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Inhaled sulfur dioxide causes pulmonary and systemic inflammation leading to fibrotic respiratory disease in a rat model of chemical-induced lung injury

Elisabeth Wigenstam[a], Linda Elfsmark[a], Anders Bucht[a,b], Sofia Jonasson[a,*]

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

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ABSTRACT

Inhalation of high concentrations of sulfur dioxide (SO_2) affects the lungs and can be immediately dangerous to life. We examined the development of acute and long-term effects after exposure of SO_2 in Sprague-Dawley rats, in particular inflammatory responses, airway hyperresponsiveness (AHR) and lung fibrosis. Animals were subjected to a single exposure of 2200 ppm SO_2 during 10 min and treated with a single dose of the anti-inflammatory corticosteroid dexamethasone 1 h following exposure.

Exposed rats showed labored breathing, decreased body-weight and an acute inflammation with neutrophil and macrophage airway infiltrates 5 h post exposure. The acute effects were characterized by bronchial damage restricted to the larger bronchi with widespread injured mucosal epithelial lining. Rats displayed hyperreactive airways 24 h after exposure as indicated by increased methacholine-induced respiratory resistance. The inflammatory infiltrates remained in lung tissue for at least 14 days but at the late time-point the dominating granulocyte types had changed from neutrophils to eosinophils. Analysis of immunoregulatory and pro-inflammatory cytokines in serum and airways implicated mixed macrophage phenotypes (M1/M2) and T helper cell activation of both T_H1 and T_H2 subtypes. Increased expression of the pro-fibrotic cytokine TGFβ1 was detected in airways 24 h post exposure and remained increased at the late time-points (14 and 28 days). The histopathology analysis confirmed a significant collagen deposition 14 days post exposure. Treatment with dexamethasone significantly counteracted the acute inflammatory response but was insufficient for complete protection against SO_2-induced adverse effects, i.e. treatment only provided partial protection against AHR and the long-term fibrosis.

1. Introduction

Sulfur dioxide (SO_2) is a colorless toxic gas with a pungent odor. In urban environments, SO_2 in the air results primarily from activities associated with the burning of fossil fuels, mainly coal and oil. In nature, SO_2 can be released to the air in massive amounts from volcanic eruptions. Recent studies in humans have investigated the effects of exposure to relatively low concentration of SO_2, reporting respiratory changes following acute exposure, particularly in asthmatics (Guarnieri and Balmes 2014; Reno et al., 2015). However, anthropogenic emissions and natural processes generate SO_2 concentrations that do not cause damage to the extent described in the present study. SO_2 is extensively produced in numerous industrial applications and is often transported in high volumes as pressurized liquid which may be accidently released after tanker disruptions forming a widespread cloud of highly concentrated gas. In humans, inhalation of SO_2 primarily affects the lungs and exposure to very high concentrations can be immediately dangerous to life (Wang et al., 2014).

Human case reports after SO_2-inhalation have described burning of the nose and throat, tightness in the chest, dyspnea, and severe airway obstructions (Atkinson et al., 1993; Charan et al., 1979; Harkonen et al., 1983; Huber and Loving 1991; Rabinovitch et al., 1989). Some of these symptoms may partially be reversed two years after the exposure. Other reported symptoms are bronchitis, sloughing of the mucosa of large and small airways along with hemorrhagic alveolar edema (Rabinovitch et al., 1989; Skalpe 1964). Bronchial hyperactivity can develop after a single exposure to a very high concentration of SO_2 (Brooks et al., 1985;

* Corresponding author at: CBRN Defence and Security, Swedish Defence Research Agency, SE-901 82 Umeå, Sweden.
E-mail address: sofia.jonasson@foi.se (S. Jonasson).

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Harkonen et al., 1983). The mechanisms by which SO_2 act on smooth muscle to induce bronchoconstriction has not conclusively been described, but it is hypothesized that the parasympathetic reflex pathways in bronchoconstriction is activated in response to SO_2 (Nadel et al., 1965; Sheppard et al., 1980) and more recent data suggest that SO_2 stimulates mast cell secretions of pro-inflammatory mediators and potent smooth muscle constrictors from the tissue such as leukotrienes (Lazarus et al., 1997) or prostaglandins (Sang et al., 2011).

Due to the fairly high water solubility inhaled SO_2 is hydrated in moist airway mucosa producing a mixture of sulfite, bisulfite, sulfonate and hydrogen ions (Balchum et al., 1960; Speizer and Frank 1966; Yokoyama et al., 1971) and may rapidly enter the bloodstream through the epithelium. The reaction products of SO_2 are rapidly absorbed from the upper respiratory passages and are readily distributed throughout the body. It has been shown that SO_2 and its *in vivo* derivatives can produce toxic effects both in the respiratory system and in the cardiopulmonary system (Meng, 2003; Meng et al., 2003b). Finally, SO_2 may oxidize to sulfate and leave through the urine (Yokoyama et al., 1971). Decreased glutathione levels in the lungs of rats exposed to SO_2 suggest that glutathione may be involved in the detoxification process (Langley-Evans et al., 1996).

