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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

medium (SAGM, Clonetics) supplemented with bovine pituitary extract (30 μg/ml), hydrocortisone (0.5 μg/ml), human recombinant epidermal growth factor (0.5 mg/ml), epinephrine (0.5 μg/ml), transferring (10 μg/ml), insulin (5 μg/ml), retinoic acid (0.1 mg/ml), triiodothryonine (6.5 mg/ml), gentamicin (50 μg/ml), amphotericin (50 mg/ml), and 5% fatty-acid free bovine serum albumin. All cell cultures were maintained in a 5% CO_2/37 °C humid atmosphere. Cells were passaged by trypsin-EDTA treatment (Clonetics), and passage number was kept to less than 10 passages from original stocks. SAEC cells are derived from primary cultures of normal, non-cancerous human small airway epithelium of healthy donors, and retain the normal function of bronchial epithelium.

Aqueous stock solutions of chromium(VI) (as $K_2Cr_2O_7$) and elemental chromium, nickel, and manganese (Chem Service, West Chester, PA) were used to prepare 1000-fold dosing solutions of each metal in distilled-deionized water. The dosing solutions were added to the cell culture medium to give desired test concentrations. Vehicle (distilled-deionized water) concentrations were 0.1%. Metals were tested over the concentration range 0.2–200 μM.

2.2. Cytotoxicity assay

SAEC cells were seeded at 2500 cells/cm^2 in 100 μl culture medium in 96-well microtiter plates and cultured to confluence. Cells were then exposed to metals as described above for 1 h or 48 h prior to assessing viability. Control cells were dosed with vehicle only. Viability was determined via mitochondrial cleavage of the tetrazolium salt MTS (CellTiter96, Promega, Madison, WI). Following the exposure period, MTS reagent was added to the culture medium and incubated 2 h. Production of the soluble formazan cleavage product of MTS was quantitatively measured on a microtiter plate reader at 450 nm wavelength.

2.3. Immunoblot analysis of phosphorylated protein

SAEC cells were seeded at 2500 cells/cm^2 in a six-well plate and cultured to confluence. Cells were then exposed to metals for 1 h prior to assessing MAPK phosphorylation status. Control cells were dosed with vehicle only. Cell extracts for immunoblot analysis were prepared by rinsing the cells in cold HEPES buffer (50 mM HEPES, pH 7.5, 1.5 mM $MgCl_2$, 10 mM KCl) and lysing in RIPA buffer (50 mM Tris, pH 7.5, 1% Nonidet P-5, 40, 0.1% deoxycholate, 150 mM NaCl, 2 mM DTT, 50 mM Tris–HCl, 1 mM PMSF, 2 μg/ml aprotinin, 0.5 μg/ml leupeptin, 1 μg/ml pepstatin A) on ice for 15 min. The RIPA lysate was clarified at 16,000 × g for 10 min and the supernatant recovered, snap-frozen in liquid nitrogen and stored at −80 °C until analysis. Protein concentrations were determined by a modified Lowry assay (DC Protein Assay, BioRad, Hercules, CA). Aliquots equal to 40 μg protein per lane were electrophoresed on a 10% SDS-PAGE mini-gel and electro-transferred to PVDF membrane (BioRad). Membranes were blocked in 5% non-fat dried milk. The transferred proteins were probed with antibodies specific for tyrosine-, serine-, threonine-phosphorylated protein (P-Tyr-100, phospho-(Ser) 14-3-3 and P-Thr-polyclonal, respectively, Cell Signaling Technology; Beverly, MA), the phosphorylated and non-phosphorylated forms of ERK 1/2, SAPK/JNK and p38 (phospho-p44/42 Thr202/Tyr204; p44/42 Thr202/Tyr204; phospho-SAPK/JNK Thr183/Tyr185; SAPK/JNK Thr183/Tyr185; phospho-p38 (D-8); p38 (H-147), respectively, Cell Signaling Technology; Beverly, MA). The secondary antibodies were goat anti-rabbit IgG or goat anti-mouse IgG antibody conjugated to horseradish peroxidase (Santa Cruz Biotechnology; Santa Cruz, CA). Protein bands were visualized via chemiluminescence (SuperSignal West Femto, Pierce, Rockford, IL). Images were digitally captured with a Kodak Imagestation 440CF.

