Chromium (VI) induced oxidative damage to DNA: increase of urinary 8-hydroxydeoxyguanosine concentrations (8-OHdG) among electroplating workers

H W Kuo, S F Chang, K Y Wu, F Y Wu

Aims: To investigate the concentration of urinary 8-hydroxydeoxyguanosine (8-OHdG) among electroplating workers in Taiwan.

Methods: Fifty workers were selected from five chromium (Cr) electroplating plants in central Taiwan. The 20 control subjects were office workers with no previous exposure to Cr. Urinary 8-OHdG concentrations were determined using high performance liquid chromatography with electrochemical detection.

Results: Urinary 8-OHdG concentrations among Cr workers (1149.5 pmol/kg/day) were higher than those in the control group (730.2 pmol/kg/day). There was a positive correlation between urinary 8-OHdG concentrations and urinary Cr concentration ($r = 0.447$, $p < 0.01$), and urinary 8-OHdG correlated positively with airborne Cr concentration ($r = 0.285$). Using multiple regression analysis, the factors that affected urinary 8-OHdG concentrations were alcohol, the common cold, and high urinary Cr concentration. There was a high correlation of urinary 8-OHdG with both smoking and drinking, but multiple regression analysis showed that smoking was not a significant factor. Age and gender were also non-significant factors.

Conclusion: 8-OHdG, which is an indicator of oxidative DNA damage, was a sensitive biomarker for Cr exposure.

It is widely recognised that hexavalent chromium (Cr(VI)) is a carcinogen and that exposure to Cr(VI) significantly increases the risk of respiratory tract cancer.1 Previous studies2–5 have shown that exposure to Cr(VI) causes chromosome aberrations, sister chromatid exchanges, gene mutation, and cell death, but the mechanisms by which these processes occur are not yet fully understood. Cr(VI) is reduced through short lived Cr intermediates (Cr(V) and Cr(IV)) to the ultimately kinetically stable trivalent species (Cr(III)). This process generates several reactive oxygen species (ROS). ROS are known to cause DNA damage by inducing breaks in the DNA strand.6 Kasai et al were the first to analyse a form of oxidative DNA damage by assessing concentrations of 8-hydroxydeoxyguanosine (8-OHdG), which is a biomarker of cellular oxidative stress during carcinogenesis.2 Kasai investigated the mechanisms by which 8-OHdG is formed in vitro by various agents, such as Fenton-type reagents, x rays, cigarette smoking, asbestos, and diesel exhaust particles. The results showed that 8-OHdG is formed by a wide variety of agents with different mechanisms of action.8

The earliest study to show the production of 8-OHdG in isolated DNA by Cr(VI) and Cr(V) was by Faux and colleagues.9 Their results showed that there was oxidative damage to isolated DNA with Cr(VI) and Cr(V), which indicated a mechanism independent of thios and involvement of hydroperoxide, probably via a Fenton-type reaction. Tsou et al investigated the capacity of Cr(III) to induce DNA lesions generated by oxidative damage by examining the formation of 8-OHdG in calf thymus DNA by CrCl3 and/or H2O2 in 10 mM phosphate buffer. The results showed that Cr(III) is capable of inducing carcinogenic lesions through interaction with cellular oxygen species.9 Previous studies have shown that 8-OHdG concentrations can measure oxidative DNA lesions in vitro and in mammalian cell cultures. However, Kim et al reported that exposure to Cr(VI) does not increase 8-OHdG concentrations in DNA extracted from sputum and white blood cells of chromate pigment workers.10

There are many factors which can affect 8-OHdG, such as animal species, gender, age, exercise, alcohol, smoking, weight, and nutrition. Therefore, there is a high degree of variation in results obtained from human subjects. Accurate assessment of 8-OHdG concentrations is difficult because concentrations are very low. Furthermore, there may be interference by a variety of substances in urine during measurement of 8-OHdG. Ames suggested that high pressure liquid chromatography (HPLC) and electrochemical detection (ECD) could be used to determine urinary 8-OHdG.10 Germadnik and colleagues used a two step solid phase extraction (SPE) for extracting 8-OHdG from human urine; analysis was performed using HPLC and ECD. However, the recovery efficiency of 8-OHdG in the extraction procedure from 2 ml of urine was found to be 45.8%.11 Currently, there are no available data regarding worker exposure to Cr and urinary 8-OHdG concentrations. In addition, there are no baseline data for urinary 8-OHdG in Taiwan. The objective of the current study, therefore, was to determine urinary 8-OHdG concentrations in electroplating workers and investigate the factors that affect 8-OHdG concentrations.

