Toxicology 287 (2011) 153–159

Contents lists available at ScienceDirect

Toxicology

journal homepage: www.elsevier.com/locate/toxicol

Relationship between pulmonary and systemic markers of exposure to multiple types of welding particulate matter

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ARTICLE INFO

Article history:
Received 13 April 2011
Received in revised form 2 June 2011
Accepted 11 June 2011
Available online 17 June 2011

Keywords:
Systemic inflammation
Cardiovascular
Bronchoalveolar lavage
Gene expression
Serum proteins
Aorta

ABSTRACT

Welding results in a unique and complex occupational exposure. Recent epidemiological studies have shown an increased risk of cardiovascular disease following welding fume exposure. In this study, we compared the induction of pulmonary and systemic inflammation following exposure to multiple types of welding fumes. Mice were exposed to 340 μg of manual metal arc stainless steel (MMA-SS), gas metal arc-SS (GMA-SS) or GMA-mild steel (GMA-MS) by pharyngeal aspiration. Mice were sacrificed at 4 and 24 h post-exposure to evaluate various parameters of pulmonary and systemic inflammation. Alterations in pulmonary gene expression by a custom designed TaqMan array showed minimal differences between the fumes at 4 h. Conversely at 24 h, gene expression changes were further increased by SS but not GMA-MS exposure.

These findings were associated with the surrogate marker of systemic inflammation, liver acute phase gene induction. Interestingly, stress response genes in cardiovascular tissues were only increased following MMA-SS exposure. These effects were related to the initial level of pulmonary cytotoxicity, as measured by lactate dehydrogenase activity, which was greatest following MMA-SS exposure. In conclusion, varying types of welding fumes elicit quantitatively different systemic inflammatory and/or stress responses.

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1. Introduction

Epidemiological studies have shown increased cardiovascular mortality and morbidity following particulate matter exposure (Brook et al., 2010; Dockery et al., 1993; Pope et al., 1992; Samet et al., 2000; Schwartz and Morris, 1995). Subsequent human and animal studies support these findings: therefore, biological mechanisms have been proposed to explain the effects. First, the direct mechanism hypothesizes that particles, most likely ultrafine in size, and/or soluble metals or organic compounds enter the circulation and induce biological effects (Brook et al., 2010; Nemmar et al., 2001, 2002; Oberdorster et al., 2002). Second, an indirect mechanism is activation of the autonomic nervous system resulting in an imbalance between sympathetic and parasympathetic stimulation (Brook et al., 2010; Gold et al., 2000; Magari et al., 2001; Pope et al., 1999). Finally, another proposed indirect effect is that particle exposure results in a pulmonary inflammatory response and release of mediators into the general circulation (Brook et al., 2010; Seaton et al., 1995). These mechanisms may combine to produce effects including endothelial dysfunction, decreased heart rate variability, enhanced coagulation potential and increased progression of atherosclerosis (Brook et al., 2010). While the indirect hypothesis of spill-over of pulmonary inflammation has the strongest mechanistic support, to some degree, all three mechanisms may work in concert to produce systemic effects (Brook et al., 2010).

Welding results in a unique and complex occupational exposure. The aerosol generated from welding contains both gases and a fume which consists of metal oxide particulate matter (Antonini, 2003). The contents of these fractions will vary depending on the type of welding performed. Therefore, the potential exists for varying pulmonary effects for different welding processes. In addition, extrapulmonary effects of welding have been described including an increased risk for cardiovascular disease (Hilt et al., 1999; Ibfelt et al., 2010; Moulin et al., 1993; Newhouse et al., 1985; Sjogren et al., 2002; Suadicani et al., 2002). Supportingly, adverse cardiovascular effects in response to metal-rich particulate matter exposure, such as residual oil fly ash (Campen et al., 2000, 2002; Farraj et al., 2011;

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0300-483X/$ – see front matter. Published by Elsevier Ireland Ltd.
doi:10.1016/j.tox.2011.06.008

Kodavanti et al., 2002; Nurkiewicz et al., 2004; Watkinson et al., 1998), strengthen the likelihood of welding fume-induced systemic effects. In this study we compared three different welding fumes including manual metal arc stainless steel (MMA-SS), gas metal arc-SS (GMA-SS) or GMA-mild steel (GMA-MS). The MMA-SS welding fume contains soluble metals, primarily chromium, while the GMA-SS contains primarily insoluble metals that persist in the lung. The GMA-MS, a common welding fume used in industry and considered the least toxic of the three described here, also has limited solubility.

Recently we showed that pulmonary exposure to the engineered nanoparticle, carbon nanotubes (CNT), resulted in adverse cardiovascular effects (Erdely et al., 2009; Li et al., 2007; Simeonova and Erdely, 2009). Pulmonary exposure to CNT caused distressed aortic mitochondrial homeostasis and increased plaque lesion area in apolipoprotein E knockout mice which indicated systemic oxidative stress and inflammation (Li et al., 2007). Subsequent studies revealed a systemic inflammatory response, measured as blood gene expression, elevated serum cytokines and chemokines and vascular inflammation (Erdely et al., 2009). These data demonstrated a lung and systemic crosstalk in response to the exposure illustrating that indirect, pulmonary-derived inflammation contributed significantly to cardiovascular effects. Here, similar methodology as our CNT study was applied to investigate the differences between the systemic inflammatory responses of three welding fumes. Also, in anticipation of systemic effects, various parameters of pulmonary inflammation and toxicity were examined to yield mechanistic insight into these effects.

2. Methods

2.1. Animals and exposure conditions

Male C57BL/6 mice (Jackson Laboratory, Bar Harbor, ME) 10–12 weeks of age were used in this study. All mice were provided food (Teklad 7913) and tap water *ad libitum* in ventilated cages in a controlled humidity and temperature environment with a 12 h light/dark cycle. Animal care and use procedures were conducted in accordance with the "PHS Policy on Humane Care and Use of Laboratory Animals" and the "Guide for the Care and Use of Laboratory Animals" (NIH publication 86-23, 1996). These procedures were approved by the National Institute for Occupational Safety and Health Institutional Animal Care and Use Committee.

Mice were exposed by pharyngeal aspiration to three types of welding fume: MMA-SS, GMA-SS and GMA-MS. A description of each fume composition and solubility is shown in Table 1 (Antonini et al., 1999). The particles were dispersed in phosphate buffered saline with an exposure dose of 340 μg in 50 μl. This dose was used for consistency in comparison to previous studies and represents approximately 58 days of exposure from the previous threshold limit value of 5 mg/m^3 factored for alveolar surface area (Erdely et al., 2011). Mice were sacrificed by carbon dioxide asphyxiation at 4 h (n = 4 Sham; n = 5 MMA-SS; n = 5 GMA-SS; n = 5 GMA-MS) and 24 h post-exposure (n = 4 Sham; n = 5 MMA-SS; n = 4 GMA-SS; n = 3 GMA-MS). An additional set of mice were exposed to soluble chromium (S-Cr) at a dose equal to that found in the MMA-SS (102 μg; n = 6 Sham and n = 6 S-Cr).