2.4. Statistical analysis

Immunoblot experiments were run in triplicate, and cytotoxicity experiments were run in quadruplicate and repeated two to four times. Data were compared by one-way ANOVA followed by Dunnett's t-test. The criterion for significance was a p value < 0.05 for all comparisons.

3. Results

We determined the effect of the principle toxic metals found in welding fumes on the ERK1/2, SAPK/JNK and p38 MAP kinases, as signaling pathways acting through these kinases are key regulators of cellular stress responses, apoptotic and anti-apoptotic signals and cellular growth and proliferation. Normal human lung epithelial cells (SAEC) were exposed to elemental and hexavalent chromium, manganese and nickel at concentrations ranging from 0.2 to 200 μM. These concentrations were not acutely toxic, with the exception of the highest concentration (200 μM) of hexavalent chromium (Table 1).

Hexavalent chromium caused a dose-dependent increase in tyrosine- and threonine-phosphorylated protein in SAEC human lung epithelial cells following a 1 h exposure, as determined by immunoblot analysis (Fig. 1). Prominent phosphoprotein bands occurred between approximately 25 and 55 kDa on the phosphotyrosine and phosphothreonine blots, which is consistent with the ERK1/2, SAPK/JNK and p38 kinases (42/44, 46/54 and 38 kDa, respectively). The ERK1/2 kinase in its activated state is dually phosphorylated at the threonine 202 and tyrosine 204 residues; SAPK/JNK is dually phosphorylated at threonine 183 and tyrosine 185; and p38 is dually phosphorylated at the threonine 180 and tyrosine 182 residues. As no effect on serine phosphorylation was observed (data not shown), the phosphoryla-

Table 1
Viability of SAEC lung epithelial cells following short-term exposure to metals

Metal	Exposure period (h)	Metal concentration (μM)			
		0.2	2	20	200
Chromium 6+	1	100.9 ± 6.7	107.7 ± 2.8	105.5 ± 3.5	68.8 ± 2.0*
	24	105.6 ± 16.2	88.9 ± 10.2	23.0 ± 1.7*	28.4 ± 4.7*
Manganese	1	120.9 ± 2.7	110.2 ± 2.2	88.6 ± 2.9	94.3 ± 2.5
	24	97.2 ± 10.1	101.9 ± 0.1	77.2 ± 9.2*	65.3 ± 8.6*
Nickel	1	–	–	–	–
	24	143.0 ± 18.3*	93.2 ± 7.5	113.2 ± 9.5	104.4 ± 19.0

Values are percent viability relative to untreated controls. –: not determined.
* Statistically significant from controls at $p < 0.05$.

tion specificity (threonine and tyrosine residues) is also consistent with these MAP kinases.

Immunoblot analysis for each specific kinase (i.e., ERK 1/2, SAPK/JNK and p38) confirmed a dose dependent increase in phosphorylation status, indicating activation by hexavalent chromium (Fig. 1). Probes for the non-phosphorylated forms of each kinase showed no changes relative to controls, indicating that the effect of hexavalent chromium manifesting after 1 h exposure was on activation status alone and not on protein expression.

We compared the relative intensity of the activation effect among the three kinases. Most notable was the response of the SAPK/JNK and p38 kinases relative to ERK1/2 (Fig. 2). The three kinases responded similarly (approximately two-fold) at the lower hexavant chromium concentrations of 0.2 and 2 μM. At the higher 20 and 200 μM concentrations, p38 kinase was most strongly activated, showing an approximately 23-fold increase in phospho-p38 relative to untreated controls. The SAPK/JNK kinase also responded strongly, showing an approximately 17-fold increase in phospho-SAPK/JNK relative to controls. In comparison to these dramatic increases, the ERK1/2 kinase showed a four-fold increase in the phosphorylated form relative to controls.

