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Metal-sulfide mineral ores, Fenton chemistry and disease – Particle induced inflammatory stress response in lung cells

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ABSTRACT

The inhalation of mineral particulates and other earth materials, such as coal, can initiate or enhance disease in humans. Workers in occupations with high particulate exposure, such as mining, are particularly at risk. The ability of a material to generate an inflammatory stress response (ISR), a measure of particle toxicity, is a useful tool in evaluating said exposure risk. ISR is defined as the upregulation of cellular reactive oxygen species (ROS) normalized to cell viability. This study compares the ISR of A549 human lung epithelial cells after exposure to well-characterized common metal-sulfide ore mineral separates. The evaluation of the deleterious nature of ore minerals is based on a range of particle loadings (serial dilutions of 0.002 m^2/mL stock) and exposure periods (beginning at 30 min and measured systematically for up to 24 h). There is a wide range in ISR values generated by the ore minerals. The ISR values produced by the sphalerite samples are within the range of inert materials. Arsenopyrite generated a small ISR that was largely driven by cell death. Galena showed a similar, but more pronounced response. Copper-bearing ore minerals generated the greatest ISR, both by upregulating cellular ROS and generating substantial and sustained cell death. Chalcopyrite and bornite, both containing ferrous iron, generated the greatest ISR overall. Particles containing Fenton metals as major constituents produce the highest ISR, while other heavy metals mainly generate cell death. This study highlights the importance of evaluating the chemistry, oxidation states and structure of a material when assessing risk management.

Introduction

From flint in the Neolithic period, gold and silver in the Iron Age, lead during Antiquity, to Rare Earth minerals in the present, mining of mineral resources has enabled important technological advances, fostered trade, as well as provided the raw materials for the creation of art and artifacts. Presently, mining of metals and mineral ores is a multibillion dollar industry, with metals and aggregates/industrial minerals generating a $230 billion annual profit (MJO, 2012). While a crucial pillar of our society, mining of mineral resources has significant environmental and health issues associated with it. Exposure to mine dust has long been recognized as an occupational health hazard. Historically, exposure to quartz, asbestos particles and coal dust has received considerable attention given the prevalence of silicosis, asbestosis and coal workers' pneumoconiosis worldwide. However, far less is known about the inhalation risks associated with other earth materials that are mined and processed. While there have been some studies about the role of Fenton chemistry in the development or enhancement of disease (Winterbourn, 1995), there is much to be explored. The purpose of this study is to investigate the toxicity of common metal-sulfide minerals (copper-sulfides, copper-iron-sulfides, arsenic-iron-sulfide, lead-sulfide, and zinc-sulfides) that are mined or present in ore deposits as gangue minerals. Each of the materials evaluated is briefly described below.

Selected metal-sulfides

Sphalerite (ZnS) is the main zinc ore mineral and commonly contains 26 mol% iron in its crystal structure ($Zn_{1-x}Fe_xS$) (Labrenz

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et al., 2000; Wright and Gale, 2010), with reports of up to 56 mol% (Di Benedetto et al., 2005; Pattrick et al., 1998). Often associated with sphalerite, galena (PbS) is the main lead ore mineral. The cations for both ores are present in the divalent oxidation state. While arsenic is no longer mined, its main ore mineral, arsenopyrite (FeAsS), is often found in gold ores. As a result, a considerable amount of arsenopyrite is processed in the mining of gold. The oxidation state of iron in arsenopyrite is predominantly divalent (i.e. $Fe^{2+}(AsS)^{-2}$) with a minor contribution of trivalent iron (i.e. $Fe^{3+}(AsS)^{3-}$). Although the presence of either an arsenic and sulfur dianionic or trianionic group in the crystal structure is debated, when dispersed in solution, arsenopyrite releases ferrous iron (Asta et al., 2010). This suggests that iron in the arsenopyrite crystal structure is mostly in the divalent oxidation state. The oxidation states of copper and iron in chalcopyrite ($CuFeS_2$), the primary copper ore mineral, has been extensively studied. Most studies indicate that the copper present is monovalent, although in oxidized surface layers divalent copper may be prevalent. To balance charge, trivalent iron is formally present in chalcopyrite, but charge transfer between iron and sulfur gives iron a strong divalent character (Goh et al., 2006; Todd et al., 2003). Furthermore, as with arsenopyrite, chalcopyrite releases ferrous iron into solution, which is important in the context of this study as the release of ferrous iron will allow the Fenton reaction to go forward:

$$H_2O_2 + Fe^{2+}(aq) \rightarrow OH^- + {}^{\bullet}OH + Fe^{3+}(aq) \quad (1)$$

Pyrite (FeS_2), as the most common sulfide mineral on Earth (and a potent reactive oxygen species generator (Cohn et al., 2006; Schoonen et al., 2006, 2010)), is often associated in varying abundances with all the ores described above.

Chalcocite (Cu_2S) and bornite (Cu_5FeS_4) are also important copper ores and are common weathering products of chalcopyrite. While copper in chalcocite is monovalent, it is possible that bornite contains a small amount of divalent copper as well. Iron in bornite has a formal trivalent oxidation state (Goh et al., 2006).

