DNA strand breakage and lipid peroxidation after exposure to welding fumes in vivo

Cheng-Hung Chuang, Chong-En Huang and Hsiu-Ling Chen

Department of Food Science and Applied Biotechnology and 1Institute of Occupational Safety and Hazard Prevention, Hung Kuang University, Taichung, 34 Chung Chie Road, Sha Lu, Taichung 433, Taiwan

A remarkable number of complex aerosols are generated from welding processes. The objective of this study was to compare DNA damage and lipid peroxidation in plasma and in lung and in liver tissue of rats exposed to welding fumes in an exposure chamber with those of control animals. Three air samples from the chamber were also collected to assess the exposure dose for each exposure (total samplings = 18). Eight male Sprague–Dawley rats were exposed to welding fumes at a concentration of 1540.76 mg/m³ for 10 min/day six times on day 1, day 3, day 7, day 15, day 30 and day 40. Lung, liver and kidney injury was measured following exposure, as well as in unexposed control rats (n = 4 at the beginning of the study). DNA strand breakage [tail moment (TMOM)] in exposed animals showed significant differences at day 1, day 4, day 7 and day 15 relative to the levels in control animals. Malondialdehyde (MDA, a lipid peroxidation product) levels increased gradually post-welding to 0.4 μM at 7 days. MDA and TMOM both reached maximum levels 7 days after the first exposure. At the start, an increasing trend in DNA strand breakage was more obvious than increases in MDA levels; MDA seemed to reflect long-term effects of exposure to welding fumes since it increased after 7 days and was sustained to 40 days in vivo. Significant differences in both MDA levels and DNA strand breakage were seen in lung, liver and kidney 40 days after the first fume inhalation. We conclude that acute exposure of rats to welding fumes causes noticeable oxidative damage and lipid peroxidation effects and that DNA damage may recover after long and repeat exposure. More chronic inhalation and low-dose studies are needed in order to further assess the effects of inhalation of welding fumes on DNA and to elucidate the possible causal mechanisms associated with the biologically damaging effects of welding fumes.

Introduction

Many toxic substances, including heavy metals, ozone, carbon monoxide, carbon dioxide and nitrogen oxides, are generated during welding (1). Some hazardous by-products of welding, such as chromium and nickel, are present in very large amounts in fumes released during stainless steel welding (2). Other studies have shown that iron (80.6%) and manganese (14.7%) are the major components of welding particles with a mass median aerodynamic diameter of 0.31 μm (3). A study of the characteristics of aerosols presented in welding fumes reports that ~30% of welding fumes are respirable dust (4) and that sub-micrometre aerosols formed during gas metal arc welding probably result from a larger droplet surface area for vaporisation of metallic species (5).

Since 1991, welding fumes have been recognized by the International Agency for Research on Cancer as class 2B carcinogens. Potential health problems, including bronchitis and wheezing, occur even in welding workers using the manual metal arc technique <1 day per week (6); altered pulmonary function (7), increased lung cancer risk (8), metal fume fever, cancer (1,9), neurotoxicity (1,10) and genotoxicity (11) are also thought to be associated with welding exposure. Previous studies have shown that heavy metals, including Cd, Mn, Cr and Fe, all of which have been shown to be related to the generation of reactive oxygen species (ROS), are major components of welding particles (12–14). Increased production of ROS can trigger other signals that further increase oxidative stress and lead to disease. Therefore, the assessment of oxidative damage is important in evaluating the occupational hazards of welding. Iarmarcovai et al. (15) presented evidence for significant induction of DNA strand breaks in 20 of 30 welders at the end of 1 week, while Botta et al. (16) showed increased levels of DNA damage in lymphocytes from welders as compared to controls. Han et al. (17) found a significant correlation between blood Mn levels and oxidative stress in asymptomatic shipyard welders. A statistically significant pre-to post-shift change in 8-OH-dG levels was found in 41 welders, and acute welding exposure was associated with a post-shift blunting systemic inflammation (18). In in vivo studies, rats treated with manual metal arc welding fumes experienced increased DNA damage and death of lung macrophages (19).

