Article

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Effects of mild steel welding fume particles on pulmonary epithelial inflammation and endothelial activation

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Abstract

Welders have an increased risk for cardiovascular disease (CVD) following exposure to welding fumes. The underlying mechanisms are largely unknown; however, oxidative stress, systemic inflammation, and endothelial dysfunction have been suggested as contributing factors to particle-induced CVD. We investigated effects of mild steel welding fume (MSWF) on three target cell types: macrophages, pulmonary epithelial, and vascular endothelial cells. Cells were exposed to MSWF at nontoxic doses for 6 h/day, for five consecutive days. The expression of 40 genes involved in inflammation, fibrosis, and endothelial activation was analyzed. Moreover, changes in the reactive oxygen species production and migration capacity of cells were assessed. The expression of matrix metallopeptidase 1 (*MMP1*) was induced in both epithelial and endothelial cells following repeated exposure to MSWF. Although *MMP1* is important in inflammatory responses *in vivo*, this effect was not concurrent with changes in the inflammatory status, cell proliferation, and migration capacities, nor did it induce oxidative stress in the cells. Thus, repeated exposure with low doses of MSWF was sufficient neither for inducing inflammatory stress in epithelial cells and macrophages nor for endothelial activation, and higher concentrations of MSWF or the nonparticle fraction of MSWF may be critical in causing the increased risk of CVD observed among welders.

Keywords

Pulmonary inflammation, endothelial activation, oxidative stress, welding fume, cardiovascular disease, welders

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Introduction

Welding is an industrial process that employs heat to join metal pieces through melting, leading to generation of complex aerosols of metal fumes, gases, and solid particles. The reaction between air and vaporized metals produces metal oxides, which condense and form complex fume particles of respirable sizes (Antonini et al., 2003). Health effects of exposure to welding fume (WF) are complex, as the WF composition is affected by the type of welding alloy used (Leonard et al., 2010; Zheng et al., 2015). The most common feed wire types used in arc welding are mild steel and stainless steel, and the different WFs generated cause diverse responses correlated with the metal composition, the soluble ion content, and the capability to produce free radicals (Taylor et al., 2003).

An association between occupational exposure to WF and cardiovascular disease (CVD) has been suggested, and some studies report that workers exposed to welding processed particles have an elevated risk of acute myocardial infarction and angina pectoris (Ibfelt et al., 2010; Li et al., 2015; Mocevic et al., 2015). Moreover, metal arc welders have increased risk for ventricular ectopy (Cavallari et al., 2008). WF-exposed animals present pulmonary inflammation and lung injury (Presume et al., 2016; Shoeb

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et al., 2017), as well as elevated oxidative stress and systemic leukocyte dysfunction (Erdely et al., 2014) leading to increased lesions of atherosclerotic plaques (Erdely et al., 2011). Furthermore, chronic exposure to WF results in welding particle accumulation and deposition of agglomerates within lung cells, particularly inside alveolar macrophages (Antonini et al., 2013). Particles entering the lungs may provoke pulmonary oxidative stress and inflammation, leading to a state of systemic oxidative stress and inflammation. This pro-inflammatory state may in turn promote processes related to CVD, including endothelial dysfunction, atherosclerosis progression, and dyslipidemia (Chin, 2015; Du et al., 2016). Welders show inflammatory cell influx, increased oxidative stress, and significant pulmonary injury (Antonini et al., 2013; Graczyk et al., 2016). WF exposure is also associated with systemic inflammatory responses (Kauppi et al., 2015; Shen et al., 2018), and serum concentrations of cytokines, cell adhesion molecules, and the acute phase proteins have been suggested as biomarkers in WF-induced CVD (Baumann et al., 2018; Fang et al., 2010; Jarvela et al., 2013).

Here, we investigated the involvement of oxidative stress, inflammation, and endothelial dysfunction as principal cellular and molecular mechanisms of CVD development following exposure to mild steel welding fume (MSWF). To do so, the three highly relevant cell types, namely macrophages, epithelial and endothelial cells, were repeatedly exposed to low concentrations of MSWF and patterns of common and differential regulated cellular responses were investigated.

Methods

Characterization of MSWF

MSWF containing 43% (m/m) iron, 22% (m/m) zinc, 1.5% (m/m) manganese, ~1% (m/m) carbon, and <0.5% (m/m) copper was obtained from the Health and Safety Laboratory (UK) and dispersed essentially as previously described (Jensen et al., 2011). The hydrodynamic diameter was measured by dynamic light scattering and further characterization was performed by field-emission scanning electron microscopy.