MATERIALS AND METHODS

Study population Fifty subjects were selected from six electroplating plants (three hard Cr, one nickel-Cr, and two mixed plants) in...
Taiwan. The control group was comprised of 20 office workers with no previous exposure to Cr. Demographic information, work history, habits, and medical symptoms of all subjects were obtained using a questionnaire. A physician confirmed medical symptoms. Sixteen per cent of workers had nasal septa ulceration and 20% had a skin rash. Detailed information on the workers’ health can be found in the authors’ previous study.14

### Determination of urinary chromium concentration

Workers’ urine samples were taken at the end of a work shift and urinary Cr, creatinine, and 8-OHdG concentrations were determined. Urine samples were collected in clean polyethylene bottles and stored at 4°C immediately following collection; they were analysed within one week by atomic absorption spectrophotometry (AAS) with a graphite furnace and Zeeman effect background correction (Perkin-Elmer Model 5100PC) to determine urinary Cr concentration. The detection limit of Cr was 0.12 ppb, and the reproducibility (coefficient of variation (CV)) was 2.2% (within series) and 3.8% (between series). There was a high level of accuracy: the concentration of the standard reference material was 20.9 ppb, which was very similar to that in the current study (21.1 ppb).

### Determination of urinary 8-OHdG concentrations

The pretreatment procedure used in the current study was based on the methods described by Germadnik and colleagues10 and Loft and colleagues.11 The pH of 4 ml urine was adjusted to 4.5 by adding 2M HCl. The mixture was then centrifuged at 4000 rpm for 10 minutes. A 2 ml aliquot of the supernatant was taken and cleaned up by SPE. The Bond Elut centrifuged at 4000 rpm for 10 minutes. A 2 ml aliquot of was adjusted to 4.5 by adding 2M HCl. The mixture was then based on the methods described by Germadnik and controls was used to correlate urinary 8-OHdG, urinary Cr, and airborne Cr concentrations. ANOVA was then used to compare the urinary 8-OHdG concentrations among the variables. Multiple regression analysis was used to analyse the factors that affected 8-OHdG concentration.

#### RESULTS

Table 1 compares the demographic data of the control group and the Cr workers. The average age of Cr workers (36 years) was higher than that of the control group (31 years). There was no significant difference between the groups for gender and body mass index (BMI). The education level of the controls was higher than that of the Cr workers. A total of 38.8% of all Cr workers worked with the electroplating tank. Smoking and alcohol consumption was more prevalent among Cr workers. A total of 24.1% of the Cr workers had a common cold compared to 0% in the control group.

Table 2 compares urinary 8-OHdG, Cr, and creatinine concentrations in Cr exposed and control groups. There was a significant difference between the two groups, even after log transfer. For urinary 8-OHdG (nM), there was a significant difference (1.6-fold) between Cr workers (73.2 nM) and the control group (44.6 nM). After adjusting for body weight, urinary 8-OHdG concentration for Cr workers was 1149.5 pmol/100 ml and column temperature was 40°C. Prior to each measurement of urinary 8-OHdG, a 55 minute wash run was performed as follows: 0–25 minutes, 100% solvent A; 26–35 minutes, 0–50% solvent B; 36–40 minutes, 50% solvent B; 41–50 minutes, 50–0% solvent B; 51–55 minutes, 100% solvent A. The wash step was followed by an electric treatment for preparing the surface of the glassy carbon electrode, pulsing the potential between +0.6 V, +1.5 V, and −1.0 V in 60 cycles within one minute. The ECD full scale range was 500 nA and the ECD potential was 0.6 V. For each measurement 100 µl was injected into the HPLC system.

For each batch of measurements two calibration curves were set up using the five concentrations of urinary 8-OHdG: aqueous concentrations (27.5 nM, 110 nM, 220 nM, 330 nM, 440 nM, 880 nM, 1760 nM) and urinary 8-OHdG standard concentrations (27.5 nM, 110 nM, 220 nM, 330 nM, 440 nM, 880 nM, 1760 nM); for both curves the correlation coefficients (r) were over 0.999. The average recovery efficiencies for aqueous and urinary 8-OHdG solutions, at four concentrations (triplicates), were 84% and 76%, respectively. The reproducibility (CV) for three urinary 8-OHdG concentrations (tested seven times) was <3%. The stability was tested over three weeks stored at −20°C. 8-OHdG was found to be very stable. After 21 days 98% of 8-OHdG was recovered, compared to 100% at the beginning (0 day).

