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N-acetyl cysteine improves the effects of corticosteroids in a mouse model of chlorine-induced acute lung injury

Elisabeth Wigenstam [a], Bo Koch [a], Anders Bucht [a,b], Sofia Jonasson [a,*]

[a] Swedish Defence Research Agency, Division of CBRN Defence and Security, Umeå, Sweden
[b] Department of Public Health and Clinical Medicine, Division of Respiratory Medicine, Umeå University, Sweden

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ABSTRACT

Chlorine (Cl_2) causes tissue damage and a neutrophilic inflammatory response in the airways manifested by pronounced airway hyperreactivity (AHR). The importance of early anti-inflammatory treatment has previously been addressed. In the previous study, both high-dose and low-dose of dexamethasone (DEX) decreased the risk of developing delayed effects, such as persistent lung injuries, while only high-dose treatment could significantly counteract acute-phase effects. One aim of this study was to evaluate whether a low-dose of DEX in combination with the antioxidant *N*-acetyl cysteine (NAC) and if different treatments (Triptolide, Reparixin and Rolipram) administered 1 h after Cl_2-exposure could improve protection against acute lung injury in Cl_2-exposed mice.

BALB/c mice were exposed to 300 ppm Cl_2 during 15 min. Assessment of AHR and inflammatory cells in bronchoalveolar lavage was analyzed 24 h post exposure. Neither of DEX nor NAC reduced the AHR and displayed only minor effects on inflammatory cell influx when given as separate treatments. When given in combination, a protective effect on AHR and a significant reduction in inflammatory cells (neutrophils) was observed. Neither of triptolide, Reparixin nor Rolipram had an effect on AHR but Triptolide had major effect on the inflammatory cell influx. Treatments did not reduce the concentration of either fibrinogen or plasminogen activator inhibitor-1 in serum, thereby supporting the theory that the inflammatory response is not solely limited to the lung.

These results provide a foundation for future studies aimed at identifying new concepts for treatment of chemical-induced lung injury. Studies addressing combination of anti-inflammatory and antioxidant treatment are highly motivated.

1. Introduction

Inhaled chlorine (Cl_2) can lead to a wide variety of respiratory injuries in both upper and lower airways (Evans 2005; White and Martin, 2010; Williams 1997). The onset of acute symptoms ranges from minutes to hours and due to the high reactivity of Cl_2, the occurrence of effects is generally limited to the exposed tissues (Evans 2005; Kales and Christiani, 2004).

Acute damages after Cl_2-exposure shown in mice include for instance epithelial sloughing, increased protein content in bronchoalveolar lavage (BAL) fluid, decreased respiratory function, and an inflammatory response with neutrophil and macrophage recruitment into the airways (Demnati et al., 1998; Johansson and Curstedt, 1997; Koohsari et al., 2007; Martin et al., 2003; Morris et al., 2005; Tuck et al., 2008). In a previous mouse study conducted in our laboratory, many of the findings are similar to symptoms described for reactive airways dysfunction syndrome (RADS) in humans. Examples of similar symptoms are the inflammatory response in lung tissue and regeneration of the lung epithelium during the first seven days after exposure to 200 ppm Cl_2 as well as the persistence of hyperreactive airways for at least 28 days together with a deposition of collagen in the lung (Jonasson et al., 2013a; Jonasson et al., 2013b). The importance of early anti-inflammatory treatment in this mouse model of chemical-induced lung injury has previously been addressed. In that study, both a high-dose (100 mg/kg) and relatively a low-dose (10 mg/kg) of dexamethasone (DEX)

Abbreviation: AHR, airway hyperresponsiveness; ALI, acute lung injury; BAL, bronchoalveolar lavage; Cl_2, chlorine; C_{RS}, respiratory compliance; DEX, dexamethasone (low-dose); G, tissue resistance; H, tissue elastance; HDEX, dexamethasone (high-dose); i.p., intraperitoneal; i.t., intratracheal; MCh, methacholine; NAC, *N*-acetyl cysteine; PAI-1, plasminogen activator inhibitor-1; RADS, reactive airway dysfunction syndrome; R_{RS}, respiratory resistance.

* Corresponding author at: Division of CBRN Defence and Security, Swedish Defence Research Agency, SE–901 82 Umeå, Sweden. Tel.: +46 90 10 67 45; fax: +46 90 10 68 00.
E-mail address: sofia.jonasson@foi.se (S. Jonasson).

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decreased the risk of developing delayed effects e.g., persistent lung injuries, while only high-dose treatment could significantly counteract acute-phase effects. These results provide a foundation for future studies aimed at identifying new concepts for treatment of chemical-induced lung injury. In particular, studies addressing combination of anti-inflammatory and antioxidant treatment are highly motivated (Jonasson et al., 2013b). In the mouse model of alkylating nitrogen mustard exposure we have shown that treatment with the antioxidant vitamin E (α-tocopherol) can reduce the acute inflammatory cell influx, and suppress the collagen formation in lung tissue to some extent (Wigenstam et al., 2009). Based on those data we suggest that antioxidants can be used in combination with corticosteroids to improve the protection against chemical-induced lung injury.

A paper by De Lange et al. (de Lange and Meulenbelt, 2011) points to that clinical data on corticosteroid efficiency after human exposure to lung-damaging agents (e.g., Cl_2, ammonia and ozone) are inconclusive and that there has been a lack of human-controlled studies of high-dose exposure to lung-damaging agents. Previous small animal studies in mice and rats have focused on other types of treatments than corticosteroids to counteract the acute toxic effects. For example, antioxidants have been reported to be effective as rescue treatment for Cl_2-induced airway hyperresponsiveness (AHR), airway inflammation, injury-induced airway epithelial cell regeneration, and oxidative stress. Antioxidants also improved survival following lethal doses of Cl_2 (Akdur et al., 2008; Chang et al., 2012; McGovern et al., 2010; Zarogiannis et al., 2011).