2.2. Tissue, blood and bronchoalveolar lavage collection

Blood was collected for serum antigen analysis by RodentMAP v2.0 (Rules Based Medicine, Austin, TX). The left lobe of the lung was ligated, removed and frozen in liquid nitrogen. The right lobes were lavaged, and the bronchoalveolar lavage (BAL) was used to determine cellular differentials and lactate dehydrogenase (LDH) activity as previously described (Zeidler-Erdely et al., 2008). A consistent section of the liver and the right kidney was harvested for metal analysis by inductively coupled plasma atomic emission spectroscopy as previously described (Antonini et al., 2007). The aorta, heart, and liver were harvested and frozen in liquid nitrogen. All samples were stored at −80 °C prior to analysis.

2.3. Determination of relative mRNA levels

For real-time RT-PCR, lung and liver RNA was isolated using the RNeasy Mini Kit (Qiagen) and heart and aorta by the fibrous RNeasy Mini Kit (Qiagen). Evaluation of gene expression was determined by standard 96-well technology using the StepOne™ (Applied Biosystems) with pre-designed Assays-on-Demand™ TaqMan® probes and primers (Applied Biosystems) and by a custom designed TaqMan® array as previously described (Erdely et al., 2009).

2.4. Statistics

All data are presented as means ± standard error. Analyses were performed using JMP® Statistical Discovery Software. Serum protein analysis and real-time RT-PCR of the TaqMan® arrays and any additional genes were analyzed by one-way analysis of variance generating a least squares mean table by Student's t-test. Differences were considered statistically significant at $p < 0.05$.

3. Results

3.1. Metal translocation

Following pulmonary exposure to welding fume there was rapid translocation of metals from the lung. The primary soluble component of the MMA-SS fume, chromium, was evident in the kidney (2.20 ± 0.38 μg/g PBS vs 13.87 ± 1.17* MMA-SS; *$p < 0.05$) and liver (2.51 ± 0.11 μg/g PBS vs 4.40 ± 0.46* MMA-SS; *$p < 0.05$) at 4 h post-exposure. Increased levels of manganese were found in the kidney after GMA-SS exposure (5.43 ± 0.24 μg/g PBS vs 7.56 ± 0.56* GMA-SS; *$p < 0.05$). There were no changes in other metals, including copper, iron, zinc and aluminum, in the liver and kidney and for any metal after GMA-MS exposure (data not shown).

3.2. Parameters of bronchoalveolar lavage

As expected, welding fume resulted in significant pulmonary inflammation. At the acute time point of 4 h post-exposure, lung cytotoxicity, represented as acellular BAL fluid LDH, was greatest in the MMA-SS group (62 ± 3U/L Sham; 128 ± 11# MMA-SS; 88 ± 2* GMA-SS; 73 ± 4 GMA-MS; #$p < 0.05$ vs all; *$p < 0.05$ vs Sham). MMA-SS-exposed mice also had the highest level of lavage LDH activity at 24 h post-exposure (57 ± 7 Sham; 127 ± 14* MMA-SS; 96 ± 3* GMA-SS; 99 ± 26* GMA-MS; *$p < 0.05$ vs Sham). Previously shown in both rats and mice, instillation/aspiration of welding fumes results in a significant neutrophilic influx (Taylor et al., 2003; Zeidler-Erdely et al., 2008). These effects were seen as early as 4 h for all fumes (8–26 BAL cellular %) and further increased by 24 h (65–88%).

3.3. Changes in pulmonary gene expression

Welding fume induced pulmonary gene expression is shown in Table 2 and Fig. 1. Acutely at 4 h, upregulated genes included markers of inflammation (*Il6*, *Cxcl1*, *Cxcl2*, *Ccl2*, *Ptgs2*), stress response genes (*Mt1*, *Mt2*, *Hif1a*), cell adhesion molecules (*Sele*, *Selp*, *Icam1*) and mediators of coagulation (*Serpine1*, *F3*). In general, the response was rather consistent between the fumes at 4 h for the genes of interest despite the differences in composition and solubility. Some differences included marked *Il13* expression with MMA-SS compared to GMA exposure while *Cxcl1* and *Cxcl2* showed greater induction in the GMA welding fume exposed mice (Table 2, Fig. 1). By 24 h, more genes from our panel were significantly increased in all fumes and many genes showed an enhanced induction compared to 4 h mostly in the SS fumes (Table 2, Fig. 1). Several genes including, *Il6*, *Sele*, *Selp*, *Cxcl1*, *Cxcl2* and *Serpine1* were further increased at 24 h in the SS fumes but were unchanged or reduced in the GMA-MS fume (Table 2, Fig. 1). Qualitatively comparing time dependent effects, *Il5*, *Il13*, *Ptgs2*, and *Gadd45a* were reduced compared to 4 h. In contrast, *Il1β*, *arg1*, *Timp4*, *Thbs1*, *Itgam* and *Igf1* were only increased at 24 h. Overall, the level of pulmonary gene induction was greatest in the GMA-SS fume compared to the other fumes at 24 h as 18 of the analyzed genes were significantly increased compared to all groups (Table 2, Fig. 1). There some differences in gene expression between MMA and GMA, but virtually all of these were expressed at 2-fold or less.

Table 1
Metal composition and solubility of welding fumes.

	Total metal composition by wt%	Soluble/insoluble ratio	wt% of metals in soluble fraction
MMA-SS	Fe – 41.1 Mn – 16.7 Cr – 28.5 Ni – 2.53 Cu – 0.40 Ti – 10.7 V – 0.11	0.345	Fe – 0.39 Mn – 11.7 Cr – 87.0 Ni – 0.65 Cu – 0.08 Ti – 0.13 V – n.d.
GMA-SS	Fe – 53.1 Mn – 23.2 Cr – 18.6 Ni – 4.85 Cu – 0.31 Ti – n.d. V – n.d.	0.006	Fe – 9.26 Mn – 68.2 Ni – 11.8 Cu – 4.5 Ti – 0.79 V – n.d.
GMA-MS	Fe – 85.9 Mn – 14.6 Cr – 0.07 Ni – 0.01 Cu – 0.41 Ti – 0.02 V – n.d.	0.020	Fe – 4.60 Mn – 93.0 Cr – 0.20 Ni – n.d. Cu – 0.70 Ti – 1.38 V – n.d.