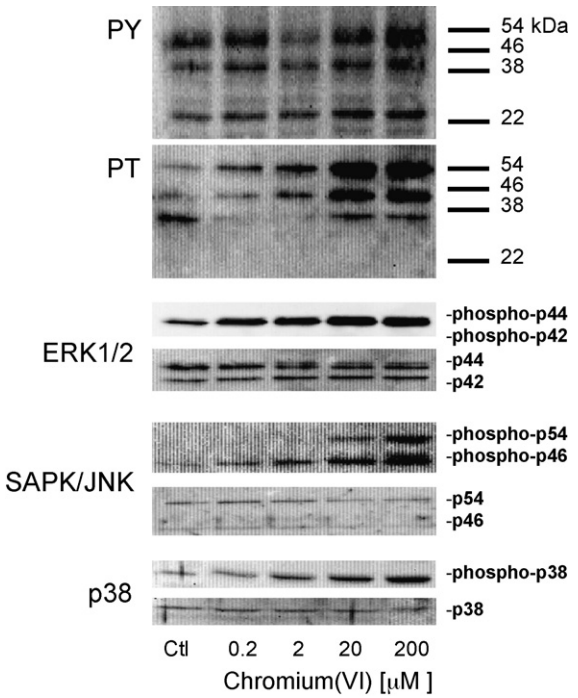

Fig. 1. Protein phosphorylation in the SAEC human lung epithelial cell line following exposure to hexavalent chromium. SAEC cells were treated with 0.2–200 μM $K_2Cr_2O_7$ for 60 min. Cellular extracts were resolved on 10% SDS-PAGE minigels, transferred to PVDF membranes, and probed for phosphotyrosine (PY), phosphothronine (PT) and the phosphorylated and nonphosphorylated forms of ERK1/2, SAPK/JNK and p38. Controls were exposed to vehicle only (distilled H_2O).

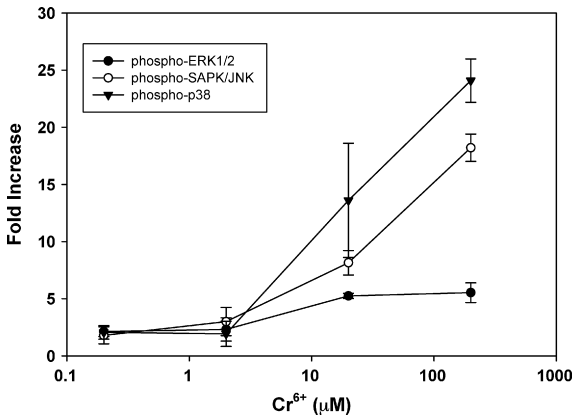

Fig. 2. Fold-increase in MAPK phosphorylation relative to controls due to hexavalent chromium exposure. Net band intensities (arbitrary units) of digitally captured chemiluminescence signals at each exposure concentration were compared to control band intensities. Data points are averages of signal comparisons from three immunoblots.

