Toxicology and Applied Pharmacology 259 (2012) 38–44

Contents lists available at SciVerse ScienceDirect

Toxicology and Applied Pharmacology

journal homepage: www.elsevier.com/locate/ytaap

Exposure to particulate hexavalent chromium exacerbates allergic asthma pathology

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ARTICLE INFO

Article history:
Received 19 September 2011
Revised 29 November 2011
Accepted 1 December 2011
Available online 9 December 2011

Keywords:
Hexavalent chromium
Asthma
Inflammation
Lung

ABSTRACT

Airborne hexavalent chromate, Cr(VI), has been identified by the Environmental Protection Agency as a possible health threat in urban areas, due to the carcinogenic potential of some of its forms. Particulate chromates are produced in many different industrial settings, with high levels of aerosolized forms historically documented. Along with an increased risk of lung cancer, a high incidence of allergic asthma has been reported in workers exposed to certain inhaled particulate Cr(VI) compounds. However, a direct causal association between Cr(VI) and allergic asthma has not been established. We recently showed that inhaled particulate Cr(VI) induces an innate neutrophilic inflammatory response in BALB/c mice. In the current studies we investigated how the inflammation induced by inhaled particulate Cr(VI) might alter the pathology of an allergic asthmatic response. We used a well-established mouse model of allergic asthma. Groups of ovalbumin protein (OVA)-primed mice were challenged either with OVA alone, or with a combination of OVA and particulate zinc chromate, and various parameters associated with asthmatic responses were measured. Co-exposure to particulate Cr(VI) and OVA mediated a mixed form of asthma in which both eosinophils and neutrophils are present in airways, tissue pathology is markedly exacerbated, and airway hyperresponsiveness is significantly increased. Taken together these findings suggest that inhalation of particulate forms of Cr(VI) may augment the severity of ongoing allergic asthma, as well as alter its phenotype. Such findings may have implications for asthmatics in settings in which airborne particulate Cr(VI) compounds are present at high levels.

Introduction

Epidemiological studies conducted in the U.K., Europe, Japan, and the U.S. show that long-term occupational exposure to certain inhaled particulate hexavalent chromium [Cr(VI)] compounds is associated with an elevated risk of developing lung cancer (reviewed in U.S. Environmental Protection Agency, 1998). These workers were also found to have increased incidences of other lung conditions, including pulmonary congestion and edema, lung tissue fibrosis, bronchiolization of the alveoli, and hyperplasia of the bronchial epithelium. Exposure to certain chromium compounds has also been associated with elevated incidences of asthma (Adams et al., 2006).

Asthma is a complex phenotype arising from genetic, environmental and social factors (Cookson, 1999). The most common form of asthma is allergic asthma. This form is initiated and perpetuated by Th2 $CD4^+$ T lymphocytes that secrete cytokines (notably IL-4, IL-5 and IL-13) that promote the generation of allergen-specific IgE (reviewed in Cohn et al., 2004). The disease manifests itself as recurrent episodes of coughing, wheezing and breathlessness, triggered by the release of pro-inflammatory factors from tissue mast cells activated by IgE-receptor cross-linking. Most of these symptoms are the result of airway obstruction, which can be attributed to a combination of factors including changes in vasculature, mucus hypersecretion, airway hyperresponsiveness, and airway remodeling within lung tissues and airways.

As early as the 1930s, reports have been published proposing a link between occupational exposure to chromium compounds and the development of asthma in industrial workers (Joules, 1932; Smith, 1931). Since then, numerous case studies (Bright et al., 1997; Fernandez-Nieto et al., 2006; Hannu et al., 2005; Leroyer et al., 1998; Moller et al., 1986; Novey et al., 1983; Olaguibel and Basomba, 1989; Park et al., 1994), as well as larger studies (reviewed

Abbreviations: AHR, airway hyperresponsiveness; Cr, chromium; Cr(VI), hexavalent chromium; Cr(III), trivalent chromium; H&E, hematoxylin and eosin; IgE, immunoglobulin E; IL, interleukin; i.p., intraperitoneal; i.n., intranasal; NKT, natural killer T-cells; OVA, ovalbumin protein; PAS, periodic acid-Schiff; Th2, helper T-cell type 2.