Table is adapted from previously published characteristics of the above welding fumes (Antonini et al., 1999).

3.4. Evidence of systemic inflammation

Changes in parameters of systemic inflammation were minimal 4 h post welding fume exposure. Of the 59 serum proteins analyzed the only protein significantly increased was IL-5 (0.50 ± 0.08 ng/ml PBS, $1.26 \pm 0.12^{*}$ MMA-SS, $0.79 \pm 0.06^{\#}$ GMA-SS, $1.39 \pm 0.13^{*}$ GMA-MS; $^{*}p < 0.05$ vs PBS and $^{\#}p < 0.05$ vs all). As a surrogate marker of systemic inflammation at 24 h, liver

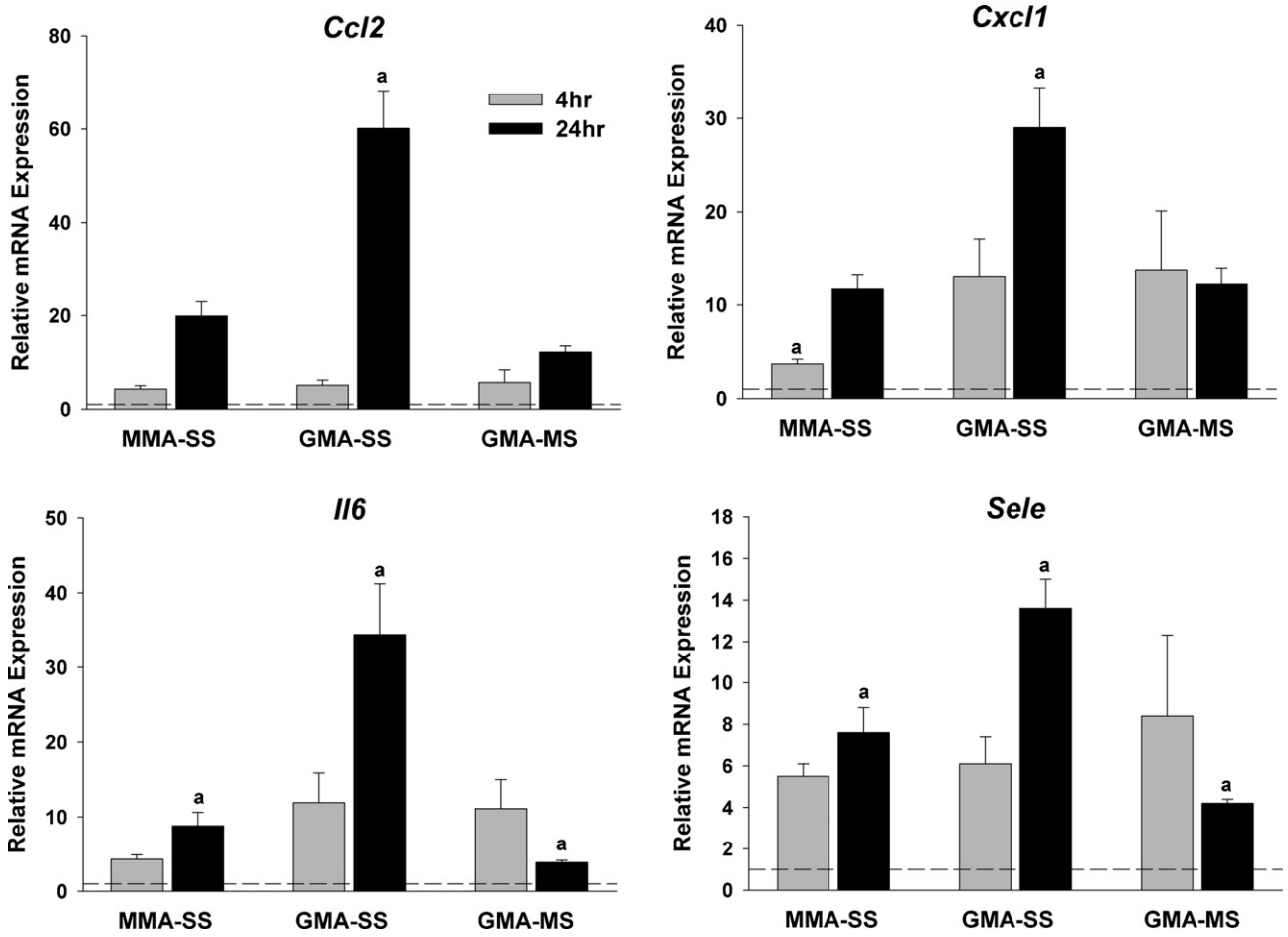

Fig. 1. Effect of welding fume exposure on pulmonary inflammatory gene expression. Results show relative expression levels at 4 h (gray bars) and 24 h (black bars) for all fume exposures. Abbreviations include *Ccl2* (chemokine (C-C motif) ligand 2 or monocyte chemotactic protein-1), *Cxcl1* (chemokine (C-X-C motif) ligand 1 or keratinocyte-derived chemokine), *Il6* (interleukin-6) and *Sele* (e-selectin). All bars shown are significantly different from respective sham ($p < 0.05$). $^{a}p < 0.05$ vs all groups for a given time point.

Table 2
Effect of welding fume exposure on pulmonary gene expression.