Although the effects of welding fumes have been studied epidemiologically and in vivo, little is known about the dose–response relationship of welding fumes (19) and oxidative damage. In a European Community respiratory health survey, the study population included 316 welders, 62% operating metal arc technique, 23% 1–3 h/day and 15% >4 h/day; therefore, only 7% of the workers in the study were actually full-time welders (6). In Taiwan, welders who should use respiratory preventive masks at all times; however, they often remove them due to impairment of vision and discomfort, especially during seasonal periods of high temperature and high humidity that occur from May to October. Thus, the working status and respiratory protection of welders seem to be additional influencing factors on the oxidative damage caused by welding fumes.

In this study, we compared oxidative damage in rats exposed to welding fumes in an exposure chamber (exposed group) with that in rats in a control group by measuring the levels of...
C.-H. Chuang et al.

malondialdehyde (MDA) and DNA strand breakage in blood and in lung and liver tissue from these animals. Air samples from the exposure chamber were also collected in order to assess correlations between particle exposure and oxidative damage.

Materials and methods

Generation of welding fumes

Welding fumes were generated by manual welding in a closed hood with windows only for hands to enter and exit. The welding fumes were transferred from the hood to the exposure chamber (90 cm in length, 65 cm in width and 90 cm in height; designed as shown in Figure 1). The rats were placed in the chamber while the welding fumes were being generated.

Welding fume exposure

All procedures involving animals were performed under protocols approved by the Hung Kuang Animal Experimental and Management Committee. Six-week-old male mice, 200 g of Sprague–Dawley rats, purchased from BiolASCO Taiwan Co., Ltd., were used. Four weeks later, their body weights were 400–450 g when the exposure experiment started. The total inhalation exposure period was 40 days. Welding fume exposure was conducted in the exposure chamber for 10 min at the beginning, 3 days later and at 1 week, 2 weeks, 1 month, and 40 days (day 1, day 3, day 7, day 15, day 30 and day 40). At the start, there were 12 rats: eight were in the exposed group and four were in the control group. We drew blood samples at the start before any exposure and at the end of the experiments. The livers and kidneys were rinsed in cold phosphate-buffered saline (pH 7.4) and blotted dry on filter paper. A portion of liver and kidney tissues were assayed by the method of Buege and Aust (26). Liver and kidney tissues were homogenized with nine volumes of EDTA solution, and 10.5 l of whole blood with 250 l of 1% NMA and applied 130 l of 1% NMP (140 l). The slides were then placed in an electrophoresis tank in alkaline solution (300 mM NaOH and 1 mM Na2 EDTA) for 15 min to allow the DNA to unwind. Electrophoresis was then carried out at 300 mA for 20 min at room temperature in the same alkaline solution. The slides were then neutralized by addition of 0.4 M Tris–HCl buffer (pH 7.5) and stained with ethidium bromide. DNA strand breakage was visually analyzed using comet scores according to the method of Chia et al. (23). The tail moment (TMOM) was determined using Comet Assay IV (Perceptive Instruments Ltd., Haverhill, Suffolk, UK) according to the formula:

\[ \text{TMOM} = \frac{\text{TDNA}}{(\% \text{ of total DNA})} \times \text{TDx (tail length)} \]

The tail moment is considered one of the best indices of comet formation obtained in computerized analysis.

In samples in which breakage of DNA strands occurred due to oxidative damage, a ‘comet’ was formed by electrophoresis. The longer DNA tail lengths represent higher percentages of breakage. The TMOM obtained by computerized analysis is considered one of the best indices of comet formation (24).