Particle toxicity

The toxicity of particles can be derived by particle overload or from the reactivity of the material (Fubini, 1997; Morrow, 1988; Oberdorster, 1995). When particle overload occurs, the alveolar macrophage (AM)-mediated clearance is impaired, which leads to an accumulation of particles within the lungs. An excessive particle burden can cause an array of pulmonary issues, ranging from chronic inflammation and fibrosis, to the development of tumors (Oberdorster, 1995). Once inhaled, a mineral particle might undergo surface-mediated reactions, including dissolution reactions (Fubini, 1997; Ghiazza et al., 2011; Harrington et al., 2012a; Schoonen et al., 2006). The reactivity of an inhaled particle is a function of its stability in the lung environment, its solubility, and modifications of its surface, such as the presence of defects or surface coatings (Fubini, 1997; Ghiazza et al., 2011; Schoonen et al., 2006). The incomplete reduction of oxygen on the surface of the particles or by metals released from the particles will generate reactive oxygen species (ROS) (Schoonen et al., 2006). Furthermore, although tightly regulated, cells also generate ROS when under stress, possibly contributing to the oxidative stress (Araujo and Nel, 2009; Gloyne et al., 2011; Knight, 2000). Apart from oxidative damage, reactive materials also impair AM-mediated clearance at a lower lung burden, thus adding to the inflammation. The concomitant disruption of efferocytosis (clearance of apoptotic cells via phagocytosis) creates a delayed "secondary" necrosis (Hodge et al., 2003; Oberdorster, 1995; Schmidt and Tuder, 2010). Unlike apoptosis (programmed cell death), which controls potentially damaging inflammation by not releasing the contents of the cell into the surrounding environment, necrotic cell death adds to the burden on the body and can have a negative cascading effect (Nanji and Hiller-Sturmhofel, 1997; Schmidt and Tuder, 2010). Therefore, while exposure to reactive foreign material alone can also generate necrotic cell death (Harrington et al., 2012b), inhibition or reduced efficiency in efferocytosis exacerbates the problem.

A number of toxicity markers can be utilized to evaluate the effect of particle exposure on lung cells (Antonini et al., 2009; Chamnongpol et al., 2002; Gloyne et al., 2011; Kida et al., 2005). The approach developed by Harrington et al. (2012b) takes into account the possible complexity of immune responses to foreign materials in the body by capturing the immediate and short-term inflammatory stress response (ISR) generated by exposure to a contaminant. The ISR is the normalization of the cellular upregulation of ROS with respect to the cell viability. It not only allows for overall stress levels to be evaluated but each component as well. Due to the relatively short time course of the exposure, the cell viability is a measure of necrotic cell death. Whereas the amount of cellular ROS upregulation is not only a marker of immediate cellular stress, but also may indicate future apoptotic cell death (Fleury et al., 2002; Yoo et al., 2012).

By focusing on the ISR, a better understanding of the inflammatory nature of a material can be established, although the specific biological pathways interrupted by the material cannot be resolved without additional studies. In this study, the protocol developed by Harrington and coworkers is used to compare the ISR for eight common metal-sulfide ore minerals. The results allow for direct comparison between materials, which is the basis for an evaluation on whether the structure and chemistry of (earth) materials are important determining factors in toxicity.

The cell culture work in this study is complemented by an assessment of the generation of $^{\bullet}OH$ and H_2O_2 by the metal-sulfides when dispersed in water in the absence of an epithelial lung cell line. This acellular test provides insight in purely mineral-driven ROS formation, which can be useful in the interpretation of ISR data. It is hypothesized that the ability of metal-sulfide minerals to generate ROS when dispersed in water will contribute to an elevated ISR. Furthermore, we expect that minerals containing ferrous iron, and to a lesser extent monovalent copper, will produce more ROS when dispersed in water as a result of their ability to promote the Fenton reaction (Eq. (1)). It should be pointed out, however, that materials that do not form ROS when dispersed in water, e.g. glass beads, will elicit a modest cellular upregulation of ROS within epithelial cells (Harrington et al., 2012b). This is the result of the immune response of epithelial cells to any foreign substance. Metals released from the mineral particles may also lead to interference of biochemical processes in the cell and contribute to a loss of cell viability (Valko et al., 2005), which would lead to higher ISR values independent of the ability of the mineral to generate ROS when dispersed in water. The completion of this study will allow for a better understanding of the inflammatory nature of a number of common metal-sulfide ore minerals.

Materials and methods

Mineral sample preparation

Eight natural metal-sulfide ore minerals, obtained from Wards (Rochester, NY), were investigated. The samples include three zinc sulfides with variable iron contents, one copper sulfide, two copper iron sulfides, a lead sulfide, and an iron arsenic sulfide (Table 1). All samples were ground and sieved to obtain a suitable respirable fraction. The materials were characterized using a suite of complementary techniques.

The mineralogical composition of samples was determined by X-ray diffractometry (XRD) using a Scintag PAD X diffractometer