	MMA-SS		GMA-SS		GMA-MS	
	4 h ($n=4$)	24 h ($n=4$)	4 h ($n=4$)	24 h ($n=5$)	4 h ($n=4$)	24 h ($n=3$)
Inflammation						
Il1a				2.4 ± 0.3[b]		
Il1b		3.1 ± 0.7		10.0 ± 1.2[a]		4.1 ± 0.2
Il5	9.0 ± 1.1[c]	2.8 ± 0.3[b]	4.6 ± 1.0		4.4 ± 1.9[c]	
Il10	2.6 ± 0.3[b]			3.1 ± 0.6[a]	2.4 ± 0.5	
Il13	22.6 ± ±1.7[a]	5.9 ± 0.5[b]	3.7 ± 0.6	3.1 ± 1.1	8.5 ± 5.6	3.6 ± 0.9
Cxcl2	5.2 ± 1.2[a]	10.8 ± 1.9	15.5 ± ±5.1	58.5 ± 2.1[a]	18.3 ± 7.0	15.7 ± 2.8
Cxcl12						1.7 ± 0.0
Ccl4	3.1 ± 0.3	5.5 ± 1.5	6.1 ± 1.2	59.1 ± 6.9[a]	6.2 ± 2.9	8.4 ± 0.9
Ccl5				1.9 ± 0.4[a]		
Ccl11	2.6 ± 0.4	2.5 ± 0.2		2.7 ± 0.1	4.1 ± 1.8	1.9 ± 0.1[a]
Ccl22		3.4 ± 0.3		3.2 ± 0.3		3.9 ± 0.5
Ccr2		1.9 ± 0.3		2.6 ± 0.4	2.1 ± 0.6[c]	2.1 ± 0.3
Csf1		1.4 ± 0.1		2.4 ± 0.3[a]		
Csf2		2.3 ± 0.3	2.9 ± 0.6[b]	3.2 ± 0.2	3.0 ± 0.8[c]	3.1 ± 0.5
Csf3	26.8 ± 2.5	18.5 ± 5.2	41.9 ± 14.1	56.3 ± 13.4[a]	30.0 ± 9.8	10.0 ± 1.8
Itgam		2.0 ± 0.3		2.8 ± 0.3		2.5 ± 0.1
Il8rb				3.7 ± 0.5[b]		2.6 ± 0.1
Cd44				1.7 ± 0.2[b]		1.6 ± 0.0[c]
Ifng				10.5 ± 3.7[a]		
Tnf		2.6 ± 0.5	2.9 ± 0.6	9.9 ± 1.3[a]	1.9 ± 0.5	3.4 ± 0.5
Fas	1.9 ± 0.1[a]					
Ptgs2	3.9 ± 0.1		3.5 ± 0.6	1.7 ± 0.1	2.3 ± 0.5	
Spp1	1.7 ± 0.1[a]	4.2 ± 1.0[c]		2.8 ± 0.6		
Arg1		6.7 ± 1.3		4.1 ± 0.2		4.4 ± 1.0
Arg2	1.9 ± 0.0	2.9 ± 0.3	1.9 ± 0.3	3.4 ± 0.1	1.8 ± 0.2	2.6 ± 0.1
Casp1		0.7 ± 0.1[a]		1.7 ± 0.1[a]		
Casp4	1.4 ± 0.1		1.5 ± 0.2	3.0 ± 0.4[a]	2.0 ± 0.2[a]	1.5 ± 0.0
Stress						
Hmox1	1.3 ± 0.1[c]	2.2 ± 0.2	1.6 ± 0.2	2.5 ± 0.2	1.9 ± 0.1[c]	2.3 ± 0.1
Sod2	1.4 ± 0.1	1.9 ± 0.1[c]	1.5 ± 0.1	1.9 ± 0.1	1.7 ± 0.1	1.6 ± 0.0[c]
Nqo1		1.5 ± 0.2[b]	1.7 ± 0.2[a]			
Cyba				1.9 ± 0.3[b]		1.9 ± 0.2[c]
Gadd45a	2.4 ± 0.1[a]		1.3 ± 0.1		1.5 ± 0.2	
Mt1	3.9 ± 0.3	3.6 ± 0.9[c]	2.5 ± 0.4	2.7 ± 0.4[d]	2.4 ± 0.5	
Mt2	4.3 ± 0.4	12.7 ± 2.6	2.5 ± 0.4	8.6 ± 1.2	3.0 ± 0.7	3.2 ± 0.2[a]
Hif1a	1.4 ± 0.1	1.5 ± 0.1	1.4 ± 0.1	1.9 ± 0.1	1.5 ± 0.2	1.6 ± 0.1
Hif3a	2.3 ± 0.2[a]					
Egr1				1.9 ± 0.0[b]		
Clu			1.5 ± 0.2[b]	2.0 ± 0.2[b]	1.4 ± 0.1[c]	1.7 ± 0.1[c]
Coagulation						
Serpine1	3.0 ± 0.2	3.1 ± 0.3[a]	3.3 ± 0.5	4.8 ± 0.6[a]	3.6 ± 1.3	2.2 ± 0.2[a]
F3	1.9 ± 0.2	2.4 ± 0.3	1.8 ± 0.2	3.6 ± 0.5[a]	1.7 ± 0.4	2.0 ± 0.2
Thbs1		2.7 ± 0.3		2.5 ± 0.2		1.8 ± 0.1[a]
Adhesion						
Selp	2.3 ± 0.2	3.2 ± 0.4	2.3 ± 0.4	5.2 ± 0.7[a]	3.2 ± 1.1	2.5 ± 0.3
Icam1	1.5 ± 0.1	1.8 ± 0.2	1.7 ± 0.2	1.9 ± 0.1	1.6 ± 0.2	1.8 ± 0.1
Remodeling/growth factor						
Timp1		14.0 ± 2.3	3.1 ± 0.6[b]	19.1 ± 0.6[d]	2.6 ± 0.6	8.4 ± 1.1
Timp4		1.7 ± 0.3		1.8 ± 0.1		1.6 ± 0.2
Igf1		1.9 ± 0.3		2.4 ± 0.2		2.1 ± 0.1
Ctgf	1.8 ± 0.1[a]					

Table criteria:

- A significant p-value of 0.05 by ANOVA unless otherwise stated.
- A fold change of 1.5.
- If a given gene is significant for any one fume by the above two criteria then any significance of $p < 0.05$ for the other fumes is included even if not 1.5 or greater. This allows a gene to be interpreted as specific for a given fume or part of a more general response.

All values shown are significant from sham treated mice at each respective time point.
[a] $p < 0.05$ vs all.
[b] $p < 0.05$ MMA-SS vs GMA-SS.
[c] $p < 0.05$ MMA-SS vs GMA-MS.
[d] $p < 0.05$ GMA-SS vs GMA-MS.

gene expression analysis for acute phase proteins was determined. There were increased levels of *Saa1* and *Apcs* as well as the stress response indicator *Mt1* following SS exposure only (Fig. 2).