#### Statistical analysis

SAS 6.12 was used for the statistical analyses. The χ² test was used to compare demographic information of the two groups. A t test was used to compare urinary 8-OHdG, Cr, and creatinine concentrations between the two groups. Pearson’s correlation was used to correlate urinary 8-OHdG, urinary Cr, and airborne Cr concentrations. ANOVA was then used to compare the urinary 8-OHdG concentrations among the variables. Multiple regression analysis was used to analyse the factors that affected 8-OHdG concentration.

#### Table 1

<table>
<thead>
<tr>
<th>Variables</th>
<th>Control group (n=20)</th>
<th>Cr worker (n=50)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td>33 (66.0)</td>
<td>11 (55.0)</td>
<td>0.390</td>
</tr>
<tr>
<td>Female</td>
<td>17 (34.0)</td>
<td>9 (45.0)</td>
<td></td>
</tr>
<tr>
<td>Age (y)</td>
<td>36.5±12.7*</td>
<td>31.6±5.7</td>
<td>0.014</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>22.7±3.9</td>
<td>22.3±3.2</td>
<td>0.714</td>
</tr>
<tr>
<td>Education (y)</td>
<td></td>
<td></td>
<td>0.019</td>
</tr>
<tr>
<td>&lt;6</td>
<td>17 (34.0)</td>
<td>0 (0.0)</td>
<td></td>
</tr>
<tr>
<td>7–9</td>
<td>16 (32.0)</td>
<td>0 (0.0)</td>
<td></td>
</tr>
<tr>
<td>10–12</td>
<td>14 (28.0)</td>
<td>2 (10.0)</td>
<td></td>
</tr>
<tr>
<td>&gt;13</td>
<td>3 (6.0)</td>
<td>18 (90.0)</td>
<td></td>
</tr>
<tr>
<td>Work duration (mth)</td>
<td>73.6±73.1*</td>
<td>48.2±20.4</td>
<td>0.108</td>
</tr>
<tr>
<td>Works with electropolating tank</td>
<td>19 (38.8)</td>
<td>0 (0.0)</td>
<td>0.001</td>
</tr>
<tr>
<td>Smoking (pack year)</td>
<td>21 (42.0)</td>
<td>3 (15.0)</td>
<td>0.032</td>
</tr>
<tr>
<td>Alcohol consumption</td>
<td>5.6±5.9</td>
<td>4.1±2.89</td>
<td>0.676</td>
</tr>
</tbody>
</table>

*Mean±SD
†p value was calculated by Fisher’s exact test.
Table 4 shows the factors that affect concentrations of urinary 8-OHdG using univariate analysis. The factors were as follows: exposure/non-exposure to Cr, gender, age, BMI, smoking, alcohol consumption, betel nut consumption, common cold, and three groups of urinary Cr concentration. It was found that urinary 8-OHdG concentrations were significantly higher in the following groups: exposure to Cr, males (1.4 times higher than for females), BMI >27 (obesity), smoking, betel nut consumption (“usually” was higher than “sometimes” and “seldom/none”), and high urinary Cr group. Subjects with a common cold and subjects who usually drank alcohol had higher urinary 8-OHdG concentrations, but these were not significant. However, there was no significant difference for age. When 8-OHdG concentrations were adjusted for weight (nM/kg), almost the same results were found, except for BMI and the three urinary Cr groups.

Table 5 shows the factors that affect urinary 8-OHdG concentration using multiple regression analysis. The factors were as follows: age, gender, smoking, alcohol, common cold, and urinary Cr concentration (three groups). The results showed that subjects who usually drank alcohol had significantly higher urinary 8-OHdG concentrations than subjects who sometimes or never/seldom drank (used as a reference). This was true also for subjects with a common cold and for subjects who had high urinary Cr (compared to low urinary Cr subjects). Males had higher urinary 8-OHdG concentrations than females but this was non-significant. Adjusted for body weight, the factors that affect urinary 8-OHdG concentrations were the same, except for subjects with a common cold (non-significant).

