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Altered ion transport in normal human bronchial epithelial cells following exposure to chemically distinct metal welding fume particles

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ABSTRACT

Welding fume inhalation causes pulmonary toxicity, including susceptibility to infection. We hypothesized that airway epithelial ion transport is a target of fume toxicity, and investigated the effects of fume particulates from manual metal arc-stainless steel (MMA-SS) and gas metal arc-mild steel (GMA-MS) on ion transport in normal human bronchial epithelium (NHBE) cultured in air-interface. MMA-SS particles, more soluble than GMA-MS particles, contain Cr, Ni, Fe and Mn; GMA-MS particles contain Fe and Mn. MMA-SS or GMA-MS particles (0.0167–166.7 μg/cm^2) were applied apically to NHBEs. After 18 h transepithelial potential difference (V_t), resistance (R_t), and short circuit current (I_{sc}) were measured. Particle effects on Na^+ and Cl^- channels and the Na^+,K^+,2Cl^--cotransporter were evaluated using amiloride (apical), 5-nitro-2-[(3-phenylpropyl)amino]benzoic acid (NPPB, apical), and bumetanide (basolateral), respectively. MMA-SS (0.0167–16.7 μg/cm^2) increased basal V_t. Only 16.7 μg/cm^2 GMA-MS increased basal V_t significantly. MMA-SS or GMA-MS exposure potentiated I_{sc} responses (decreases) to amiloride and bumetanide, while not affecting those to NPPB, GMA-MS to a lesser degree than MMA-SS. Variable effects on R_t were observed in response to amiloride, and bumetanide. Generally, MMA-SS was more potent in altering responses to amiloride and bumetanide than GMA-MS. Hyperpolarization occurred in the absence of LDH release, but decreases in V_t, R_t, and I_{sc} at higher fume particulate doses accompanied LDH release, to a greater extent for MMA-SS. Thus, Na^+ transport and Na^+,K^+,2Cl^--cotransport are affected by fume exposure; MMA-MS is more potent than GMA-MS. Enhanced Na^+ absorption and decreased airway surface liquid could compromise defenses against infection.

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1. Introduction

Inhalation of welding fume is associated with numerous morbidities in the pulmonary system of workers (Sferlazza and Beckett, 1991; Antonini et al., 2003, 2010). Several types of welding processes are utilized in manufacturing, and they give rise to fumes of different metal compositions and flux-derived materials (Antonini et al., 1999). In shielded manual metal arc-stainless steel (MMA-SS) welding, fluxes are incorporated into welding rods to protect the welds from oxidation. In gas metal arc-mild steel (GMA-MS) welding, shielding gases are used to protect against oxidation. The composition of the particles generated during MMA-SS and GMA-MS processes are different: MMA-SS particles contain Cr, Ni, Fe and Mn (28% Cr, 3% Ni, 41% Fe and 17% Mn, respectively), whereas GMA-MS particles contain Fe and Mn (85% and 14%, respectively) (Antonini et al., 1999, 2003). Antonini et al. (1999) also determined that MMA-SS fume particles are more soluble than GMA-MS particles (soluble/insoluble ratio of 0.345 for MMA-SS compared to 0.02 for GMA-MS).

A number of investigations have described differences in the toxicities of welding fumes of different composition in myriad organ systems. In a comparison of the relative toxicities of MMA-SS and GMA-MS fumes following intratracheal instillation of generated particles, Antonini et al. (2010) observed that MMA-SS evoked substantially more lung toxicity than GMA-MS, in terms of the cellular inflammatory response, and elevations in the levels in lung lavage of lactate dehydrogenase (LDH) activity and albumin; GMA-MS elicited only mild responses. These differences could have arisen from the greater water solubility of MMA-SS metals compared to GMA-MS. In a similar, comparative study addressing differences between manual metal arc-hard surfacing (MMA-HS) and GMA-MS, a greater toxicity of MMA-HS was observed in the heart, in which myocardial contractility and reactivity to the positive inotropic, β-adrenoceptor agonist, isoproterenol, were diminished to a greater degree by MMA-SS (Zheng et al., 2015). Consisting of Mn (50.9%) and Cr (8.5%), MMA-HS fume also has greater solubility than GMA-MS. Zeidler-Erdely et al. (2008) found in a comparison of two mouse strains a possible tumorigenic effect caused by gas metal arc-stainless steel (GMA-SS) fume that was not evident in