3.5. Evidence of a cardiovascular stress response

There was increased expression of several stress response genes including *Mt1*, *Mt2* and *Hif3a* in the aorta (Fig. 3A) and heart (Fig. 3B)

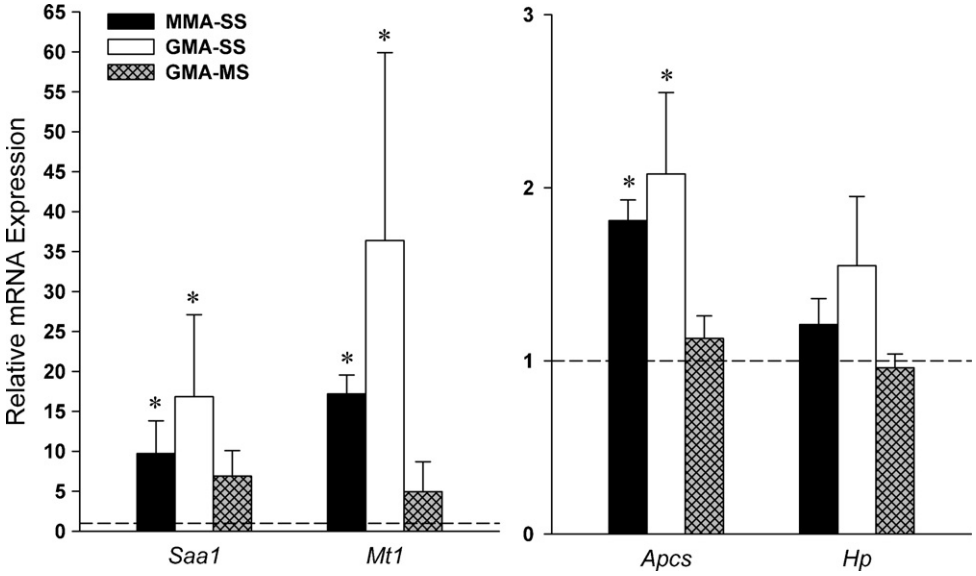

Fig. 2. Effect of welding fume exposure on liver inflammatory gene expression. Results show relative expression levels at 24 h for all fume exposures. Abbreviations include *Saa1* (serum amyloid A1), *Mt1* (metallothionein 1), *Apcs* (amyloid P component, serum) and *Hp* (haptoglobin). *$p < 0.05$ vs sham.

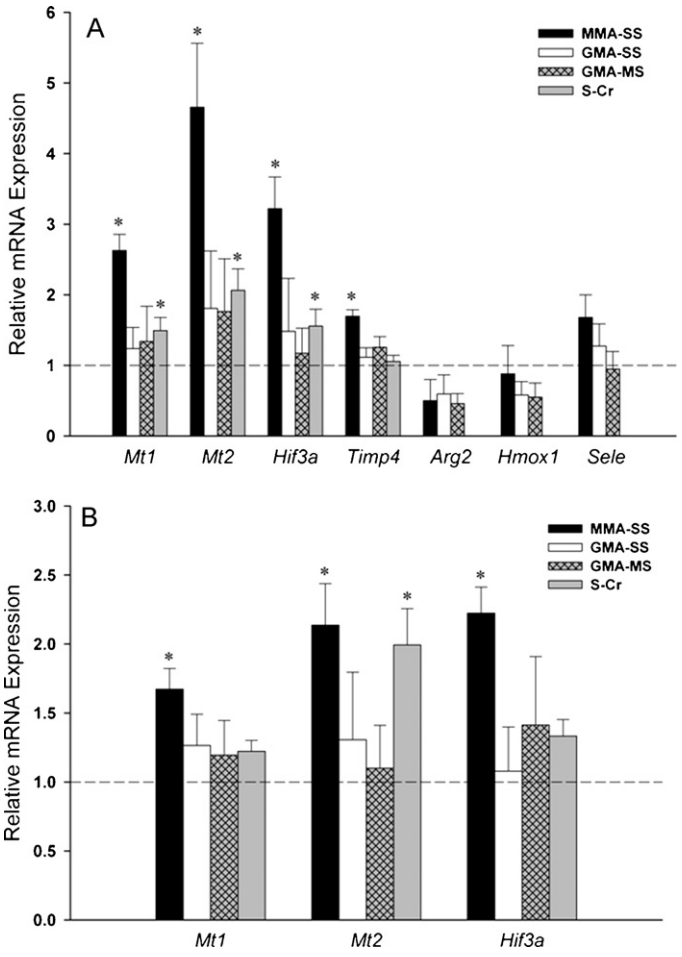

Fig. 3. Effect of welding fume exposure on cardiovascular stress response gene expression. Results show relative expression levels at 4 h for all fume exposures in aorta (A) and heart (B). Abbreviations include *Mt* (metallothionein), *Hif3a* (hypoxia inducible factor 3 alpha), *Timp4* (tissue inhibitor of metalloproteinase 4), *Arg2* (arginase II), *Hmox1* (heme oxygenase 1) and *Sele* (e-selectin). *$p < 0.05$ vs sham.

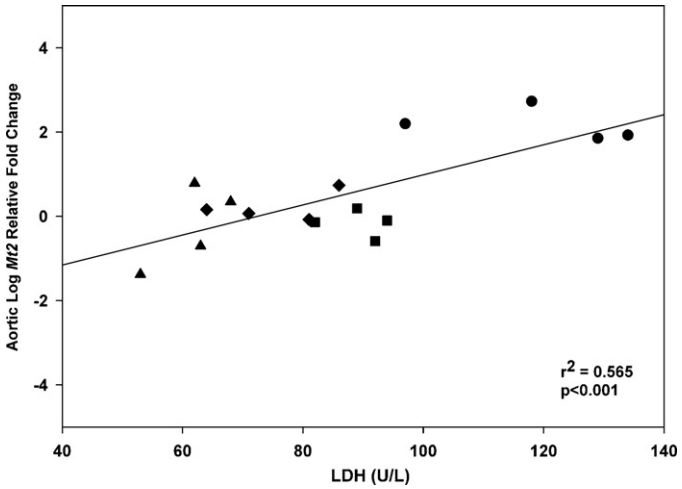

Fig. 4. Correlation between aortic metallothionein 2 (*Mt2*) relative expression and bronchoalveolar lavage lactate dehydrogenase (LDH) activity. Regression analysis of *Mt2* and LDH for all exposures is indicated by the solid line. Symbols include solid triangle (Sham), solid circle (MMA-SS), solid square (GMA-SS) and solid diamond (GMA-MS).

of MMA-SS but not GMA-exposed mice. Aortic *Timp4* was also increased, but markers related to vascular function such as *Arg2*, *Hmox1* and *Sele* were not changed at 4 h (Fig. 3A). Pulmonary exposure to S-Cr, the primary soluble component of the MMA-SS fume, induced stress response gene expression in the aorta and heart although qualitatively to a lesser extent when compared to the total MMA-SS fume (Fig. 3). Stress response gene expression in the aorta was still evident in the MMA-SS exposed mice at 24 h (*Mt1*, 1.00 ± 0.18 PBS vs $2.94 \pm 0.76^*$ MMA-SS; *Mt2*, 1.00 ± 0.20 vs $3.87 \pm 1.17^*$; *Hif3a*, 1.00 ± 0.14 vs $2.65 \pm 0.62^*$; $^*p < 0.05$). There were no gene expression changes in the heart and aorta from our selected panel of 93 target genes for the GMA-exposed mice. At 4 h there was a significant positive correlation between lavage LDH activity and aortic *Mt2* gene expression (Fig. 4).