The mechanisms by which SO_2 damages the lungs and the long-term consequences of this injury are not clarified. In particular, few studies have addressed the effects of high-level concentrations of SO_2 (Charan et al., 1979; Woodford et al., 1979). The first aim of the present study was to understand the pathogenesis of both short-term and long-term sequelae of lung injury induced by high concentration of SO_2, in particular early inflammatory responses, the development of airway hyperresponsiveness (AHR) and lung fibrosis. The second aim was to evaluate the effect of anti-inflammatory treatment administered intraperitoneally by a single high-dose injection of the corticosteroid dexamethasone (DEX), one hour after SO_2-exposure.

2. Materials and methods

2.1. Animals

Female Sprague-Dawley (SD) rats (8–9 weeks old, Envigo RMS B.V, 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 rats were weighed before subjected to SO_2, and following exposure their health condition was monitored. The experiment terminated 5 h, 24 h and 14, and 28 days post exposure (n = 5–12 rats per group). The same animals were used for both respiratory mechanics and for bronchial lavage. For histopathological evaluation, dedicated animals were used. The care of the animals and the experimental protocols were approved by the regional ethics committee on animal experiments (Umeå, Sweden) in accordance to Swedish law.

2.2. SO_2-exposure protocol

Animals were placed in individual nose-only containers (EMMS, UK) and coupled to an inhalation exposure tower (Battelle) providing equal and simultaneous exposure to SO_2 (AGA gas, Sweden; compressed gas in gas cylinders: 10 mol-% SO_2, 90 mol-% nitrogen). The compressed gas mixture was diluted with air to the final concentration of 2200 ppm. Rats were subjected to a single exposure of SO_2-gas mixture during 10 min. The SO_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. Control animals were breathing room air for 10 min.

2.2.1. Dose-response of inhaled SO_2

A small dose-response study was performed to evaluate the health effects by counting inflammatory cells (neutrophils) in BAL fluid 24 h after exposure to different concentrations of SO_2. The first group was exposed to 1200 ppm for 15 min (300 ppm h) and the number of neutrophils in BAL was estimated to $7.5 \pm 5.3\%$ neutrophils/ml. The concentration of SO_2 in the second group was 2500 ppm (625 ppm h) and after 5 min this exposure was discontinued, animals showed labored breathing and very marked discomfort and this concentration was not examined any further ($30.8.5 \pm 3.2\%$ neutrophils/ml in BAL). A third group was exposed to 1800 ppm (450 ppm h) SO_2 ($18.0.5 \pm 7.9\%$ neutrophils/ml in BAL) and the group selected for further investigation was exposed to 2200 ppm (550 ppm h, $22.7 \pm 9.2\%$ neutrophils/ml in BAL). The final exposure time was set to 10 min.

2.3. DEX-treatment

DEX (Dexamethasone 21-phosphate disodium salt, Sigma-Aldrich) was administered intraperitoneally (i.p.) (10 mg/kg body weight) 1 h following exposure to SO_2. The dose of DEX was selected based on a previous study by Luo et al. (Luo et al., 2014). The experiment terminated 24 h or 14 days post exposure (n = 6 rats per group).

2.4. Respiratory mechanics 24 following SO_2-exposure

Animals were weighed and anesthetized with pentobarbital sodium (50 mg/kg body weight, i.p.). Rats were tracheostomized with a 15-gauge cannula and mechanically ventilated in a quasi-sinusoidal fashion with a small animal ventilator (flexiVent™, SCIREQ®) at a frequency of 1,5 Hz (90 breaths/min) and a tidal volume (V_T) of 10 ml/kg body weight. A positive end-expiratory pressure of 3 cmH_2O was applied. The animal's cardiac output was monitored throughout the respiratory mechanics assessment. Rats were paralyzed with pancuronium (0.1 mg/kg body weight, i.p.) before 4 sigh maneuvers at $3 \times V_T$ were performed at the beginning of the experiment to establish stable baseline respiratory mechanics and to ensure a similar volume history before the experiments. In order to measure AHR, incremental doses of inhaled methacholine (MCh, acetyl-β-methylcholine chloride, Sigma-Aldrich (St. Louis, MO, USA)) were given at 5-min intervals. The MCh, diluted in saline to a volume of 20 μl, was given during 10 s as an aerosol (Aeroneb™ PRO, SCIREQ®). Each dose of MCh (0, 5, 15 and 45 mg/ml) was aerosolized without any interference with the ventilation pattern. The more detailed description of the method can be found in a previously published paper by Gustafsson et al. (Gustafsson et al., 2014). Respiratory mechanics was only analyzed at time-point 24 h (number of control rats n = 12, and SO_2-exposed rats n = 11).

2.5. Serum sampling

Directly after assessment of respiratory mechanics, blood samples were taken from the aorta in the abdomen. The blood was centrifuged (15 min, 20° C, 3000 rpm) and the serum was saved in −70° C until analysis.

2.6. Differential cell count in BAL

The lungs were lavaged six times via a tracheal tube with a total volume of 2 ml + 23 ml Ca^{2+}/Mg^{2+} free Hanks' balanced salt solution (HBSS, Sigma-Aldrich (St. Louis, MO, USA)). The BAL fluid was