Cell exposures and molecular analysis

Human monocytic THP1, bronchial epithelial HBEC-3KT, and microvascular endothelial HMEC-1 cells were exposed to four concentrations (0.035, 0.175, 0.875, or 4.375 μg/ml) of MSWF. Calculations and exposure setup are shown in the Supplementary material, Methods section.

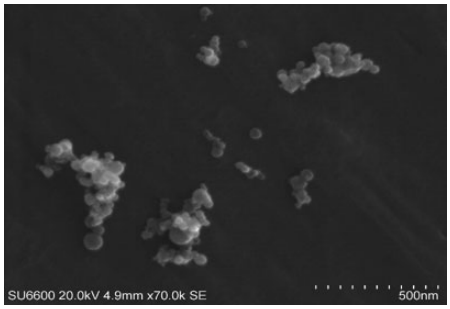

Figure 1. Representative SEM image of MSWF. SEM: scanning electron microscopy; MSWF: mild steel welding fume.

Cellular uptake of MSWF was assessed by confocal microscopy in HBEC-3KT and HMEC-1 cells. Cytotoxicity was assessed by the Cell Counting Kit 8 (CCK-8) assay (Sigma-Aldrich, St. Louis, Missouri). Intracellular reactive oxygen species (ROS) levels were measured using 2′7 ′-dichlorodihydrofluorescein diacetate (Sigma-Aldrich). Cell migration was assessed by live cell imaging using IncuCyte ZOOM (Essen BioScience, Ann Arbor, Michigan). Gene expression was analyzed by a custom RT2 gene array (Qiagen, Hilden, Germany) (Supplementary Table 1). In addition, expression of intracellular adhesion molecule 1 (*ICAM1*), vascular cell adhesion molecule 1 (*VCAM1*), and selectin E (*SELE*) was assessed by real-time polymerase chain reaction. Detailed protocols can be found in the Supplementary material, Methods section. Statistical analyses were performed in STATA v. 16. The values of $p < 0.05$ were considered significant.

Results

Characteristics of MSWF

Dispersed MSWF had a hydrodynamic diameter of 414.73 ± 44.48 nm and was composed of both single particles of approximately 50 nm in diameter and larger agglomerates/aggregates as shown in Figure 1. The MSWF particles were more dispersed in cell culture media and showed some degree of agglomeration/aggregation after 6 h (Supplementary Table 2).

Uptake and effects on cell viability

Cellular uptake of MSWF was not visible at day 1 of exposure, but at day 5 of exposure an accumulation of

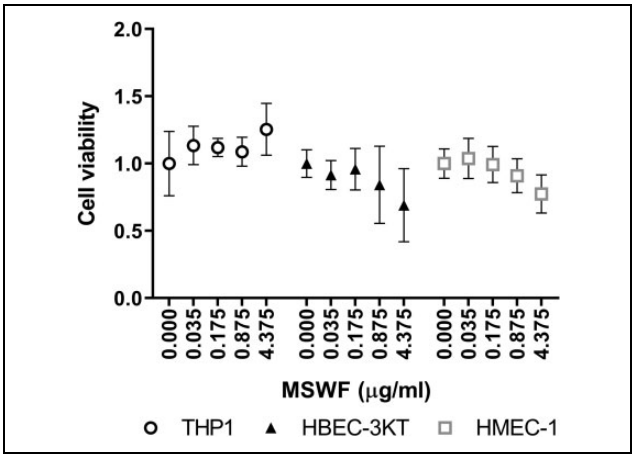

Figure 2. Effects of MSWF exposure on cell viability was assessed in THP1, HBEC-3KT, and HMEC-1 cells on day 1 of exposure. The mean viability of control cells was set to 1. Data indicate mean ± SD. MSWF: mild steel welding fume.

MSWF particles was observed in HBEC-3KT and HMEC-1 cells (Supplementary Figure 1). A trend to a reduction in cell viability was observed in HBEC-3KT and HMEC-1 at day 1 of exposure as shown in Figure 2. However, this trend was not present after 5 days of exposure (data not shown). Based on these data, the lowest occupational relevant concentration and the highest concentration were selected for further analysis.

