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Particles internalization, oxidative stress, apoptosis and pro-inflammatory cytokines in alveolar macrophages exposed to cement dust

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ABSTRACT

Exposure to cement dust is one of the most common occupational dust exposures worldwide, but the mechanism of toxicity has not been fully elucidated. Cement dust (N) and clinker (C) samples collected from Nigeria and another sample of cement dust (U) collected from USA were evaluated using alveolar macrophage (NR8383) cell culture to determine the contribution of different sources of cement dust in the severity of cement dust toxicity. Cement dust particles internalization and morphologic alterations using transmission electron microscopy (TEM), cytotoxicity, apoptotic cells induction, intracellular reactive oxygen species generation, glutathione reduction, TNF-α, IL-1β, and CINC-3 secretion in alveolar macrophages (NR8383) exposed to cement dust and clinker samples were determined. Particles were internalized into the cytoplasmic vacuoles, with cells exposed to U showing increased cell membrane blebbing. Also, NR8383 exposed to U show more significant ROS generation, apoptotic cells induction and decreased glutathione. Interleukin-1β and TNF-α secretion were significantly more in cells exposed to both cement dust samples compared with clinker, while CINC-3 secretion was significantly more in cells exposed to clinker ($p < 0.05$). Endocytosis, oxidative stress induced-apoptosis and induction of pro-inflammatory cytokines may be key mechanisms of cement dust immunotoxicity in the lung and toxicity may be factory dependent.

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Abbreviations: AM, alveolar macrophage; CINC-3, cytokine induced neutrophil chemoattractant-3; GSH, glutathione; HOBr, hypobromous acid; HOCl, hypochlorous acid; IL-1β, interleukin-1 beta; MIP-2, macrophage inflammatory protein 2; MMP2, matrix metallopeptidase 2; MMP9, matrix metallopeptidase 9; MTT, (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; NLRP-3, nod-like receptor protein-3; NR8383, alveolar macrophage cell line; ROS, reactive oxygen species; TEM, transmission electron microscopy; TGF-β, transforming growth factor-beta; TNF-α, tumor necrosis factor-alpha.

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1. Introduction

Exposure to cement dust that is rich in silica and heavy metals is one of the most common occupational dust exposures worldwide. Cement dust is known to be a mixture of calcium oxide (60–67%), silicon oxide (17–25%), aluminum trioxide (3–8%), and ferric oxide (0–5%) (Fell et al., 2003). Cement factory can be divided into three sections: crushing, milling and packing units. The intermediate component of cement dust production is clinker which is pulverized with gypsum or mixed with additives (metals) in an energy intensive process (Eckert et al., 1999). In an effort to reduce production cost, most cement dust manufacturers have resulted to use of alternative raw feeds and secondary fuels derived from industrial by-products (Yan et al., 2010). Examples of such alternative source of fuels are solvents, used tires, waste oil, paint residues, biomass such as woodchips, sewage sludge, etc. (Mokrzycki and Uliasz-Bochenczyk, 2003; Kaantee et al., 2004). The use of variable energy sources may increase the variability/severity of cement dust toxicity and cement dust metal content Ogunbileje et al., 2013). Recent data from our laboratory showed that the concentration of metals and chromium (VI) in cement dust is factory dependent (Ogunbileje et al., 2013).

Respiratory tract, especially lung is often the target site of injury in cement dust occupationally exposed workers (Zeleke et al., 2010). Alveolar macrophages (AM) within the alveolus serve as the first line of host defense against inhaled particulate matters (Holian and Scheule, 1990, Holt et al., 2008). Alveolar macrophages uptake of particles is believed to induce a number of cell-damaging processes which include reactive oxygen species (ROS) generation, protein and lipid peroxidation, alteration of calcium homeostasis, pro-inflammatory cytokines secretion, and increased cell death (Hohr et al., 2002; Li et al., 2002). Current evidence suggests that pro-inflammatory cytokines (TNF-α and IL-1β) and inflammatory mediators in the pulmonary systems play vital roles in particulates induced lung injury (Driscoll et al., 1997; Rao et al., 2004; Nel et al., 2006).