Protein analysis

The Bio-Rad Protein Assay, based on the method of Bradford, is a simple and accurate procedure for determining concentrations of solubilized protein. It involves the addition of an acidic dye to protein solution and subsequent measurement at 595 nm with a spectrophotometer. A high shears rotor/stator was used to mix tissue (0.2 g lung and 0.2 g kidney) with 1.8 ml EDTA.

MDA analysis

MDA levels were used as an indicator of lipid peroxidation. MDA assay of serum was performed as described by Hong et al. (25) and tissue MDA was assayed by the method of Buege and Aust (26). Liver and kidney tissues were homogenized with nine volumes of EDTA solution, and 10.5 l of 50 mM butylated hydroxytoluene (BHT) was added to 1 ml of this mixture to avoid the interference caused by MDA generated by strong acid and heat during this analytical process. The MDA standard solution used 1,1,3,3-tetraethoxypropane (Fluka, No. 87670) at concentrations of 0.075, 0.1, 0.25, 0.4, 0.5, 0.75 and 1.0 µM. One-milliliter of tissue solution was added to 10 ml of collagenase dissolved in 1× Hank’s Balanced Salt Solution (Ca2+- and Mg2+-free), as modified from that reported previously (20–22). The mixture was incubated with shaking at 37°C for 50 min, followed by centrifugation at low speed (40 × g, 5 min) to remove undigested tissue debris. The supernatant was further centrifuged (700 × g, 10 min) to precipitate the cells, which were immediately used for the comet assay. Cell numbers and viability of isolated cells were determined using Trypan Blue dye.

Comet assay for DNA strand breakage

The comet assay protocol was described in a previous report (22,23). First, 140 µl of 1% normal melting point agarose (NMA) was applied to a slide. We then mixed 50 µl of whole blood with 250 µl of 1% NMA and applied 130 µl of that mixture to the slide as a second layer. After application of a third layer of 1% NMP (140 µl), we immersed the slides in cold lysing solution (2.5 M NaCl, 100 mM Na2 EDTA, 10 mM Tris, 1% N-sodium lauroyl sarcosinate) for 1 h at 4°C. The slides were then placed in an electrophoresis tank in alkaline solution (300 mM NaOH and 1 mM Na2 EDTA) for 15 min to allow the DNA to unwind. Electrophoresis was then carried out at 300 mA for 20 min at room temperature in the same alkaline solution. The slides were then neutralized by addition of 0.4 M Tris–HCl buffer (pH 7.5) and stained with ethidium bromide.

Enzymatic digestion, the minced tissues (1.0 g liver and 1.0 g kidney) were added to 10 ml of collagenase dissolved in 1× Hank’s Balanced Salt Solution (Ca2+- and Mg2+-free), as modified from that reported previously (20–22). The mixture was incubated with shaking at 37°C for 50 min, followed by centrifugation at low speed (40 × g, 5 min) to remove undigested tissue debris. The supernatant was further centrifuged (700 × g, 10 min) to precipitate the cells, which were immediately used for the comet assay. Cell numbers and viability of isolated cells were determined using Trypan Blue dye.

Preparation of animal tissues and isolation of cells from fresh liver and kidney

Blood samples (0.5 ml) were drawn from the rats after each exposure; liver, lung and blood were also collected after the rats were sacrificed by decapitation at the end of the experiments. The livers and kidneys were rinsed in cold phosphate-buffered saline (pH 7.4) and blotted dry on filter paper. A portion of the fresh tissue was minced thoroughly on aluminium foil on ice before homogenization or collagenase treatment. Minced liver was suspended at 1 ml/g in phosphate-buffered saline (pH 7.4) and blotted dry on filter paper. A portion of liver and kidney tissues were homogenized smoothly in ice using a Potter–Elvehjem glass homogenizer (GLAS-COL, GKH-GT Apparatus, Terre Haute, IN, USA). For enzymatic digestion, the minced tissues (1.0 g liver and 1.0 g kidney) were
The rats were sacrificed after the last exposure. In both liver and kidney, TMOM was significantly higher in the exposed group than in the control group (Figure 4). In kidney and lung, higher levels of MDA were found in the exposed group than in the control group (Figure 5).