Effects on gene expression

MSWF exposure only induced minor alterations in the expression patterns of inflammation and endothelial activation markers in exposed HBEC-3KT, HMEC-1, and THP1 cells as illustrated in Figure 3(a) and (b). However, matrix metallopeptidase-1 (*MMP1*) expression showed a significant twofold increase following repeated exposure for 5 days with 4.375 μg/ml MSWF in HBEC-3KT and HMEC-1 cells, in Figure 3(c) and (d). No effects in *MMP1* expression were observed after day 1 of exposure or with lower concentrations. Fold changes in gene expression levels can be found in Supplementary Table 3. Furthermore, MSWF exposure did not affect the expression of *ICAM*, *VCAM*, and *SELE* (data not shown).

Effects on oxidative stress and cell migration

Neither direct exposure with MSWF nor exposure to conditioned media from THP1 cells affected ROS production in HBEC-3KT and HMEC-1 cells as shown in Figure 4. Similarly, exposure to MSWF-conditioned media did not affect the migration of epithelial and endothelial cells as shown in Figure 5.

Discussion

The toxicity of WF is dependent on particle size, distribution, morphology, and chemical composition, as well as on concentration and exposure time. Moreover, metal release, rather than total metal content, is a critical determinant of toxicity (McCarrick et al., 2019). In this study, MSWF exposure did not induce significant cytotoxic effects on macrophages and epithelial and endothelial cells; however, the utilized concentrations were low and of occupational relevance. Generally, toxic effects of WF exposure have been observed at higher concentrations than those included in the current study (Lai et al., 2016; Leonard et al., 2010; McCarrick et al., 2019).

WF may induce pulmonary inflammation, and stainless steel welding fume (SSWF) appears to be more pneumotoxic and has greater inflammatory potential than MSWF, plausibly through differences in the metal constituents (Badding et al., 2014; Taylor et al., 2003). In this study, the expression of several inflammatory markers was not affected by MSWF exposure. However, an increased expression of *MMP1* was observed in epithelial cells after repeated exposure with MSWF. MMPs play important roles in pulmonary inflammation, fibrosis, and COPD and are involved in the recovery from lung damage (Gueders et al., 2006). MMPs are activated by several pro-inflammatory cytokines and growth factors, and *MMP1* expression is increased in alveolar epithelial cells during pulmonary fibrosis, and they represses mitochondrial respiration and oxidative stress, while promoting cell proliferation and migration (Herrera et al., 2013). Enhanced *MMP1* expression was, however, not concurrent with increased ROS production or alterations in the expression of superoxide dismutase 2, nor were changes in expression of TIMP metallopeptidase inhibitor 1 (TIMP1) observed. TIMP1 is a known regulator of MMP activity as it binds to and inhibits MMPs (Brew and Nagase, 2010). The expression of MMPs and TIMPs is coordinately regulated and dependent on the inflammatory status, and dysregulation may potentially lead to tissue injury (Shapiro, 2009). Furthermore, epithelial cell proliferation and migration were not affected by direct MSFW exposure or exposure to conditioned media. Size and age of the particles may affect the generation of free radicals and ROS (Leonard et al., 2010) and, thus,