Increased ROS generation and a concurrent reduction of glutathione which result in oxidative stress appeared to be central in particulates induced toxicity (Nel et al., 2006). Intracellular oxidative stress is believed to induce intracellular stress which has been reported to contribute to NLRP3 inflammasome activation (Dostert et al., 2008). The activation of NLRP3 has been shown to be important in IL-1β production, a highly inflammatory cytokine (Dostert et al., 2008; Church et al., 2008). Additionally, intracellular ROS generation plays an important role in induction of apoptosis and DNA damage (Simon et al., 2000) with resultant lung pathology. Increased accumulation of apoptotic cells in the lung may overwhelm the clearance mechanism and leads to apoptotic cells being converted to secondary apoptotic cells (Erwig and Henson, 2008). This might results in the release of their toxic content and chronic inflammatory response may ensue (Kuwano, 2007). Increased lung cells apoptosis has also been associated with pulmonary fibrosis (Matute-Bello and Martin, 2003) and it is known to be central in lung pathology (Martin et al., 2003).

Neutrophils accumulation in the lung in response to the presence of dust particles play important role in tissue damage during lung injury (Meszaros et al., 2000; Guo and Ward, 2002). Cytokine-induced neutrophils chemoattractant-3 (CINC-3) or macrophages inflammatory protein-2 (MIP-2) is a potent chemotactic factor for neutrophils (Driscoll, 2000,). Increased numbers of neutrophils do not only result in increased release of their lytic content but also result in the release of cytokines and hypohalous acids (Mantovani et al., 2011). Hypohalous acids (HOCl and HOBr) have been reported to react with DNA resulting in inflammatory-mediated DNA damage (Kang and Sowers, 2008). Hence increase CINC-3 may further exacerbate lung injury.

The mechanisms of cement dust induced lung injury have not been fully defined. Recent epidemiological studies revealed that exposure to cement dust particles is associated with development of lung related cancer in occupationally exposed individuals (Smailyte et al., 2004; Dietz et al., 2004), but these observations have not been consistent (Dab et al., 2011). Therefore, this study is aimed at determining the effects of internalization of cement dust particles by alveolar macrophages, morphological changes following internalization, rate of apoptotic cells induction by cement dust and clinker, DNA fragmentation, ROS production and GSH reduction, and rate of secretion of TNF-α, IL-1β and CINC-3 and their implications. The study also aims to clarify the role of sources of cement dust in the severity of cement dust toxicity using cement dust collected from Nigeria and USA.

2. Materials and methods

2.1. Cement dust and clinker particles

Cement dust (N) and clinker (C), were obtained from Lafarge Cement Factory, Sagamu, Nigeria. The USA cement dust (U) sample was produced by TXI Cement Factory, Midlothian, Texas. Lafarge and TXI cement factories are the largest sources of cement dust in Nigeria and Texas, respectively. Cement dust samples and clinker were heated in oven at 200 °C for 24 h to eliminate interferences from endotoxins. Size of cement dust from Nigeria ranges 0.2–4 μM, cement dust form USA was 0.2–5 μM, while the size of clinker was 1–6 μM as determined by examination under the microscope after sonication. Cement dust obtained from USA appeared to contain more particles of size less than 1 μM.

2.2. Cell culture

Alveolar macrophages (NR8383) cell line was purchased from American Type Culture Collection (ATCC, Manassas, VA). NR8383 was derived from lung lavage of a normal adult male Sprague-Dawley rat. The alveolar macrophages were cultured in RPMI-1640 supplemented with 2 mM glutamine, 15% fetal bovine serum, 1.5 g/L sodium bicarbonate, 1% penicillin-streptomycin in a humidified incubator with 5% CO_2 at 37 °C.