**Welding fumes and oxidative damage**

Figures 6 and 7 show the particle concentrations in welding fumes and TMOM and MDA levels during different exposure periods. The air particle levels ranged from 4.5 to 5.5 mg/m³, and the variation of air particle levels was smaller than MDA levels and TMOM of exposed rats. Meanwhile, the study found no consistent relationship between the exposure and the effects; with respect to this parameter, inconsistent results were also obtained for TMOM. However, MDA and TMOM both reached maximum levels on day 7 after the first exposure.

**Discussion**

**Welding fume exposure dose**

This study was undertaken to assess the association between oxidative stress-based biomarkers and exposure to welding fumes in an *in vivo* study. Exposure to welding fumes causes a variety of biological effects. However, a definitive relationship between welding exposure and health effects has not been demonstrated in cross-sectional and longitudinal studies of occupational groups (29–32). This lack of definitive correlation has been attributed to small sample size or matrix exposure to welding fumes. For example, in an *in vivo* study, Antonini et al. (3) indicated that mild steel welding fumes had no effect on lung inflammation and injury. In a case study, Hannu et al. (33) showed that challenge tests with both mild steel and stainless steel welding using a common electrode did not cause bronchial obstruction. In the present work, we attempted to set up an *in vivo* study in order to avoid confounding factors that can affect apparent causal relationships between welding exposure and measurable precursors of health effects. In this study, the particle concentration of welding fumes was 1540.76 mg/m³. The total exposure dose was 12.32 mg/kg body weight, almost equal to that reported by Yu et al. (11,27) in 2003 and 2004 and 2-fold below the dose of 2.35 mg/kg body weight administered in 2000 (28) (Table 1). The above were all high-dose and acute experimental studies. Meanwhile, the mean cumulative exposure concentration (1000 mg/m³/day) was evaluated in shipyard welders with lung cancer in a nested case–control study (34). Acute studies all found a positive association between welding fumes and oxidative damage or lung cancer. In addition, some studies have investigated welders’ air metal exposure. Seel et al. (34) measured Ni exposure levels of 140 μg/m³/day and Cr levels of 73 mg/m³/day.

<table>
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<th>References</th>
<th>Air concentration (mg/m³)</th>
<th>Exposure time (min)</th>
<th>Exposure days</th>
<th>Body weight (g)</th>
<th>Total exposure dose (mg/kg)</th>
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<tr>
<td>Antonini et al., 2007</td>
<td>21.8 ± 4.7</td>
<td>180</td>
<td>3</td>
<td>300</td>
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<tr>
<td>Yu et al., 2004</td>
<td>116.8 ± 3.9</td>
<td>120</td>
<td>3</td>
<td>218 ± 9</td>
<td>11.56</td>
</tr>
<tr>
<td>Yu et al., 2003</td>
<td>107.1 ± 6.3</td>
<td>120</td>
<td>3</td>
<td>218 ± 10</td>
<td>14.13</td>
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<tr>
<td>Yu et al., 2000</td>
<td>65 ± 3</td>
<td>240</td>
<td>5</td>
<td>200</td>
<td>23.78</td>
</tr>
<tr>
<td>Our study</td>
<td>1540.76</td>
<td>10</td>
<td>6</td>
<td>450</td>
<td>12.32</td>
</tr>
</tbody>
</table>

Exposure dose (mg/kg) = (air concentration (mg/m³) × exposure time (min) × exposure days (m³/day))/rat body weight.