4. Discussion

The findings of this study show that various types of welding fume result in different toxicities in the lung which thereby may

influence systemic inflammation. While all fumes had increased pulmonary inflammatory gene expression within hours after exposure, the response was greater at 24 h in the SS fumes but not in the GMA-MS fume. In a corresponding fashion the SS fumes showed evidence of systemic inflammation. Interestingly, only the MMA-SS fume was able to induce stress response genes in the heart and aorta. The soluble component of the MMA-SS fume was partially responsible for the induction of cardiovascular stress response genes. Lastly, the data suggests that the level of initial pulmonary cytotoxicity in the MMA-SS compared to GMA fumes positively correlated to the early stress response.

With the exception of a few genes, expression changes in the lungs were rather uniform across welding fume types within hours after exposure. Therefore, early changes in gene expression were not a good determinant for cytotoxicity especially when comparing the MMA-SS and the GMA-MS welding fumes. The response 24 h after exposure showed that the expression changes in the lungs continued to increase in the SS fumes unlike the GMA-MS fume. Generally, the GMA-MS fume is considered the least toxic when compared to the other two fumes and relatively high inhalation exposure to GMA-MS produced little to no lung inflammation or injury in rats (Antonini et al., 2010; Taylor et al., 2003). At 24 h, the inflammatory gene expression changes were the greatest in the GMA-SS exposed mice rather than mice exposed to MMA-SS further supporting these changes as a whole did not reflect lung cytotoxicity.

An aim of this study was the comparison of systemic inflammation between different fume exposures. Previous studies in young human welders have shown increased markers of systemic inflammation including elevated circulating neutrophils in non-smoking welders and C reactive protein for all welders (Fang et al., 2009; Kim et al., 2005). As a surrogate marker of systemic inflammation, acute phase gene expression in the liver was analyzed. At 24 h, liver acute phase genes were increased following SS exposure. IL-6, a classic mediator of the acute phase response and known effector cytokine following a pulmonary exposure (Brook et al., 2010; Kido et al., 2011), was increased in the lung of exposed mice providing the potential for spillover into the systemic circulation. In support, pulmonary gene expression of *Il6* continued to increase from 4 to 24 h following SS exposure but decreased with GMA-MS exposure providing a qualitative explanation of the observed nonsignificant change in liver acute phase genes in response to GMA-MS. A spillover of inflammatory cytokines into the systemic circulation can occur within hours following a pulmonary exposure (Erdely et al., 2009). In this data set, only IL-5 was increased 4 h post-exposure. IL-5 suggests an eosinophilic response although this was not a marked finding in lavage differentials in previous instillation exposures (Taylor et al., 2003) or in this study as eosinophils only ranged from 0.5 to 3%. In addition, increased pulmonary *Il5* gene expression was absent by 24 h in GMA and reduced in MMA-SS exposed mice which indicated an acute, unsustained, response. Given the induction of the acute phase response in the liver, primary cytokines were likely increased in the serum at some point prior to 24 h but not at the 4 h time point.

Only the MMA-SS fume induced stress response genes in the aorta and heart. The results showed a very rapid recognition by the periphery to a cytotoxic pulmonary exposure. Also, the data showed acute pulmonary cytotoxicity at 4 h, but not inflammatory pulmonary gene expression, was correlative. These effects were not unique to MMA-SS as pulmonary CNT or LPS exposures induce metallothioneins in extrapulmonary tissues (Erdely et al., 2009; Simeonova and Erdely, 2009). Therefore, the rapid translocation of metals from the MMA-SS likely does not explain the increase in metallothioneins supporting a previous conclusion that the soluble metals in residual oil fly ash were not a prime factor in vascular dysfunction (Nurkiewicz et al., 2004). Increased levels of metallothioneins can occur in response to primary cytokines (e.g. IL-6, IL-1, and TNF) and stress (Coyle et al., 2002). In support, pulmonary exposures, including this study, were associated with systemic inflammation and vascular oxidative stress (Brook et al., 2010; Erdely et al., 2009; Nurkiewicz et al., 2009) and the stress response was evident in various extrapulmonary organs (Erdely et al., 2009). In addition to metallothionein, *Timp4*, the vascular Timp, was acutely affected after a pulmonary exposure previously (Erdely et al., 2009) and in this study. *Timp4* was increased following multiple cardiovascular disorders and has been proposed as a marker for vascular inflammation (Dollery et al., 1999; Koskivirta et al., 2006). Together the results suggest pulmonary exposures with acute pulmonary toxicity induce systemic inflammation and/or stress which can be monitored in extrapulmonary tissues.

The extrapulmonary stress response in the vasculature due to MMA-SS exposure was partly due to S-Cr. As discussed above, translocation of soluble metals seems unlikely to be the cause for the vascular response. In that respect, previous studies in both rats and mice have shown that the soluble fraction of the MMA-SS welding fume contributed to approximately 80% of the pulmonary toxicity when compared to the total fume (Taylor et al., 2003; Zeidler-Erdely et al., 2008). These results suggest that while the S-Cr contributes greatly to the pulmonary toxicity, insoluble components also have an effect. Similar findings were observed in the qualitative comparison in this study as induction of vascular metallothioneins following S-Cr exposure was not at the level seen from the total MMA-SS fume exposure.

One limitation of this study was the examination of the extrapulmonary effects, especially in relation to cardiovascular effects, of welding fume utilizing aspiration as the route of exposure. Exposure by routes other than inhalation often become the optimal choice for reasons including cost, fume availability and the quantitative comparison of numerous types of welding fumes. It has been shown that freshly generated welding fume induces greater lung inflammation than aged fume (Antonini et al., 1998). These effects were related to reactive oxygen species associated with the freshly generated fume. Also, aspirated particles that have been collected onto filters in bulk can have greater agglomeration with larger diameters, while particles generated during inhalation generally have significantly smaller diameters (Antonini et al., 1998; Antonini et al., 2007). These results suggest less reactivity and surface area for aspiration compared to inhalation; two qualities suspected to affect the pulmonary response. In support, qualitative comparisons in mice from inhalation and aspiration exposures to GMA-SS suggest that one third the dose by inhalation caused approximately 2–3 times the toxicity in the lung (Zeidler-Erdely et al., 2008, 2011). Therefore, the results in this study based on mass dose may underestimate the effects in welders and the 340 μg dose by aspiration may be reflective of an inhalation dose of only approximately 38 μg relative to pulmonary toxicity.