In the present study, a low dose of the corticosteroid both with concomitant antioxidant *N*-acetyl cysteine (NAC) or in combination with Reparixin and other anti-inflammatory substances (Rolipram or Triptolide) was administered intraperitoneally (i.p.) to evaluate whether a combination treatment or different kind of anti-inflammatory substances could improve protection against acute lung injury in Cl_2-exposed mice. A low dose of DEX was administered as a repeated treatment (1 h + 6 h following Cl_2- exposure), as an aerosol or as an intratracheal (i.t.) treatment. The study focused on acute lung injury and inflammation that occurs 24 h after exposure. To address this aim we used a murine nose-only exposure system together with a small animal ventilator (flexiVent™, SCIREQ®, Montreal, Canada) in order to evaluate respiratory mechanics and airway reactivity in response to the various treatments after Cl_2-exposure. In addition to airway physiology measurements, we performed extensive evaluation of inflammatory cells in BAL fluid, analysis of a coagulation marker, fibrinogen, and analysis of the fibrinolytic protease inhibitor, plasminogen activator inhibitor-1 (PAI-1), both in serum.

2. Materials and methods

2.1. Animals

Female BALB/c mice (8–10 weeks old, Harlan laboratories, Netherlands) were used in this study. Animals were housed in plastic cages with absorbent bedding material and were maintained on a 12 h daylight cycle, 22 °C, with a 30% relative humidity. Food (R36, Lantmännen, Sweden) and water were provided ad libitum. All mice were weighed before subjected to Cl_2, and following exposure their health condition was monitored. All experiments were terminated 24 h post exposure ($n = 6$–8 mice per group with the exception of i.t. instillation treatment group; $n = 2$). The care of the animals and the experimental protocols were approved by the regional ethics committee on animal experiments in Umeå, Sweden

2.2. Cl_2-exposure protocol

Animals were placed in individual nose-only containers (EMMS, UK) and coupled to an inhalation tower (Battelle) providing equal and simultaneous exposure to Cl_2 (Air Liquide, Germany; compressed gas in gas cylinders: 1 mol% Cl_2, 99 mol% nitrogen). The compressed gas mixture was diluted with air to the final concentration of 300 ppm. Mice were subjected to a single exposure of Cl_2-gas mixture during 15 min. The Cl_2 concentration in the inhalation tower was monitored throughout the exposure time and the experiments were conducted in a designated fume hood for toxic gas exposures. The Cl_2 concentration was controlled with a mass flow regulator and the generated concentration was monitored with a chlorine detector. Control animals were breathing room air for 15 min.

2.3. Interventions

2.3.1. Dexamethasone

The dose of DEX (Dexamethasone 21-phosphate disodium salt, Sigma–Aldrich (St. Louis, MO, USA) 10 or 100 mg/kg body weight) was determined and modified from our previous study where DEX was administered 1 h following Cl_2-exposure (Jonasson et al., 2013b). A low dose of DEX (10 mg/kg body weight) was also administered as a repeated treatment (1 h + 6 h following Cl_2-exposure) and as an i.t. administration. DEX was dissolved in 200 μl phosphate-buffered saline (PBS, Sigma–Aldrich, USA) for i.p. injections and in 50 μl PBS for i.t. treatments.

The aerosol dose was determined from the mouse model of alkylating nitrogen mustard exposure (melphalan-exposed) and the end-point was to have a dose that was as effective as DEX 10 mg/kg i.p. to suppress neutrophils in BAL fluid (Wigenstam et al., 2012). In order to obtain an effective aerosol dose of DEX; 1, 2 or 5 mg/ml of DEX was diluted in saline to a volume of 200 μl and was given during 30 s as an aerosol (Aeroneb™ PRO, SCIREQ®). The animals were placed in individual nose-only containers and were via a connecting tube exposed to aerosolized DEX in various concentrations. (Neutrophils ($\times 10^4$) in BAL fluid: 100 ± 25 neutrophils/ml in placebo-treated melphalan-exposed mice. Three concentrations of DEX via aerosol: 1 mg/ml: 71 ± 41 neutrophils/ml; 2 mg/ml: 64 ± 20 neutrophils/ml and, 5 mg/ml: 29 ± 13 neutrophils/ml. BAL fluid from melphalan-exposed mice administered DEX i.p. (10 mg/kg) displayed 26 ± 11 neutrophils/ml. (Aerosol DEX concentration 5 mg/ml vs. placebo $p < 0.01$.))

2.3.2. N-*acetyl cysteine*

The dose of the antioxidant NAC (*N*-acetyl cysteine, Sigma–Aldrich (St. Louis, MO, USA), 500 mg/kg body weight) was determined and modified from our previous study (Rocksen et al., 2000). NAC was dissolved in a volume of 200 μl PBS and was administered 1 h following Cl_2-exposure as a single i.p. treatment or in a combination with either low-dose or high-dose DEX.

2.3.3. Rolipram

Rolipram (selective phosphodiesterase 4 inhibitor, Sigma–Aldrich (St. Louis, MO, USA), 10 mg/kg body weight) was dissolved in a volume of 400 μl PBS (95%) and DMSO (7.5%) and administered as a single i.p. treatment 1 h following Cl_2-exposure.

2.3.4. Triptolide

Triptolide (has been used as a NFκB inhibitor, Triptolide is extracted from *Tripterygium wilfordii*, Sigma–Aldrich (St. Louis, MO, USA), 500 μg/kg body weight) was dissolved in a volume of 200 μl PBS (95%) and DMSO (5%) and administered as a single i.p. treatment 1 h following Cl_2-exposure.

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