Exposure dose = (1540.76 mg/m³ × 0.04167 day × 0.0864 m³/day)/0.45 kg = 12.32 mg/kg.
45 μg/m³/day, while Borská et al. (35) reported Ni levels (0.340–10.129 mg/m³) and Cr levels (0.557–16.343 mg/m³) in an occupational atmosphere that exceeded maximum permitted concentrations (0.10–1.0 mg/m³). High Cr and Ni levels were also demonstrated in body fluids of welders (16,36,37). Therefore, metal exposure should be given serious attention in future studies.

**DNA damage in welders**

In this study, obvious DNA strand breakage was demonstrated in welding-exposed rats within a month’s exposure, compared to that of control animals. At the end of the study, however, the level of DNA strand breakage declined with respect to the referent in the beginning. Moreover, MDA levels increased over three exposures (at day 7) and persisted to day 40. In *in vivo* studies, acute exposure to welding fumes can cause noticeable oxidative damage. DNA damage might recover after long and repeated exposure, whereas for lipid peroxidation this may not be the case. Significant changes in MDA levels can reflect lipid peroxidation in the erythrocyte membrane (38,39).

The results of the MDA assay are especially significant in light of the evidence showing that the oxidation of low-density lipoproteins is important in the pathogenesis of atherosclerosis (40) and that welders exposed to chromium (VI) have increased plasma lipid oxidation (41). It has been shown that stainless steel welding fumes can produce biologically reactive hydroxyl radicals (**OH** and that *in vitro* treatment with welding fumes results in a concentration-dependent increase in DNA damage and lung macrophage death (19). In epidemiological studies, Iarmarcovai et al. (15) evaluated metals in body fluid and DNA damage in welders. Higher chromium, lead and nickel and frequency of chromosomal damage were found in blood and urine of welders as compared to controls. In an *in vivo* study, the alkaline comet assay showed a significant increase at the...
end of week (post-shift) compared with that at the beginning: 20 of 30 welders also showed similar results in 8-OH-dG levels (16). A 2-fold higher incidence of chromosomal breaks was found in welders chronically exposed to chromium (VI) compared with that of controls (42), and increased DNA damage was seen with occupational exposure to welding fumes (19,36,37,43). The present results are in good agreement with epidemiological studies confirming the production of DNA damage in welders potentially exposed to welding fumes. Previous studies have indicated that increased metal concentrations in biological samples in welders may be related to DNA damage (16,37). Significant differences were found in metal concentrations in biological samples from welders equipped with smoke extraction systems and those working without any collective protective devices. Installation of collective protective devices in work areas may help to significantly improve the air quality of the occupational environment. Therefore, installation and improvement of the efficiency of environmental protective devices are important for future attention.

DNA damage and exposure duration
The present study demonstrated an apparent increase in DNA strand breakage in liver and kidney and in MDA levels in kidney and lung of rats exposed to welding fumes six times within 40 days. Jelmlert et al. (43) showed that an increase in chromatid breaks is associated with cumulated welding fume exposure for more than 1 year in welders, both with and without the use of respirators. Beyond 4 months of exposure, the welders showed slight, but significant, increases in chromatid breaks (44). Lillenberg et al. (6) showed that welders employing the manual metal arc technique <1 day/week experienced a prevalent risk of wheezing. In an in vivo study, a time-dependent increase of apoptosis in lung cells was seen (19). Clearly, additional studies are required in order to fully understand the acute and chronic toxicological effects of welding fume exposure. The study found no consistent relationship between the levels of respirable particles and oxidative effects; however, from literature reviews, Dick et al. (45) have shown that ultra fine particles may cause adverse effects via oxidative stress. The experimental and epidemiological data also indicated that airborne particulate matter with an aerodynamic diameter of <2.5 μm is easily deposited in the human lung and has a severe impact on health (46,47). Therefore, the ultra fine particles in welding fumes should be elucidated to assess the adverse health effects on welding exposure. Meanwhile, due to 62% workers operating welding equipment <1 h/day, further study should be focused on assessing the health effects including lung, liver and kidney of welders working intermittently but exposed to high doses without the use of respiratory masks.

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References


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