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animals treated with MMA-SS and GMA-MS fumes; GMA-SS has greater bio-persistence than the other two fumes. Lastly, in a comparison of the effects of MMA-SS, GMA-SS and GMA-MS, pulmonary inflammatory gene induction was greatest after treatment with MMA-SS, whereas the largest increase in expression of stress genes in aorta and heart occurred in response to MMA-SS, which was attributable, as least in part, to soluble Cr (Erdely et al., 2011).

Welders experience greater susceptibility to respiratory infections and an increase in mortality due to lobar pneumonia (Coggon et al., 1994; Antonini et al., 2004; Coggon and Palmer, 2016; Marongiu et al., 2016; Suri et al., 2016). A number of proposals have been advanced to explain this finding, including a recent one from a report by Suri et al. (2016) in which it was observed that adherence of pneumococcal bacteria and infection is increased in cultured cells and in vivo in mice. Differences have been observed in the effects of MMA-SS and GMA-MS on the clearance of bacteria from the lungs. Compared to GMA-MS, the clearance of *L. monocytogenes* after infection was reduced substantially following treatment of rats with fumes generated from MMA-SS, in association with inflammation and immune suppression in the lung (Antonini et al., 2004). MMA-SS and GMA-MS fumes also differed in their detrimental effects on macrophage function and viability: MMA-SS produced greater toxicity than GMA-SS (Antonini et al., 1999).

We hypothesized that clearance of bacteria from the airways by the mucociliary escalator may be retarded by exposure to welding fumes, which is an alteration that would prolong bacterial residence and foster greater adherence to airway cells. This could arise if the fume exerts a toxic effect on airway epithelium, so as to interfere with ion transport and the establishment of an airway surface liquid (ASL) that has a depth that is sufficient to allow adequate beating of cilia and clearance of bacteria from the lungs by the escalator. Such a dehydration of the ASL is thought to account for the chronic bacterial infection seen in cystic fibrosis patients (Haq et al., 2016). It is possible that some differences in lung toxicity caused by MMA-SS and GMA-MS fumes could reflect differences in the metal compositions and solubilities of the fumes. Thus, the present study was conducted to investigate the effects of MMA-SS and GMA-MS fume particles on ion transport in human primary cultured airway epithelial cells. Fume particle effects on concomitant LDH release also was investigated to ascertain effects on ion transport and cell damage occur concomitantly with respect to fume dose. The results indicate that welding fume particles interfere with ion transport in such a way as to favor increased transepithelial Na^+ absorption through ENaC. Associated with dehydration of the airway surface liquid, this effect could hinder efficient clearance of bacteria from the airways.

2. Materials and methods

2.1. Culture of normal human bronchial epithelial (NHBE) cells

NHBE cells (Lonza Inc.; Walkersville, MA) cells were seeded in plastic T-75 flasks and were grown in submersion in B-ALI medium (Lonza) until 80% confluent. The confluent monolayer was trypsinized for 4–6 min, and cells were seeded onto permeable inserts (0.4 μm pores; Corning; Corning, NY) that had been coated with rat tail collagen (BD Biosciences; San Jose, CA); cell density was 50,000 cells per insert. Cells were maintained at 37 °C in an air/5% CO_2 mixture in an incubator. NHBE cells were submerged for three days in B-ALI growth medium (100 μl apical chamber; 500 μl basal chamber) before 500 μl of B-ALI differentiation medium was added to the basal chamber and the apical medium removed to initiate the air-liquid interface (ALI) culture conditions. Medium was changed every 48 h. During cell growth and differentiation transepithelial resistance (R_t) was measured with EVOM^2 epithelial volt-ohm meter STX^2 electrodes (World Precision Instruments; Sarasota, FL) to assess growth to confluence from the increase in the R_t. Cells were ready for use at 24 d, when R_t reached a value of at least 1000 Ω cm^2.