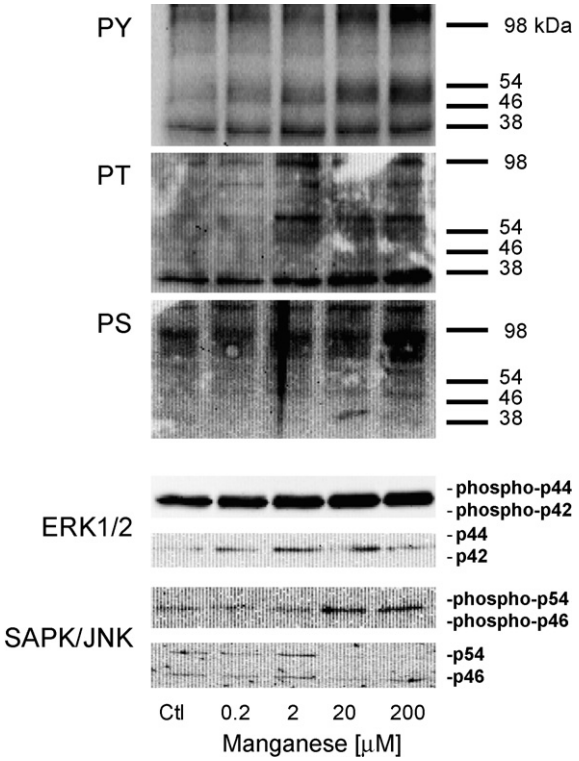

Fig. 3. Protein phosphorylation in the SAEC human lung epithelial cell line following exposure to manganese. SAEC cells were treated with 0.2–200 μM manganese for 60 min. Cellular extracts were resolved on 10% SDS-PAGE minigels, transferred to PVDF membranes, and probed for phosphotyrosine (PY), phosphothronine (PT) and phosphoserine (PS) and the phosphorylated and nonphosphorylated forms of ERK1/2 and SAPK/JNK. Controls were exposed to vehicle only (distilled H_2O).

Within the 1 h timeframe of the protein phosphorylation experiments, hexavalent chromium was not acutely cytotoxic to SAEC cells except at the highest (200 μM) dose (Table 1). Further cytotoxicity testing showed that after 48 h the lowest dose (0.2 μM) had no effect on SAEC viability, while higher doses of 2–200 μM showed significant cytotoxic effects.

Manganese caused a dose-dependent increase in tyrosine-, serine- and threonine-phosphorylated protein following a 1 h exposure, as determined by immunoblot analysis (Fig. 3). Prominent phosphoprotein bands occurred between approximately 25 and 52 kDa on the phosphotyrosine and phosphothreonine blots, which is consistent with the ERK1/2, SAPK/JNK and p38 kinases (42/44, 46/54 and 38 kDa, respectively). Protein bands of approximately 90–100 kDa were also observed on the phosphotyrosine and phosphothreonine blots as well as on a phosphoserine blot.

Immunoblot analysis for each specific kinase confirmed a dose dependent increase in phosphorylation status for ERK1/2 and SAPK/JNK, indicating activation by manganese (Fig. 3). Probes for the non-phosphorylated forms of these kinases showed no changes relative to controls, indicating that the effect of manganese manifesting after 1 h exposure was on activation status alone and not on protein expression. No effect on p38 was observed. Although the phosphothronine blot did show a band at 38 kDa with a dose dependent increase in phosphorylation, the corresponding band on the phosphotyrosine blot did not show such a dose dependent increase. This may explain in part the lack of a detectable response in specific probes for phospho-p38.

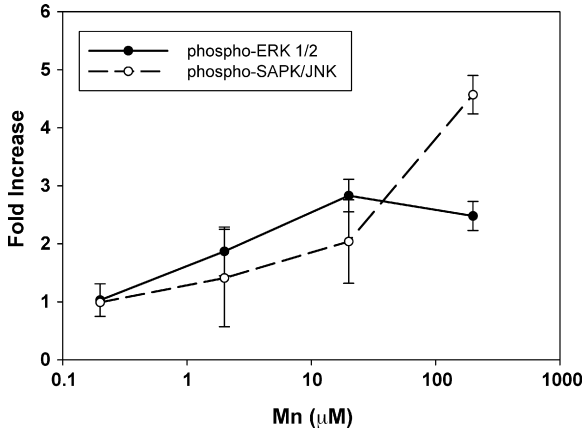

Fig. 4. Fold-increase in MAPK phosphorylation relative to controls due to manganese exposure. Net band intensities (arbitrary units) of digitally captured chemiluminescence signals at each exposure concentration were compared to control band intensities. Data points are averages of signal comparisons from three immunoblots.

The relative increase in phosphorylation of ERK1/2 and SAPK/JNK was between two- and four-fold relative to controls (Fig. 4). The relative increase of ERK1/2 was similar to that observed with hexavalent chromium (Fig. 2). However, the relative increase in SAPK/JNK phosphorylation was much less than the 10–25-fold increases seen with hexavalent chromium at the same exposure concentrations.

Within the 1 h timeframe of the protein phosphorylation experiments, manganese was not acutely cytotoxic to SAEC cells at any exposure concentration (Table 1). Further testing after 48 h exposure showed cytotoxicity only at the two higher concentrations (20–200 μM).