In conclusion, the data as a whole suggests low grade systemic inflammation and stress response to welding fume exposure. Specifically, evidence of systemic inflammation reflected a temporal change in pulmonary inflammatory gene expression following SS exposure. Interestingly, while all three fumes induced early increases in pulmonary inflammatory gene expression, only the MMA-SS fume caused increased levels of stress response genes in the aorta and heart. These genes indicate vascular inflammation and/or systemic stress that were correlative to initial lung cytotoxicity. These findings show systemic effects from exposure to welding fumes, particularly SS fumes, complement previous epidemiologic findings of extrapulmonary effects in welders.

Conflict of interest

There are none.

Acknowledgements

Special thanks to Dr. Vincent Castranova, Dr. Jane Ma and Dr. Paul Nicolaysen for their review of the manuscript.

References

Antonini, J.M., 2003. Health effects of welding. Crit. Rev. Toxicol. 33, 61–103.
Antonini, J.M., Clarke, R.W., Krishna Murthy, G.G., Sreekanthan, P., Jenkins, N., Eagar, T.W., Brain, J.D., 1998. Freshly generated stainless steel welding fume induces greater lung inflammation in rats as compared to aged fume. Toxicol. Lett. 98, 77–86.
Antonini, J.M., Lawryk, N.J., Murthy, G.G., Brain, J.D., 1999. Effect of welding fume solubility on lung macrophage viability and function in vitro. J. Toxicol. Environ. Health A 58, 343–363.
Antonini, J.M., Roberts, J.R., Stone, S., Chen, B.T., Schwegler-Berry, D., Chapman, R., Zeidler-Erdely, P.C., Andrews, R.N., Frazer, D.G., 2010. Persistence of deposited metals in the lungs after stainless steel and mild steel welding fume inhalation in rats. Arch. Toxicol..
Antonini, J.M., Stone, S., Roberts, J.R., Chen, B., Schwegler-Berry, D., Afshari, A.A., Frazer, D.G., 2007. Effect of short-term stainless steel welding fume inhalation exposure on lung inflammation, injury, and defense responses in rats. Toxicol. Appl. Pharmacol. 223, 234–245.
Brook, R.D., Rajagopalan, S., Pope 3rd, C.A., Brook, J.R., Bhatnagar, A., Diez-Roux, A.V., Holguin, F., Hong, Y., Luepker, R.V., Mittleman, M.A., Peters, A., Siscovick, D., Smith Jr., S.C., Whitsel, L., Kaufman, J.D., 2010. Particulate matter air pollution and cardiovascular disease: an update to the scientific statement from the American Heart Association. Circulation 121, 2331–2378.
Campen, M.J., Costa, D.L., Watkinson, W.P., 2000. Cardiac and thermoregulatory toxicity of residual oil fly ash in cardiopulmonary-compromised rats. Inhal. Toxicol. 12, 7–22.
Campen, M.J., Nolan, J.P., Schladweiler, M.C., Kodavanti, U.P., Costa, D.L., Watkinson, W.P., 2002. Cardiac and thermoregulatory effects of instilled particulate matter-associated transition metals in healthy and cardiopulmonary-compromised rats. J. Toxicol. Environ. Health A 65, 1615–1631.
Coyle, P., Philcox, J.C., Carey, L.C., Rofe, A.M., 2002. Metallothionein: the multipurpose protein. Cell. Mol. Life Sci. 59, 627–647.
Dockery, D.W., Pope 3rd, C.A., Xu, X., Spengler, J.D., Ware, J.H., Fay, M.E., Ferris Jr., B.G., Speizer, F.E., 1993. An association between air pollution and mortality in six U.S. cities. N. Engl. J. Med. 329, 1753–1759.
Dollery, C.M., McEwan, J.R., Wang, M., Sang, Q.A., Liu, Y.E., Shi, Y.E., 1999. TIMP-4 is regulated by vascular injury in rats. Circ. Res. 84, 498–504.
Erdely, A., Hulderman, T., Salmen-Muniz, R., Liston, A., Zeidler-Erdely, P.C., Chen, B.T., Stone, S., Frazer, D.G., Antonini, J.M., Simeonova, P.P., 2011. Inhalation exposure of gas-metal arc stainless steel welding fume increased atherosclerotic lesions in apolipoprotein E knockout mice. Toxicol. Lett. 204, 12–16.
Erdely, A., Hulderman, T., Salmen, R., Liston, A., Zeidler-Erdely, P.C., Schwegler-Berry, D., Castranova, V., Koyama, S., Kim, Y.A., Endo, M., Simeonova, P.P., 2009. Cross-talk between lung and systemic circulation during carbon nanotube respiratory exposure. Potential biomarkers. Nano Lett. 9, 36–43.
Fang, S.C., Cavallari, J.M., Eisen, E.A., Chen, J.C., Mittleman, M.A., Christiani, D.C., 2009. Vascular function, inflammation, and variations in cardiac autonomic responses to particulate matter among welders. Am. J. Epidemiol. 169, 848–856.
Farraj, A.K., Hazari, M.S., Haykal-Coates, N., Lamb, C., Winsett, D.W., Ge, Y., Ledbetter, A.D., Carll, A.P., Bruno, M., Ghio, A., Costa, D.L., 2011. ST depression, arrhythmia, vagal dominance, and reduced cardiac micro-RNA in particulate-exposed rats. Am. J. Respir. Cell Mol. Biol. 44, 185–196.
Gold, D.R., Litonjua, A., Schwartz, J., Lovett, E., Larson, A., Nearing, B., Allen, G., Verrier, M., Cherry, R., Verrier, R., 2000. Ambient pollution and heart rate variability. Circulation 101, 1267–1273.
Hilt, B., Qvenild, T., Romyhr, O., 1999. Morbidity from ischemic heart disease in workers at a stainless steel welding factory. Norsk. Epidemiol. 9, 21–26.
Ibfelt, E., Bonde, J.P., Hansen, J., 2010. Exposure to metal welding fume particles and risk for cardiovascular disease in Denmark: a prospective cohort study. Occup. Environ. Med. 67, 772–777.
Kido, T., Tamagawa, E., Bai, N., Suda, K., Yang, H.H., Li, Y., Chiang, G., Yatera, K., Mukae, H., Sin, D.D., Van Eeden, S.F., 2011. Particulate matter induces translocation of IL-6 from the lung to the systemic circulation. Am. J. Respir. Cell Mol. Biol. 44, 197–204.
