detail previously (Liu et al., 2013). It was shown that the diagnostic sensitivity of eNO in spontaneously tidal breathing and conscious rats could be markedly improved when accounting for the normalization of the following physiological variables: exhaled carbon dioxide (CO_2) as an indirect measurement of lung perfusion and NO-reabsorption as well as breathing frequency as a substitute for exhaled volume. The latter is important to consider as measurements in small animals require an additional flow of sheath air to overcome the limitation of small tidal volumes. Time course measurements of eNO following exposure were reported elsewhere (Liu et al., 2013) and were omitted in this comparative study.

Briefly, NO was analyzed real-time using a chemi-luminescence analyzer (Sievers 280B NOA; Sievers Instrument, Inc., Denver, CO). Synthetic air was used as sheath air during measurements of eNO. The actual concentration of eNO was not accounted for the residual back ground level of NO which was approximately 15 ppb. Measurements were made in two-compartment head-out volume displacement plethysmographs (in-house design). The exhaust flow from the head-out compartment was split *via* a manifold allowing for a

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