Nickel also caused a dose dependent increase in tyrosine- and serine-phosphorylated protein with a qualitatively different response than that observed following hexavalent chromium and manganese exposure. Prominent phosphotyrosine bands appeared in the range of approximately 20–200 kDa, while a single phosphoserine band at approximately 100 kDa (Fig. 5). Only

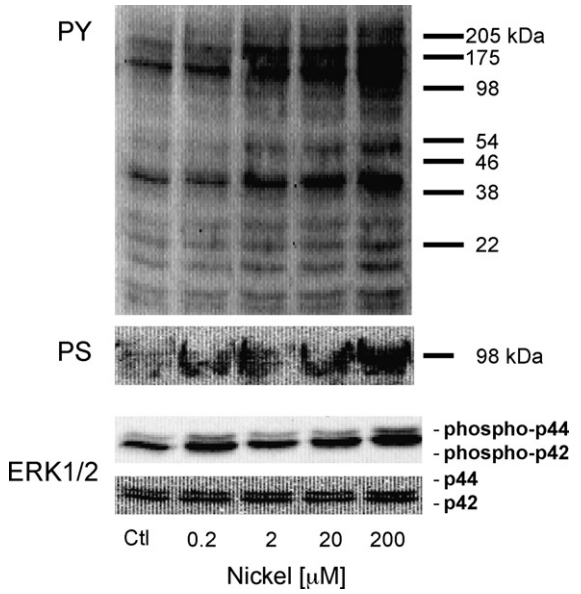

Fig. 5. Protein phosphorylation in the SAEC human lung epithelial cell line following exposure to nickel. SAEC cells were treated with 0.2–200 μM nickel for 60 min. Cellular extracts were resolved on 10% SDS-PAGE minigels, transferred to PVDF membranes, and probed for phosphotyrosine (PY), and phosphoserine (PS) and the phosphorylated and nonphosphorylated forms of ERK1/2. Controls were exposed to vehicle only (distilled H_2O).

ERK1/2 was specifically phosphorylated in response to nickel exposure. Nickel was not cytotoxic to SAEC cells at any exposure concentration after 48 h, and the lowest exposure concentration (0.2 μM) was stimulatory to SAEC proliferation (Table 1).

The dose concentrations used in the present study are relevant to welding fume exposures. To demonstrate this quantitatively, chromium is used as an example. The dose range used in all experiments was 0.2–200 μM, equivalent to mass concentrations of 0.01–10 μg/ml in cell culture media for chromium (atomic mass 52). Experiments were conducted in 100 cm^2 Petri dishes containing 12 ml media, giving a total dose range of 0.12–120 μg chromium per 100 cm^2 cell monolayer area or 0.012–12 mg/m^2. Welding fume concentrations have been estimated at 100–400 mg/m^3 (Ulfarson, 1981), and Taylor et al. (2003) measured chromium concentrations in welding fumes as high as 31 μg/mg fume. With these values, chromium concentrations would range from 3.1 to 12.4 mg/m^3. To estimate a human exposure dose, we used an average breathing rate for moderate activity of 1.6 m^3/h (Layton, 1993) and an 8 h work day where dose is given by [metal concentration (mg/m^3)] × [breathing rate (m^3/h)] × [time (h)] for a daily dose range of approximately 40–160 mg. Assuming an alveolar surface area of 100 m^2, the dose per unit surface area would be 0.4–1.6 mg/m^2, which is bracketed by the *in vitro* exposure doses. This simplified calculation does not purport to represent advanced dosimetry estimates, but rather to show that the *in vitro* concentrations used in the present study are within a meaningful range.

4. Discussion

The MAP kinase protein phosphorylation cascades regulate cellular processes ranging from proliferation and differentiation to inflammatory cytokine release and apoptosis. The ERK1/2 pathway positively regulates cellular growth and differentiation through proliferative and anti-apoptotic signals. The SAPK/JNK and p38 pathways regulate a variety of stress responses, principally inflammatory mediator production and release and the initiation of apoptotic processes.