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doi:10.1016/j.taap.2011.12.001

in Leikauf, 2002), suggest an association between occupational exposure to chromates and asthma. It should be noted that for most of these studies the type of asthma (allergic versus non-allergic) was not identified. Interestingly, in the few studies in which skin prick tests and/or IgE levels were measured, the results were either mixed or showed no correlation with bronchial hyperresponsiveness, the primary parameter used to diagnose asthma in most studies. Such findings suggest that chromate compounds may be poor sensitizers for allergic asthma. Alternative explanations for the reported associations between chromium exposure and asthma include a capacity to mediate non-allergic forms of asthma, or the capacity to exacerbate pre-existing asthmatic conditions (also known as irritant-induced asthma). In the latter, chromium would act as a secondary inflammatory agent or adjuvant rather than a primary sensitizing agent.

Using a mouse model of intranasal chromium exposure we recently showed that a single inhaled dose of particulate Cr(VI) mediates an extensive inflammatory response characterized by an acute influx of neutrophils followed by macrophages, elevated levels of IL-6 and GROα (IL-8 homolog) in airways, and progressive interstitial pneumonitis (Beaver et al., 2009a). A similar profile of inflammation was induced by repetitive exposures to the same compound, although the severity of the inflammation and injury within tissues was markedly increased (Beaver et al., 2009b). These earlier reports established that intranasal delivery of zinc chromate suspension efficiently targets the pulmonary tract of BALB/c mice, resulting in widespread distribution within lung tissues, with a preferential accumulation around small central airways. Such "hot spots" of particle accumulation have been reported in the same anatomical locations in the lungs of chromate workers (Ishikawa et al., 1994). The results from these previous studies suggest that inhaled particulate Cr(VI) is an efficient inducer of innate inflammatory responses. In the current studies we examined how exposure to Cr(VI) might impact on an ongoing allergic asthmatic response. Specifically, we investigated how the marked innate inflammation induced by inhaled particulate Cr(VI) might alter the pathology of the allergic response. We postulated that co-exposure to particulate chromate would either result in an exacerbated form of the ongoing allergic response, or might change the immunological phenotype of the response.

For the present studies, we made use of a well-established animal model of allergic asthma previously described by our laboratory (Balsley et al., 2010; Gwinn et al., 2006). Mice are initially primed with ovalbumin protein (OVA) in Alum adjuvant and then given repeated intranasal challenges of OVA to induce an inflammatory response dominated by an influx of eosinophils, elevated levels of Th2-associated cytokines (notably IL-13) in airways, goblet cell hyperplasia, mucus secretion, and bronchial hyperreactivity. In the current studies, groups of OVA-primed mice were challenged either with OVA alone, or with a combination of OVA and Cr(VI), and various parameters associated with asthmatic responses were measured.

Materials and methods

Chromium preparation. Endotoxin-free particulate basic zinc chromate [$ZnCrO_4$ $4Zn(OH)_2$] was obtained from Rockwood Pigments (Beltsville, MD). The particles (previously determined to be 4.7 μm in size, Beaver et al., 2009b) were suspended in sterile 0.9% sodium chloride solution, pH 7.39, (saline) by sonication and then allowed to spin on a stir plate for 1 h prior to use to ensure a homogeneous mixture.