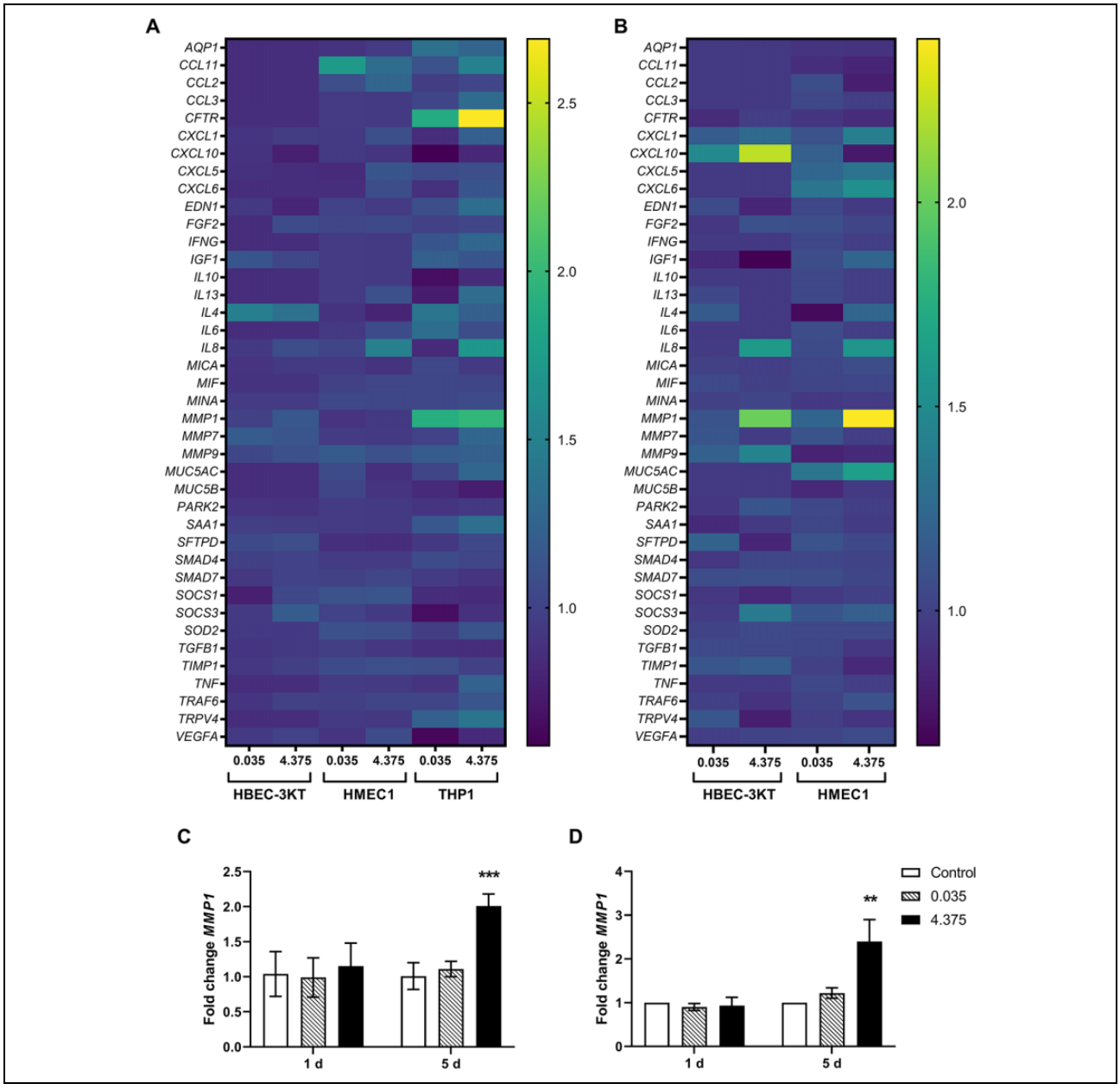

Figure 3. Effects of MSWF exposure on the expression of inflammation, fibrosis, and endothelial activation markers. Mean fold changes in expression were illustrated by heat map at (a) 1 day and (b) 5 days of exposure. Changes in *MMP1* expression in (c) HBEC-3KT and (d) HMEC-1 cells. Data indicate mean fold changes ± SD. Controls were set to 1. Significance was determined by one-way ANOVA with Dunnett's test, ***$p < 0.001$; **$p < 0.01$. MSWF: mild steel welding fume; *MMP1*: matrix metallopeptidase 1; ANOVA: analysis of variance.

effects of exposure to newly produced WF could possibly induce a more prominent effect on ROS production.

Endothelial dysfunction is a key event in the development of CVD, as it actively participates in the process of lesion formation, predisposing to vasoconstriction, platelet activation, leukocyte adhesion, oxidative stress, thrombosis, coagulation and inflammation (Verma et al., 2003). However, endothelial cells may be activated without being dysfunctional, and intact activated endothelium can contribute to disease initiation and progression (Galley and Webster, 2004). Particle exposure can induce endothelial activation by increasing intracellular levels of pro-inflammatory cytokines and chemokines and inducing oxidative stress (Hu et al., 2016). Here,

