Interindividual Variability in Response to Sodium Dichromate–Induced Oxidative DNA Damage: Role of the Ser\textsuperscript{326}Cys Polymorphism in the DNA-Repair Protein of 8-Oxo-7,8-Dihydro-2′-Deoxyguanosine DNA Glycosylase 1

Amanda J. Lee, Nikolas J. Hodges, and James K. Chipman

School of Biosciences, The University of Birmingham, Birmingham, United Kingdom

Abstract

Although the genotoxic mechanism(s) of hexavalent chromium (CrVI) carcinogenicity remain to be fully elucidated, intracellular reduction of CrVI and concomitant generation of reactive intermediates including reactive oxygen species and subsequent oxidative damage to DNA is believed to contribute to the process of carcinogenesis. In the current study, substantial interindividual variation (7.19-25.84% and 8.79-34.72% tail DNA as assessed by conventional and FPG-modified comet assay, respectively) in levels of DNA strand breaks after in vitro treatment of WBC with sodium dichromate (100 μmol/L, 1 hour) was shown within a group of healthy adult volunteers (n = 72) as assessed by both comet and formamidopyrimidine glycosylase–modified comet assays. No statistically significant correlation between glutathione S-transferases M1 or T1, NADPH quinone oxidoreductase 1 (codon 187) and X-ray repair cross complementation factor 1 (codon 194) genotypes and individual levels of DNA damage were observed. However, individuals homozygous for the Cys\textsuperscript{326} 8-oxo 7,8-dihydro-2′-deoxyguanosine glycosylase 1 (OGG1) polymorphism had a statistically significant elevation of formamidopyrimidine glycosylase–dependent oxidative DNA damage after treatment with sodium dichromate when compared with either Ser\textsuperscript{326}/Ser\textsuperscript{326} or Ser\textsuperscript{326}/Cys\textsuperscript{326} individuals (P = 0.008 and P = 0.003, respectively). In contrast, no effect of OGG1 genotype on background levels of oxidative DNA damage was observed. When individuals were divided on the basis of OGG1 genotype, Cys\textsuperscript{326}/Cys\textsuperscript{326} individuals had a statistically significant (P < 0.05, one-way ANOVA followed by Tukey test) higher ratio of oxidative DNA damage to plasma antioxidant capacity than either Ser\textsuperscript{326}/Ser\textsuperscript{326} or Ser\textsuperscript{326}/Cys\textsuperscript{326} individuals. The results of this study suggest that the Cys\textsuperscript{326}/Cys\textsuperscript{326} OGG1 genotype may represent a phenotype that is deficient in the repair of 8-oxo-7,8-dihydro-2′-deoxyguanosine, but only under conditions of cellular oxidative stress. We hypothesize that this may be due to oxidation of the Cys\textsuperscript{326} residue. In conclusion, the homozygous Cys\textsuperscript{326} genotype may represent a biomarker of individual susceptibility of lung cancer risk in individuals that are occupationally exposed to CrVI. (Cancer Epidemiol Biomarkers Prev 2005;14(2):497–505)

Introduction

Epidemiologic studies, supported by animal and in vitro data, suggest that hexavalent chromium (CrVI) compounds are carcinogenic, and the IARC has classified CrVI as a group I carcinogen (1). CrVI compounds are used industrially as catalysts, corrosion inhibitors, and as alloying metal for making stainless steel and various superalloys. Subsequently, CrVI is present in various welding fumes (2-5). In the workplace, exposure to chromium can be oral and dermal; however, the respiratory tract is the major site of exposure and, consequently, there is an increased risk of cancers of the respiratory system in exposed individuals (2).