Mouse model of allergic lung inflammation and intranasal delivery of particulate chromium. All experiments were conducted under the approval of The George Washington University Institutional Animal Care and Use Committee. A previously described (Balsley et al., 2010; Gwinn et al., 2006) mouse model of acute allergic lung inflammation was used. Briefly, female BALB/c mice aged 8–10 weeks (purchased from the Jackson Laboratory, Bar Harbor, Maine) were primed by intraperitoneal (i.p.) injection of 100 μg ovalbumin (OVA) protein in saline mixed 1:1 with Imject alum adjuvant (Pierce, Rockford, IL). Alum was included as an adjuvant to help promote priming of OVA-specific Th2 CD4 + T cells. Seven days later the mice were challenged under light anesthesia (isoflurane) with 50 μg OVA in saline via intranasal (i.n.) delivery for four consecutive days (days 7–10). Most analyses were conducted 2 days later (day 12). In some experiments, mice were given i.n. challenges with saline alone, or were challenged with OVA plus 24 μg chromate solution (Cr) included on days 7 and 9, or were challenged with only Cr on days 7 and 9. Of importance is the fact that Cr was only included during the challenge phase of each regimen so that we could specifically observe its impact on effector inflammatory responses, rather than immune sensitization. Fig. 1 shows a summary of the four different treatment groups.

Bronchoalveolar lavage and analysis of collected cells and fluid. Immediately following euthanasia (by exposure to carbon dioxide), bronchoalveolar lavage (BAL) was performed to isolate airway cells and fluid. For this, a cannula was inserted into the trachea and three 1-ml washes of cold PBS infused in and out of the airways. The fluid and cells were then separated by centrifugation and used for different analyses. The separated cells were treated with ammonium chloride lysis buffer and then quantified by hemocytometry. Eosinophils and neutrophils were identified using forward light scatter/side light scatter distribution as previously described (Balsley et al., 2010; Gwinn et al., 2006) and verified using cytospin analysis. To measure levels of airway cytokines, BAL fluids were pooled within treatment groups and then concentrated 4-fold using 4-kDa cut-off Centricon columns (Millipore, Billerica, MA). Levels of IL-4, IL-5, IL-6, IL-13, IL-17 and IFN-γ cytokines were measured using FlowCytomix™ bead-based simplex kits (eBioscience, San Diego, CA).

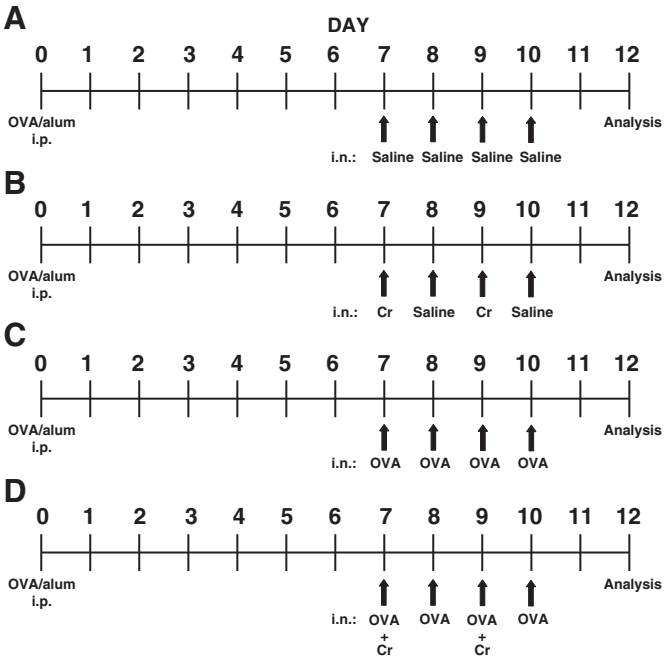

Fig. 1. Regimen of acute allergic lung inflammation and intranasal delivery of particulate chromium. On day 0, female BALB/c mice were primed i.p. with 50 μg OVA mixed 1:1 with Imject alum adjuvant. On days 7–10, mice were challenged intranasally (i.n.) with 50 μl of the indicated agent: (A) saline-treated mice (control) received saline challenges. (B) Cr-treated mice were challenged with 24 μg Cr on days 7 and 9 and saline on days 8 and 10. (C) OVA-treated mice were administered 50 μg OVA for all challenges. (D) OVA + Cr-treated mice were challenged with 50 μg OVA + 24 μg Cr on days 7 and 9 and 50 μg OVA on days 8 and 10. All mice were euthanized on day 12 and analyses were conducted.