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Pulmonary functional and morphological damage after exposure to tripoli dust

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ABSTRACT

Tripoli is a microcrystalline siliceous rock used to polish metals and precious stones. Its inhalation has been associated with increased prevalence of breathing complaints and pneumoconiosis. However, its acute human exposure has not been so far studied. We aimed at evaluating the putative mechanical, morphological, biochemical and inflammatory lung damage in mice acutely exposed to Tripoli dust. BALB/c mice were randomly assigned to 2 groups: In control group (CTRL, $n = 6$) animals received intratracheally (i.t.) 0.9% NaCl (50 μl), while Tripoli group (TRIP, $n = 15$) received 20 mg of Tripoli powder diluted in 50 μL of saline i.t. The experiments were done 15 days later. TRIP mice showed higher pulmonary mechanical impedance, polymorphonuclear cells, TNF-α, IL1-β and IL-6 than CTRL. TRIP presented granulomatous nodules containing collagenous fibers that occupied 35% of the lung tissue area. In conclusion, acute exposure to Tripoli dust triggered important lung damage in mice lungs that if found in human workers could trigger severe illness.

Introduction

The chronic inhalation of crystalline silicon dioxide (SiO_2) is associated with the occurrence of silicosis. Despite being one of the firstly recognized occupational lung diseases, silicosis remains an important cause of morbidity and mortality worldwide (Martínez et al., 2010). This pneumoconiosis displays persistent inflammation, fibroblast proliferation, and excessive collagen deposition (Thakur et al., 2009). Furthermore, the cytotoxic effects of silica in lung tissue yield macrophage death, subsequent release of inflammatory cytokines, such as TNF-α, IL-1β and IL-6, and many other substances. As a net result fibrosis (Piguet et al., 1990; Davis et al., 1998; Mossman and Churg, 1998; Srivastava et al., 2002; Rimal et al., 2005; Hamilton et al., 2008; Sirajuddin and Kanne, 2009) and apoptosis (Borges et al., 2002; Srivastava et al., 2002; Langley et al., 2010) ensue. The continuous recruitment and activation of macrophages and granulocytes contributes to the chronic inflammatory process and, thus, to tissue remodeling (Scabilloni et al., 2005; Delgado et al., 2006). As part of the fibrotic process silicotic nodules or granulomas (Scabilloni et al., 2005) are formed. Increased pulmonary mechanical impedance represents the functional counterpart of the morphological changes (Ebihara et al., 2000; **Borges** et al., 2001; Faffe et al., 2001; **Hertzberg** et al., 2002).

Crystalline silica is found in sand and several rocks, like sandstone, granite and silex and presents polymorphisms, the principal naturally occurring crystalline silica being quartz (Moore, 1999). Cristobalite, tridymite and Tripoli constitute the three other forms of crystalline silica. Tripoli presents unique applications as an abrasive owing to its hardness and because its grain structure lacks distinct edges and corners. It is a mild abrasive, making it suitable for use in toothpaste and tooth polishing compounds, industrial soaps, metal/jewelry polishing mixtures, resins, ceramics, paints, rubber, and cement (Keller, 1978). Along the processing and use of Tripoli powder the dust generated can be inhaled by human beings, not only workers but the general population as well, and may cause an inflammatory lung disease. It should be noted that Tripoli dust contains more components than SiO_2, what could induce a different harmful outcome. However, no study on the detailed acute functional respiratory impairment secondary to exposure to Tripoli dust has been reported so far either in human beings or in experimental animals. Furthermore, no epidemiological work on the prevalence

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or incidence of Tripoli-triggered disease has been published. In other words, Tripoli powder may be generating more harm than so far recognized by physicians and public health authorities.

The aim of the present study was to describe the physical and chemical characteristics of the Tripoli dust used, and to verify whether the exposure to Tripoli dust induces lung morphological, inflammatory and mechanical burdens that could resemble those of chronic silicosis, thus calling physicians and environmental health officers' attention to the Tripoli issue.

Methods

Animal and experimental protocol

Twenty-one female BALB/c mice (20–25 g) were randomly divided into 2 groups. In control group (CTRL, $n = 6$) mice were intratracheally (i.t.) instilled with 0.05 mL of sterile saline solution (0.9% NaCl), whereas Tripoli-administered animals (TRIP, $n = 15$) received intratracheally 20 mg of Tripoli dust vortexed in 0.05 mL of saline, respectively, as previously described in murine models of acute silicosis (Faffe et al., 2001; Borges et al., 2001, 2002). Before the administration of the latter suspension, a stock solution of 600 mg of Tripoli was placed in a 2-mL Eppendorf tube and saline solution was added to reach a final volume of 1.5 mL. The suspension was vortex-mixed for 10 min and 0.05 mL of it was then collected with a Gilson precision micropipette (Gilson, Inc., Middleton, WI, USA) and immediately given to the animal. The vortexing was repeated for every TRIP animal. Fifteen days after saline or Tripoli administration the animals were analyzed.