**DISCUSSION**

In order to study conditions of “oxidative stress” and possible correlations between diseases and the exposure of the individual to ROS inducing agents, fast, sensitive, and easy to use methods for the analysis of oxidative damage are needed. 8-OHdG is one of the major oxidative adducts formed by radical induced damage to DNA. Of the 20 known oxidative adducts in DNA, 8-OHdG has aroused most interest owing to its mutagenic potential. However, there is little data concerning the measurement of 8-OHdG in urine using HPLC. The process of urinary 8-OHdG determination using HPLC-ECD, first described by Ames et al, is very complicated and is susceptible to interference. Loft et al developed an automatic triple column switching HPLC technique with isocratic separation and electrochemical detection for the determination of urinary 8-OHdG, and found that the detection limit was 0.2 nM. Neither of the above mentioned studies determined recovery efficiency or stability of urinary 8-OHdG. Germadnik et al used a two step SPE procedure (for extraction of 8-OHdG from urine), followed by analysis with HPLC-ECD to determine urinary 8-OHdG concentrations. The limit of detection for urinary 8-OHdG was found to be 0.9 nM and recovery of urinary 8-OHdG was 45.8%. The recovery efficiency in the study of Germadnik et al was lower than in the current study (75%). In the current study, the extraction efficiency of three cartridges (C18 ENVI (Supelcosil), C18 (Supelcosil), and C18/OH (Varian)) was tested prior to the commencement of the study. The results of this test showed that the C18/OH cartridge was the most efficient. During the two step SPE procedure, the flow rate of the pump was maintained at a
stable level in order to minimise loss of 8-OHdG. The duration of methanol removal by evaporation had no effect on concentrations of 8-OHdG. The data from the current study showed high precision and stability (samples were kept at −20°C for three weeks).

Although Cr(VI) compounds are well documented carcinogens, their mechanism of action is still not well understood. Many studies have shown that when Cr(VI) reduces to Cr(V) and the trivalent form (Cr(III)), free radicals are produced. The formation of the reactive Cr(V) complex can be detected by electron spin resonance in mitochondria, microsomes, and intact cells. 8-OHdG is a key biomarker relevant to carcinogenesis in both animal models and human studies, and may be detected when it is excreted in urine. Faux et al measured the concentrations of 8-OHdG in calf DNA incubated in vitro when simultaneously exposed to Cr(VI), Cr(V), and H₂O₂ and reported that concentrations of 8-OHdG were higher than when DNA was incubated with either compound alone. This may help to explain the mechanism of Cr induced DNA oxidation involving H₂O₂ via a Fenton type or Haber-Weiss type reaction.°

The current study found that urinary 8-OHdG concentrations in the electroplating worker group exposed to airborne chromic acid were significantly higher (1.6-fold) than in the control group. Furthermore, urinary 8-OHdG concentrations were positively correlated to urinary Cr and airborne Cr concentrations. Urinary Cr was classified into three groups (high, medium, and low). Based on multiple regression analysis, the results showed that there was a significant difference between the high urinary Cr group (>6.41 µg/g creatinine) and low urinary Cr group (<1.13 µg/g creatinine) with regard to urinary 8-OHdG concentrations. However, there was no significant difference between the

<table>
<thead>
<tr>
<th>Table 4</th>
<th>Univariate analysis of the factors that affect urinary 8-OHdG concentrations</th>
</tr>
</thead>
<tbody>
<tr>
<td>Variables</td>
<td>8-OHdG (nmol) p value</td>
</tr>
<tr>
<td>Group</td>
<td>n</td>
</tr>
<tr>
<td>Cr worker</td>
<td>48</td>
</tr>
<tr>
<td>Control</td>
<td>19</td>
</tr>
<tr>
<td>Gender</td>
<td>42</td>
</tr>
<tr>
<td>Male</td>
<td>25</td>
</tr>
<tr>
<td>Female</td>
<td>17</td>
</tr>
<tr>
<td>Age (y)</td>
<td>16</td>
</tr>
<tr>
<td>&lt;27</td>
<td>34</td>
</tr>
<tr>
<td>27–40</td>
<td>17</td>
</tr>
<tr>
<td>&gt;40</td>
<td>58</td>
</tr>
<tr>
<td>BMI</td>
<td>58</td>
</tr>
<tr>
<td>Smoking</td>
<td>Yes</td>
</tr>
<tr>
<td>No</td>
<td>43</td>
</tr>
<tr>
<td>Alcohol consumption</td>
<td>Usually</td>
</tr>
<tr>
<td>Seldom/never</td>
<td>60.6±45.6</td>
</tr>
<tr>
<td>Betel nut consumption</td>
<td>Usually</td>
</tr>
<tr>
<td>Seldom/never</td>
<td>56.9±37.8</td>
</tr>
<tr>
<td>Common cold</td>
<td>Yes</td>
</tr>
<tr>
<td>No</td>
<td>39</td>
</tr>
<tr>
<td>Urinary Cr concentration†</td>
<td>Low</td>
</tr>
<tr>
<td>Moderate</td>
<td>27</td>
</tr>
<tr>
<td>High</td>
<td>14</td>
</tr>
</tbody>
</table>

*Mean±SD
†Low urinary Cr: <1.13 µg/g cre. Moderate urinary Cr: 1.13–6.41 µg/g cre. High urinary Cr: >6.41 µg/g cre.

