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Activation of MAP kinases by hexavalent chromium, manganese and nickel in human lung epithelial cells

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Abstract

Epidemiological studies indicate that workers who perform welding operations are at increased risk for bronchitis, siderosis, occupational asthma and lung cancer due to fume exposure. Welding fumes are a complex chemical mixture, and the metal composition is hypothesized to be an etiological factor in respiratory disease due to this exposure. In the present study, human lung epithelial cells *in vitro* responded to hexavalent chromium, manganese and nickel over a concentration range of 0.2–200 μM with a significant increase in intracellular phosphoprotein (a measure of stress response pathway activation). The mitogen-activated protein kinases ERK1/2, SAPK/JNK and p38 were activated via phosphorylation following 1-h exposures. Hexavalent chromium up-regulated p-38 phosphorylation 23-fold and SAPK/JNK phosphorylation 17-fold, with a comparatively modest 4-fold increase in ERK1/2 phosphorylation. Manganese caused a two- to four-fold increase in SAPK/JNK and ERK 1/2 phosphorylation, with no observed effects on p38 kinase. Nickel caused increased (two-fold) phosphorylation of ERK 1/2 only, and was not cytotoxic over the tested concentration range. The observed effects of welding fume metals on cellular signaling in lung epithelium demonstrate a potentially significant interplay between stress-response signaling (p38 and SAPK/JNK) and anti-apototic signaling (ERK1/2) that is dependant on the specific metal or combination of metals involved.

Keywords: Welding fumes; MAP kinases; Chromium; Manganese; Nickel

1. Introduction

An estimated 1 million workers worldwide perform work involving welding activities, and exposure to welding fumes can result in a variety of adverse health effects, particularly of the respiratory system (Antonini et al., 2003; Speizer, 2000). Epidemiological studies indicate that workers who perform welding operations are at increased risk for bronchitis, siderosis, asthma and lung cancer due to fume exposure (Doherty et al., 2004; Antonini et al., 2003; Yoshii et al., 2002; Jockel et al., 1998; Moulin, 1997; Beach et al., 1996; Wang et al., 1994). The International Agency on Cancer (IARC) has classified welding fumes as a possible human carcinogen (i.e., Group 2B; OSHA, 2000). Other non-respiratory effects, such as metal fume fever, infertility and chronic renal failure are also associated with exposure to welding fumes (McNeilly et al., 2004; El-Zein et al., 2003; Wong et al., 2003; Nuyts et al., 1995).

Welding fumes are a complex mixture of respireable particulates, chiefly condensed metals, metal oxides and organic compounds, which vary according to the base

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metal and the specific type of operation (e.g. metal joining versus cutting; stainless versus mild steel; shielded versus gas arc). Recent research has demonstrated that the metal components of welding fumes are a determinant of pulmonary responses (Antonini et al., 2004). Although iron is quantitatively the predominant metal, toxicologically significant metals such as chromium, manganese and nickel are also present and are most strongly associated with adverse health effects (Nemery, 1990). There is also evidence that the age of the welding fumes will determine respiratory effects, with newly formed fume reportedly causing a greater inflammatory response in exposed rats compared to aged fume (Antonini et al., 1998). This is significant in that a welder's breathing zone will contain higher levels of newly formed fume relative to the general welding environment.

In addition to its barrier function, the lung epithelium plays an active role in toxic stress and inflammatory responses following exposure to respiratory toxicants. Human lung epithelial cells produce and release proinflammatory cytokines that recruit involvement of immune cells. For example, lung epithelial cells *in vitro* release IL-6 and IL-8, which are chemotactic for macrophages and neutrophils, following exposure to hexavalent chromium and manganese (Pascal and Tessier, 2004). Similarly, following exposure to diesel exhaust particles (Abe et al., 2000) and grain dust (Park et al., 2000), cultured lung epithelium will release the cytokines IL-6, IL-8 and TNF-α. The epidermal growth factor receptor (EGFR) regulates in part production of inflammatory mediators in lung epithelial cells, and, when the toxic insult is significant enough to result in cell death, regeneration of the epithelial barrier (Davies et al., 1999; Holgate et al., 1999).

The mechanisms of epithelial repair, cytokine production and other stress responses in lung epithelial cells following toxicant exposure are initiated and regulated principally through the mitogen-activated protein kinase (MAPK) extracellular receptor kinase (ERK 1/2), stress-activated protein kinase/c-Jun NH_2-terminal kinase (SAPK/JNK) and p38 signaling pathways. The MAPKs are activated by transition metals in lung epithelial cells *in vitro* (Iryo et al., 2000; Samet et al., 1998). Samet et al. reported varying degrees of activation of ERK2, JNK and p38 in cultured BEAS-2B human lung epithelial cells following exposure to 500 μM concentrations of arsenic, chromium, copper, vanadium and zinc, with vanadium being the most potent activator. This signaling was associated with IL-6, IL-8 and TNF-α release. The epidermal growth factor receptor was later shown to be involved in lung epithelial responses to copper, vanadium and zinc (Wu et al., 1990). Other research has shown that ambient particulate matter activates the JNK pathway, which may be related to carcinogenesis (Timblin et al., 1998). Non-metallic compounds also activate MAPKs in lung epithelial cells, for example carbon monoxide, reactive oxygen species, asbestos, coal and silica dust, as well as infectious agents (Zhang et al., 2003, 2005; Iwagaki et al., 2003; Albrecht et al., 2002). Because of their central role in cellular signaling, aberrant signaling by MAP kinases is a mechanistic factor in many disease states, including respiratory disease. For example, ERK1/2 signaling is involved in malignant transformation in non-small cell lung carcinoma (Vicent et al., 2004). The p38 pathway mediates cytoskeletal changes in pulmonary endothelial cells following hypoxic stress (Kayyali et al., 2002). Signaling by p38 also appears to mediate fibrosis in asthma and chronic bronchitis and is stimulated by vanadium, a fibrogenic metal (Zhang et al., 2001). Therefore, activation of MAP kinase pathways, whether by transition metals or other components of welding fumes, is a potentially significant mechanism of toxicity relative to respiratory disease.

We have previously reported that human lung epithelial cells *in vitro* showed a significant increase in intracellular phosphoprotein, a measure of signal pathway activation, following exposure to 0.2–200 μM hexavalent chromium, manganese and nickel, and that this was associated with cytotoxicity and inflammatory cytokine release for the hexavalent chromium and manganese exposures only (Pascal and Tessier, 2004). A body of research exists on the effects of transition metals on cellular signal pathways. However to date the effects of hexavalent chromium, manganese and nickel on MAP kinase activation in human lung epithelium has not been demonstrated, nor has the relative increase in activation of the ERK1/2, SAPK/JNK and p38 MAP pathways been reported. Here we report on the activation of the ERK1/2, SAPK/JNK and p38 MAP kinases following metal exposures similar to our previous study, in support of current epidemiological and biological evidence towards the hypothesis that the metal composition of welding fumes, particularly for chromium, manganese and nickel, are etiological factors in respiratory disease caused by this exposure (Antonini et al., 2004; Keskinen et al., 1980).

2. Materials and methods

2.1. Cell culture and treatment

Normal human small airway epithelial cells (SAEC, Clonetics, San Diego, CA) were cultured in small airway growth