2.2. Exposure of NHBE cells to welding fumes

The MMA-SS and GMA-MS fume particles used in this study were provided by Lincoln Electric Co. (Cleveland, OH). Details regarding the generation and characterization of these particles have been published (Zeidler-Erdely et al., 2008; Antonini et al., 1999). Count median diameters of the particles were 0.92 and 1.38 μm for MMA-SS and GMA-MS respectively. Particles that had been collected on filters were suspended in B-ALI differentiation medium and applied (50 μl) to the apical surface of the cells in doses ranging from 0.0167 to 166.7 μg/cm^2. Control cells received only differentiation medium. The NHBE cells were incubated under these conditions for 18 h before measurements were made. The lowest dose was equivalent to ~17 mg in a human lung deposition, calculated as ~2 days of human exposure at the previous threshold limit value-time-weighted average of 5 mg/m^3.

2.3. NHBE cell electrophysiology

After incubation with welding fumes, inserts containing adherent NHBE cells were placed into Ussing chambers in order to measure transepithelial potential difference (V_t; mV), using Ag/AgCl electrodes (0.9% NaCl in 4% agar). The apical and basolateral chambers contained modified Krebs-Henseleit (MKH) solution (composition below). The cells were allowed to reach a stable V_t under open-circuit conditions before applying a 0 mV voltage-clamp in order to record short-circuit current (I_{sc}; μA/cm^2) using an EVC 4000 automatic voltage/current amplifier (World Precision Instruments; Sarasota, FL). Square-wave voltage pulses (1 mV, 5 s duration) were delivered every 55 s to yield a voltage response for calculation of R_t from Ohm's law.

2.4. NHBE cell ion channel/transport blockers

Under short-circuit conditions, responses (I_{sc} and R_t) of the cells were measured after administration of the epithelial Na^+ channel blocker, amiloride (3.5×10^{-5} M), applied to the apical chamber of the Ussing chamber; the Cl^- channel blocker, 5-nitro-2-(3-phenylpropylamino)-benzoic acid (NPPB, 10^{-4} M), applied to the apical chamber; and bumetanide (10^{-4} M), the Na^+,K^+,2Cl^--cotransport blocker, applied to the basolateral chamber. These agents evoked electrophysiological responses, which were quantified as percent change from I_{sc} values before their addition, i.e., %Δ I_{sc} and %Δ R_t.

2.5. Measurement of LDH activity

The oxidation of lactate to pyruvate and the formation of NADH was measured in samples from the apical and basolateral compartments of the transwell inserts. Medium was harvested from four inserts for each dose of fume particles or controls. 100 μl of B-ALI differentiation medium was first added to the apical compartment, which had been given 50 μl with the fume treatment, from which 150 μl was retrieved for assay of LDH activity. LDH measurements were made using a COBAS c111 analyzer (Roche Diagnostic Systems; Indianapolis, IN).

2.6. Chemicals and reagents

MKH solution (pH 7.4, 37 °C) contained 113 mM NaCl, 4.8 mM KCl, 2.5 mM $CaCl_2$, 1.2 mM KH_2PO_4, 1.2 mM $MgSO_4$, 25 mM $NaHCO_3$, and 5.7 mM glucose, and was saturated with 95% O_2/5% CO_2. Amiloride, NPPB, and bumetanide were dissolved in saline. All drugs and reagents were from Sigma-Aldrich (St. Louis, MO).

2.7. Statistical analysis

The results are expressed as means ± S.E.M of replicate determinations using separate inserts for Ussing chamber and LDH measurements.