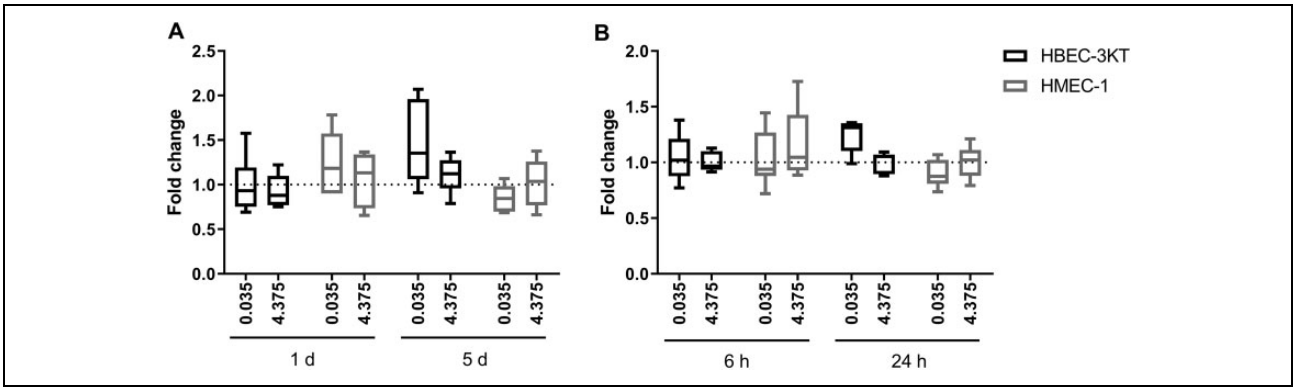

Figure 4. Analysis of oxidative cell stress after MSWF exposure. Intracellular ROS levels were measured in HBEC-3KT and HMEC-1 cells exposed to (a) MSWF for 1 and 5 days and (b) conditioned media for 6 and 24 h. Controls were set to 1. Boxes represent median and 5 and 95 percentiles. MSWF: mild steel welding fume.

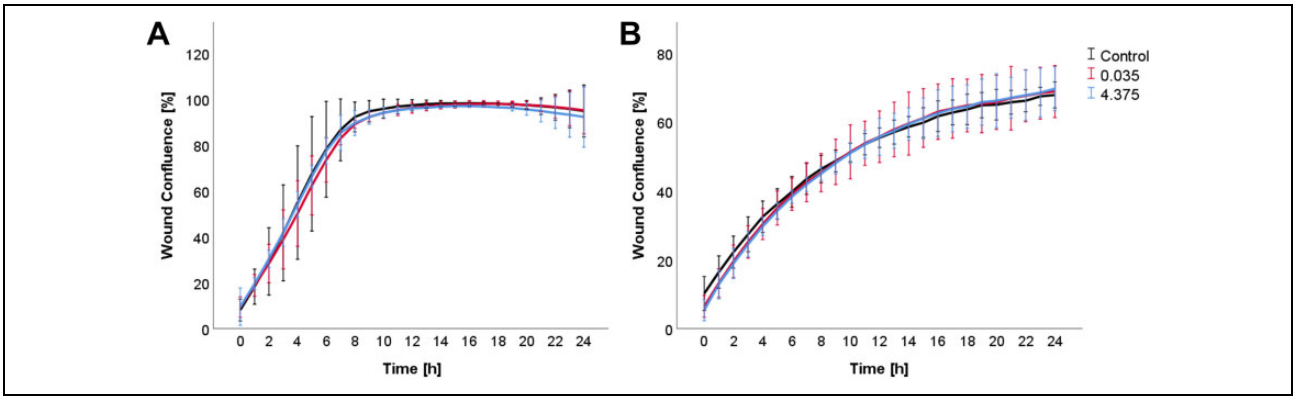

Figure 5. Analysis of cell migration. Migration of (a) HBEC-3KT and (b) HMEC-1 cells following exposure to conditioned media was assessed by live cell imaging. Data represent mean ± SD.

no alterations in the expression of adhesion molecules and endothelial makers were detected following MSWF exposure in HMEC-1 cells. Endothelial cells exposed to conditioned media also did not show any changes in migration capacity. Moreover, no notable changes in inflammatory markers and ROS production were observed, despite an increase in *MMP1* expression following repeated exposure of endothelial cells to MSWF. These data suggest that MSWF exposure is not sufficient in inducing endothelial activation at the concentrations investigated in this study. Interestingly, welders show no change in endothelial function following exposure to MSWF (Kauppi et al., 2015; Li et al., 2015). Higher reactivity and stronger inflammatory responses have been observed for SSWF than for MSWF (Leonard et al., 2010; Shoeb et al., 2017; Taylor et al., 2003), and thus, an involvement of endothelial activation in SSWF-induced CVD cannot be ruled out.

Although the expression of *MMP1* was induced in both epithelial and endothelial cells following repeated exposure to MSWF, this effect was not concurrent with changes in the inflammatory status of cells, nor with changes in the proliferation and migration capacities and oxidative stress responses of the cells. Moreover, repeated exposure with low concentrations of MSWF was not sufficient in inducing epithelial inflammation and endothelial activation. Notably, this study only focused on the particle fraction of the WF exposure and did not consider systemic effects in workers following exposure. Thus, plausibly the soluble fractions of WF may be crucial for health outcome, and WF-induced cardiovascular effects cannot be allotted to the particle fraction only.

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Author contributions

JSE and YJA contributed equally to this work.

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Supplemental material

Supplemental material for this article is available online.

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