CrVI compounds including sodium dichromate are genotoxic and cause DNA strand breaks (6, 7), chromium-DNA adducts (8), DNA–DNA (9), DNA–protein cross-links (10), and oxidative base damage including 8-oxo-7,8-dihydro-2′-deoxyguanosine (8-oxo-dG; ref. 6) in vitro. Although the mechanism(s) involved remains to be fully elucidated (2), intracellular reduction of CrVI by cellular reductants such as reduced glutathione (GSH) and ascorbic acid and the subsequent generation of partially reduced chromium species such as CrV and CrIV are believed to be important (11). In addition, reduction of CrVI may also result in the concomitant generation of reactive oxygen species including hydroxyl radicals (‘OH), singlet oxygen (O), superoxide (O\textsubscript{2}˙) and hydrogen peroxide (12-15). In addition to reductive activation of CrVI, the antioxidant nature of GSH may also play a protective role in detoxification of reactive oxygen species and partially reduced chromium species. Interestingly, recent studies have indicated that human lung cells are able to internalize particulate chromium, resulting in high local intracellular concentrations of CrVI whose effects seem to be mediated by reactive oxygen species (16, 17).

The alkaline version of the single cell gel electrophoresis assay or comet assay represents a sensitive technique for the detection of ssDNA breaks in cells exposed to genotoxic carcinogens. Collins et al. (18) developed the use of formamidopyrimidine glycosylase (FPG) in the comet assay for the specific detection of oxidized purines, predominantly 8-oxo-dG. Because of its sensitivity, many studies have used the comet assay to investigate CrVI-induced DNA damage in vitro (e.g., 6, 19-24).

Biomarkers of susceptibility are indicators of an inherent or acquired limitation of an organism’s ability to respond to the challenge of exposure to a specific xenobiotic substance (25). Examples include interindividual differences in metabolism (activation and detoxification) of carcinogenic chemicals, DNA repair, and the functions of proto-oncogenes or tumor...
suppressor genes, as well as interindividual differences in nutritional, hormonal, and immunologic factors (26).

The intracellular reduction of sodium dichromate is extremely complex and involves multiple pathways including metabolism by enzymes for which functional polymorphisms in human populations are known to exist (e.g., NQO1 and cytochrome P450s 27-29). A major defense system against the damaging effects of oxidative stress are the glutathione S-transferases (GST), which catalyze the nucleophilic addition of glutathione (GSH) to electrophilic acceptors including toxic products generated from tissue damage (e.g., glutathionealkenes, epoxides, hydroperoxides, and aldehydes) produced as a result of lipid peroxidation (30).

A wide range of low molecular weight cellular antioxidants (e.g., GSH, ascorbic acid, melatonin, and vitamins D and E) also plays a role in the reduction and metabolic activation of CrVI (31, 32). It is likely that intracellular levels of these molecules will be influenced by both genetic factors and environmental factors including diet, alcohol consumption, and smoking habit. In addition, several components of the base excision repair pathway, including the DNA glycosylase 8-oxo-7,8-dihydro-2’-deoxyguanosine glycosylase 1 (OGG1) and X-ray repair cross complementation 1 (XRCC1), are also polymorphic in the human population. Base excision repair is the principal pathway for repair of oxidative DNA damage including 8-oxo-dG in eukaryotic cells. Therefore, functional polymorphisms in components of the cellular base excision repair machinery may also be expected to influence levels of sodium dichromate–induced oxidative DNA damage.

Because of the above factors, it is likely that there will be a considerable heterogeneity in individual response to sodium dichromate–induced DNA damage. The aim of the current study, therefore, is to utilize the conventional and FPG-modified comet assay to study interindividual differences in DNA damage in human WBC after treatment in vitro with sodium dichromate. The relationship between differences in life-style factors, plasma antioxidant capacity, and genetic polymorphisms in detoxification (GSTM1, GSTT1, NQO1) and DNA repair (OGG1, XRCC1) genes and levels of sodium dichromate–induced DNA damage were investigated in an attempt to identify potential biomarkers of susceptibility to sodium dichromate–induced DNA damage.

Materials and Methods

All chemicals were obtained from (Sigma-Aldrich, Poole, Dorset, United Kingdom) and were the highest purity available unless stated otherwise.