<table>
<thead>
<tr>
<th>Table 5</th>
<th>Factors that affect the concentration of urinary 8-OHdG using multiple linear regression (n=67)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Variables</td>
<td>8-OHdG (nM) β (SE) p value</td>
</tr>
<tr>
<td>Gender (female = 0)</td>
<td>27.0 (15.2)</td>
</tr>
<tr>
<td>Age (&lt;27 years = 0)</td>
<td>−0.16 (0.64)</td>
</tr>
<tr>
<td>Smoking (yes = 0)</td>
<td>−15.5 (16.5)</td>
</tr>
<tr>
<td>Alcohol (never = 0)</td>
<td>93.2 (22.9)</td>
</tr>
<tr>
<td>Common cold (no = 0)</td>
<td>40.9 (17.4)</td>
</tr>
<tr>
<td>Urinary Cr concentration*</td>
<td>Moderate (low = 0)</td>
</tr>
<tr>
<td>High (low = 0)</td>
<td>86.5 (22.5)</td>
</tr>
</tbody>
</table>

R² 0.6097 0.0001 0.6042 0.0001

*Low urinary Cr: <1.13 µg/g cre. Moderate urinary Cr: 1.13–6.41 µg/g cre. High urinary Cr: >6.41 µg/g cre.
medium and low urinary Cr groups. The authors suggest that in order to detect significant concentrations of 8-OHdG, urinary Cr must exceed a certain level, since 8-OHdG concentrations may be affected by other factors such as gender, BMI, the common cold, smoking, alcohol, and betel nut consumption. Within the respiratory tract, Cr(VI) is reduced in the epithelial lining fluid, pulmonary alveolar macrophage, bronchial tree, and peripheral lung parenchyma cells. Hence, lung cancer can only be induced when Cr(VI) overwhelms these defence mechanisms. However, the efficient uptake and reduction of Cr(VI) in red blood cells explained its lack of carcinogenicity at a distance from the portal of entry into the body. Smoking causes oxidative DNA damage in lung tissue as there was a positive correlation between smoking (number of cigarettes smoked per day) and concentrations of urinary 8-OHdG ($r = 0.444$). Park et al studied oxidative damage in the tissue of rats exposed to cigarette smoke. The results showed that glutathione plays a crucial role in protecting proteins and DNA from oxidation caused by cigarette smoke. The current study showed that smoking was a significant factor using univariate analysis, but was not significant using multiple regression analysis. This apparent contradiction may be explained by the fact that there was a strong intercorrelation between smoking and alcohol. Six workers consumed alcohol regularly (more than three occasions per week, 250 ml per occasion) and also smoked. However, there was no interactive effect on 8-OHdG concentrations between any of the factors (such as smoking and alcohol) and urinary Cr. There are no available data with which to compare this interactive effect and, as such, this is an area for further research. Loft et al reported that, in general, concentrations of urinary 8-OHdG were higher (29%) among men than women. This finding is consistent with the current study (34%). This difference may be explained by the genetic differences between males and females. Possibly male DNA is better able to repair itself or is less susceptible to damage. Age was inversely proportional to urinary 8-OHdG concentrations among rats. However, concentrations of 8-OHdG in the rat intestinal tract, kidneys, and livers increased with age. 8-OHdG concentrations may also be affected by an individual's ability to repair DNA.

In conclusion, urinary 8-OHdG concentrations among Cr workers (73.2 nmol) were higher than the control group (44.6 nmol). Urinary 8-OHdG concentrations correlated positively with urinary Cr concentration ($r = 0.447, p < 0.01$) and there was a positive correlation between urinary 8-OHdG and airborne Cr concentration ($r = 0.305$). Thus, it has been shown that 8-OHdG, which is an indicator of oxidative DNA damage, may be used as a biomarker for Cr exposure.

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REFERENCES
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