Particle analysis

The dust sample was kindly provided by a gemstone-polishing company in São Lourenço, Brazil. It was taken from the same supply being at the polishers' disposal. It was dried in an oven at 50 °C until completely de-hydrated. Elements were determined by an energy dispersive X-ray fluorescence spectrometer (EDX 700HS, Shimadzu Corp, Analytical Instruments Division, Kyoto, Japan). Aluminum (Al), cobalt (Co), iron (Fe), potassium (K), magnesium (Mg), sodium (Na), sulfur (S), silicon (Si), aluminum oxide (Al_2O_3), calcium oxide (CaO), red iron oxide (Fe_2O_3), potassium oxide (K_2O), magnesium oxide (MgO), manganese oxide (MnO), sodium oxide (Na_2O), titanium dioxide (TiO_2) and silicon dioxide (SiO_2) were determined and the results expressed as percent composition (wt %) of particles. The trace elements bromine (Br), copper (Cu), germanium (Ge), lutecium (Lu), manganese (Mn), nickel (Ni), rubidium (Rb), selenium (Se), tin (Sn), strontium (Sr), titanium (Ti), zinc (Zn), and zirconium (Zr) were measured and the results expressed as particles per million (ppm) of particles. Three independent samples of the particulate matter were analyzed for this purpose.

The distribution of particle sizes, as measured by their volume and surface, and the diameters encompassing 90%, 50% and 10% of the particulate matter were determined by laser diffraction (Long Bench Mastersizer S, Malvern Instruments Ltd, Malvern, Worcestershire, UK). The particulate matter was visualized by scanning electron microscopy (JEOL 5310, Tokyo, Japan).

Pulmonary mechanics

Fifteen days after saline or Tripoli dust administration, the animals were sedated with diazepam (1 mg *i.p.*) and anesthetized with pentobarbital sodium (20 mg kg body weight^{-1} *i.p.*), paralyzed with pancuronium bromide (0.1 mg kg body weight^{-1} *i.v.*), and mechanically ventilated (Samay VR15, Universidad de la Republica, Montevideo, Uruguay) with a frequency of 100 breaths min^{-1}, tidal volume of 0.2 mL, flow equal to 1 mL s^{-1}, and positive end-expiratory pressure amounting to 2 cmH_2O. The anterior chest wall was surgically removed. A pneumotachograph (1.5-mm ID, length = 4.2 cm, distance between side ports = 2.1 cm) was connected to the tracheal cannula for the measurement of airflow (V'). Changes in lung volume were obtained by flow signal digital integration. The pressure gradient across the pneumotachograph was determined by means of a Validyne MP45-2 differential pressure transducer (Engineering Corp, Northridge, CA, USA). Equipment resistive pressure (=Req.V') was subtracted from pulmonary resistive pressure so that the present results represent intrinsic values. Transpulmonary pressure was measured with a Validyne MP-45 differential pressure transducer (Engineering Corp, Northridge, CA, USA). Briefly, we determined lung resistive (ΔP_1) and viscoelastic/inhomogeneous (ΔP_2) pressures, static elastance (E_{st}), and viscoelastic component of elastance (ΔE) by the end-inflation occlusion method (Bates et al., 1985). ΔP_1 selectively reflects airway resistance, and ΔP_2 represents stress relaxation or viscoelastic properties and mechanical heterogeneities of the lung (Bates et al., 1989; Saldiva et al., 1992). Lung mechanics were measured 10–15 times in each animal.

Histological study

Heparin (1000 IU) was intravenously injected immediately after the determination of respiratory mechanics. The trachea was clamped at end expiration, and the abdominal aorta and vena cava were sectioned, yielding a massive hemorrhage that quickly euthanized the mice. The right lungs were removed *en bloc* and quick-frozen by immersion in liquid nitrogen and fixed with Carnoy's solution (Nagase et al., 1992). After fixation, the tissue was embedded in paraffin. Four-μm-thick slices were cut and stained with hematoxylin-eosin or picrosirius red.

Morphometric analysis was performed with an integrating eyepiece with a coherent system made of a 100-point and 50 lines (known length) grid coupled to a conventional light microscope (Axioplan, Zeiss, Oberkochen, Germany) in granuloma free areas. The volume fraction of collapsed and normal alveoli was determined in each sample by the point-counting technique (Gundersen et al., 1988) across 10 random non-overlapping microscopic fields at ×400 magnification. The total amount of points also included those falling on tissue, airways and other non-alveolar structures.

The number of mononuclear (MN) and polymorphonuclear (PMN) cells in the pulmonary tissue was counted in each animal across 10 random non-overlapping microscopic fields at ×1000 magnification in a 10,000 μm^2 granuloma free area; in the same field the amount of points that fell on lung tissue was also counted, so that cellularity was expressed as percentage of lung tissue area (Gundersen et al., 1988; Capelozzi et al., 1997).

The fraction area of the granulomas was determined using the point-counting technique across 20 random non-coincident microscopic fields per animal at a magnification of ×200. Percentage of lung tissue occupied by granulomatous nodules was scored as following: phase 1, nodules present only in the lung parenchyma; phase 2, nodules around the airways; phase 3, nodules obstructing the airway; and, phase 4, lung nodules in various structures.

Analysis of cytokines

Samples of lung cytosol were analyzed by ELISA for the detection of the inflammatory cytokines TNF-α, IL-1β, IL-6 (ELISA kits, R&D Systems Europe, Abingdon, UK) with detection limits of 5.1 pg/mL, 1.6 pg/mL and 3.0 pg/mL respectively.