Kim, J.Y., Chen, J.C., Boyce, P.D., Christiani, D.C., 2005. Exposure to welding fumes is associated with acute systemic inflammatory responses. Occup. Environ. Med. 62, 157–163.
Kodavanti, U.P., Schladweiler, M.C., Ledbetter, A.D., Hauser, R., Christiani, D.C., McGee, J., Richards, J.R., Costa, D.L., 2002. Temporal association between pulmonary and systemic effects of particulate matter in healthy and cardiovascular compromised rats. J. Toxicol. Environ. Health A 65, 1545–1569.
Koskivirta, I., Rahkonen, O., Mayranpaa, M., Pakkanen, S., Husheem, M., Sainio, A., Hakovirta, H., Laine, J., Jokinen, E., Vuorio, E., Kovanen, P., Jarvelainen, H., 2006. Tissue inhibitor of metalloproteinases 4 (TIMP4) is involved in inflammatory processes of human cardiovascular pathology. Histochem. Cell Biol. 126, 335–342.
Li, Z., Hulderman, T., Salmen, R., Chapman, R., Leonard, S.S., Young, S.H., Shvedova, A., Luster, M.I., Simeonova, P.P., 2007. Cardiovascular effects of pulmonary exposure to single-wall carbon nanotubes. Environ. Health Perspect. 115, 377–382.
Magari, S.R., Hauser, R., Schwartz, J., Williams, P.L., Smith, T.J., Christiani, D.C., 2001. Association of heart rate variability with occupational and environmental exposure to particulate air pollution. Circulation 104, 986–991.
Moulin, J.J., Wild, P., Haguenoer, J.M., Faucon, D., De Gaudemaris, R., Mur, J.M., Mereau, M., Gary, Y., Toamain, J.P., Birembaut, Y., et al., 1993. A mortality study among mild steel and stainless steel welders. Br. J. Ind. Med. 50, 234–243.
Nemmar, A., Hoet, P.H., Vanquickenborne, B., Dinsdale, D., Thomeer, M., Hoylaerts, M.F., Vanbilloen, H., Mortelmans, L., Nemery, B., 2002. Passage of inhaled particles into the blood circulation in humans. Circulation 105, 411–414.
Nemmar, A., Vanbilloen, H., Hoylaerts, M.F., Hoet, P.H., Verbruggen, A., Nemery, B., 2001. Passage of intratracheally instilled ultrafine particles from the lung into the systemic circulation in hamster. Am. J. Respir. Crit. Care Med. 164, 1665–1668.
Newhouse, M.L., Oakes, D., Woolley, A.J., 1985. Mortality of welders and other craftsmen at a shipyard in NE England. Br. J. Ind. Med. 42, 406–410.
Nurkiewicz, T.R., Porter, D.W., Barger, M., Castranova, V., Boegehold, M.A., 2004. Particulate matter exposure impairs systemic microvascular endothelium-dependent dilation. Environ. Health Perspect. 112, 1299–1306.
Nurkiewicz, T.R., Porter, D.W., Hubbs, A.F., Stone, S., Chen, B.T., Frazer, D.G., Boegehold, M.A., Castranova, V., 2009. Pulmonary nanoparticle exposure disrupts systemic microvascular nitric oxide signaling. Toxicol. Sci. 110, 191–203.
Oberdorster, G., Sharp, Z., Atudorei, V., Elder, A., Gelein, R., Lunts, A., Kreyling, W., Cox, C., 2002. Extrapulmonary translocation of ultrafine carbon particles following whole-body inhalation exposure of rats. J. Toxicol. Environ. Health A 65, 1531–1543.
Pope 3rd, C.A., Schwartz, J., Ransom, M.R., 1992. Daily mortality and PM10 pollution in Utah Valley. Arch. Environ. Health 47, 211–217.
Pope 3rd, C.A., Verrier, R.L., Lovett, E.G., Larson, A.C., Raizenne, M.E., Kanner, R.E., Schwartz, J., Villegas, G.M., Gold, D.R., Dockery, D.W., 1999. Heart rate variability associated with particulate air pollution. Am. Heart J. 138, 890–899.
Samet, J.M., Dominici, F., Curriero, F.C., Coursac, I., Zeger, S.L., 2000. Fine particulate air pollution and mortality in 20 U.S. cities, 1987–1994. N. Engl. J. Med. 343, 1742–1749.
Schwartz, J., Morris, R., 1995. Air pollution and hospital admissions for cardiovascular disease in Detroit, Michigan. Am. J. Epidemiol. 142, 23–35.
Seaton, A., MacNee, W., Donaldson, K., Godden, D., 1995. Particulate air pollution and acute health effects. Lancet 345, 176–178.
Simeonova, P.P., Erdely, A., 2009. Engineered nanoparticle respiratory exposure and potential risks for cardiovascular toxicity: predictive tests and biomarkers. Inhal. Toxicol. 21 (Suppl. 1), 68–73.
Sjogren, B., Fossum, T., Lindh, T., Weiner, J., 2002. Welding and ischemic heart disease. Int. J. Occup. Environ. Health 8, 309–311.
Suadicani, P., Hein, H.O., Gyntelberg, F., 2002. Airborne occupational exposure. ABO phenotype and risk of ischaemic heart disease in the Copenhagen Male Study. J. Cardiovasc. Risk 9, 191–198.
Taylor, M.D., Roberts, J.R., Leonard, S.S., Shi, X., Antonini, J.M., 2003. Effects of welding fumes of differing composition and solubility on free radical production and acute lung injury and inflammation in rats. Toxicol. Sci. 75, 181–191.
Watkinson, W.P., Campen, M.J., Costa, D.L., 1998. Cardiac arrhythmia induction after exposure to residual oil fly ash particles in a rodent model of pulmonary hypertension. Toxicol. Sci. 41, 209–216.
Zeidler-Erdely, P.C., Battelli, L.A., Stone, S., Chen, B.T., Frazer, D.G., Young, S.H., Erdely, A., Kashon, M.L., Andrews, R., Antonini, J.M., 2011. Short-term inhalation of stainless steel welding fume causes sustained lung toxicity but no tumorigenesis in lung tumor susceptible A/J mice. Inhal. Toxicol. 23, 112–120.
Zeidler-Erdely, P.C., Kashon, M.L., Battelli, L.A., Young, S.H., Erdely, A., Roberts, J.R., Reynolds, S.H., Antonini, J.M., 2008. Pulmonary inflammation and tumor induction in lung tumor susceptible A/J and resistant C57BL/6J mice exposed to welding fume. Part. Fibre Toxicol. 5, 12.