We evaluated the effect of hexavalent chromium, manganese and nickel, three toxic metals found in welding fumes, on protein phosphorylation in SAEC normal human lung epithelial cells *in vitro*. General protein phosphorylation was assessed as a general measure of cellular signaling activity initiated by metal treatment, followed by determination of the specific MAP kinases. There were qualitative and quantitative differences in cellular protein phosphorylation due to each metal. Hexavalent chromium appeared to effect a more limited range of protein phosphorylation than manganese or nickel, whereas it was significantly more toxic.

Protein phosphorylation was initiated within an hour following initial exposure, at which time cells remained viable. However at 24 h postexposure, hexavalent chromium and manganese resulted in significant cell death, so the protein phosphorylation activity observable at 1 h is related either to early survival mechanisms (that fail), stress signals to other cell populations (e.g. immune cells), and/or the initiation of apoptotic processes.

Hexavalent chromium was shown to activate the ERK1/2, SAPK/JNK and p38 pathways over a 0.2–200 μM concentration range following a 1 h exposure. This activation was measured as fold-increase in levels of the phosphorylated form of each individual kinase relative to levels in untreated controls. At lower chromium concentrations all three kinases showed equivalent activation, whereas at higher chromium concentrations p38 and SAPK/JNK showed significantly higher activation relative to ERK1/2. Immunoblots for general phosphoprotein show that the effect of chromium was limited to the tyrosine- and threonine-phosphorylated protein in the molecular weight range 22–55 kDa. Although this does not indicate that the effect of chromium is limited strictly to the MAP kinase sig-

naling pathways, it does demonstrate a limited range of cellular phosphoprotein mediated activity within the cell in response to this toxicant.

The higher chromium concentrations also resulted in significant cell death after 48 h exposure, so this cytotoxic response appears to be associated with the action of the p38 and SAPK/JNK signals, the lack of ERK1/2 signaling, or a combination of the two. It is interesting to note that at 2 μM chromium, the response of all three MAPK pathways is relatively the same, and this concentration yielded a modest, non-significant cytotoxic effect. Therefore at this and lower concentrations the balance of MAPK signals favored cell survival, whereas at higher concentrations the predominance of SAPK/JNK and p38 signaling was associated with significant cell death.

Manganese was similarly shown to activate ERK1/2 and SAPK/JNK kinases, but p38 kinase was unaffected under these experimental conditions. The effect was much less pronounced than that of hexavalent chromium, and only at the highest concentration did the relative activation of SAPK/JNK exceed that of ERK1/2. In addition to this more modest effect on MAPK activation, we also observed a greater diversity of protein phosphorylation. Manganese exposure caused an increase in tyrosine- and threonine-phosphorylated protein in the range of approximately 22–100 kDa, also in serine-phosphorylated protein in the range of approximately 50–175 kDa. Taken together these data include protein bands consistent with the MAP kinases as well as proteins whose molecular weight and phosphorylation characteristics would place them outside this family of proteins. Therefore, whereas manganese has a more limited effect on MAP kinase signaling relative to chromium, it appears to initiate a greater range of cellular responses which may account for the observation that manganese is less toxic to SAEC cells than hexavalent chromium.

In the present series of experiments, nickel caused an increase in cellular tyrosine-phosphorylated protein in the molecular weight range of approximately 10–210 kDa and a single serine-phosphorylated protein band at approximately 100 kDa. Of the three MAP kinases, only ERK1/2 was effected by nickel. Nickel was not toxic to SAEC cells over the concentration range tested, and showed a significant proliferative effect after 48 h exposure, which is consistent with the positive regulation of cellular growth and proliferation by ERK1/2.

In conclusion, MAP kinases are activated by metal components of welding fumes, with qualitatively and quantitatively distinct responses by the ERK 1/2, SAPK/JNK and p38 pathways following hexavalent chromium, manganese and nickel exposure. The relative response of each MAPK pathway to a given metal exposure appears to play a significant role in the eventual cellular response. A more detailed understanding of these phenomena in lung epithelium may shed light on the mechanisms through which epithelial cytotoxicity following welding fume exposure manifests as respiratory disease.

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