Sample Collection. Ethical approval was granted from South Birmingham, United Kingdom, Local Research Ethics Committee before commencement of the study. Subjects for study were healthy adults ages 17 to 62 years (n = 72). After obtaining their informed consent, blood samples (2 mL) were obtained by venipuncture and collected in Vacutainer tubes (Haemograd, Becton Dickinson, United Kingdom) containing either EDTA or sodium heparin anticoagulant. All samples were stored on ice until required and were processed within 3 hours of sample collection. Volunteers completed a detailed questionnaire at the time of sampling in order to gather information on diet, smoking habit, age, and alcohol consumption.

Comet Assay. The comet assay was done as described by Singh et al. (33) with the following alterations. Whole blood (10 μL; EDTA anticoagulant) was suspended in 1 mL HBSS containing 100 μmol/L sodium dichromate and incubated for 1 hour at 37°C. Although cells respond to lower concentrations of sodium dichromate (34) this concentration was used to provide sufficient sensitivity to discriminate between individual responses. After treatment, cells were pelleted by centrifugation (200 × g, 5 minutes). Cell pellets were resuspended in 0.5% low-melting-point agarose (110 μL) and transferred on to a glass microscope slide (Surigipath, Peterborough, United Kingdom) that had been precoated with 0.5% normal melting point agarose. The slides were coveredslipped and placed on ice for a further 10 minutes. Coverslips were removed and the slides lysed for 1 hour at 4°C in lysis buffer (2.5 mol/L NaCl, 100 mmol/L Na2EDTA, 10 mmol/L Tris base, 1% sodium N-lauryl sarcosinate, 10% DMSO, and 1% Triton X-100). Slides were then transferred to a horizontal electrophoresis tank (Pharmacia Biotech, Little Chalfont, Buckinghamshire, United Kingdom) containing electrophoresis buffer (300 mmol/L NaOH, 1 mmol/L EDTA, pH 13). After exactly 20 minutes to allow DNA unwinding, they were subjected to electrophoresis for 20 minutes using an electrophoresis power supply (Pharmacia LKB, Cambridge, United Kingdom). After electrophoresis, slides were neutralized by flooding with three changes of neutralization buffer (0.4 mol/L Tris, pH 7.5) for 5 minutes each wash. The slides were then stained with 50 μL ethidium bromide solution (20 μg/mL).

FPG-Modified Comet Assay. The modified comet assay was carried out as described by Collins et al. (18). Briefly, The comet assay was carried out as described above, with the exception that after lysis slides were washed 3 × 5 minutes with FPG buffer (40 mmol/L HEPES, 0.1 mol/L KCl, 0.5 mmol/L EDTA, 0.2 mg/mL bovine serum albumin, pH 8). After this time, the slides were incubated with 1 unit of FPG enzyme (Trevigen, Gaithersburg, MD) in 50 μL FPG buffer at 37°C for 1 hour. After this time, the slides were incubated with 1 unit of FPG enzyme (Trevigen) in 50 mL FPG buffer at 37°C for 1 hour as optimized by Collins et al. (18). One unit FPG is defined as the amount of enzyme required to cleave 1 pmol 32P-labeled oligonucleotide probe containing 8-oxo-dG within an oligonucleotide duplex in 1 hour at 37°C. To minimize potential variation in FPG activity, the same batch number (3632LO, stored at ~80°C in aliquots) was used in all experiments. Control slides were incubated with 50 μL FPG buffer only. DNA unwinding and electrophoresis were then completed as described above.

Slide Visualization and Analysis. Slides were examined at 320× magnification (32/0.40 dry objective) using a fluorescence microscope (Zeiss, Welwyn Garden City, Hertfordshire, United Kingdom), fitted with a 515- to 560-nm excitation filter and a barrier filter of 590 nm. A video camera (Kinetic Pulnix TM-765) received the images, which were analyzed with a personal computer–based image analysis system Komet 3.0 Europe (Kinetic Imaging Ltd., Liverpool, United Kingdom). One hundred randomly selected nuclei were analyzed per slide using the equipment described. We note that our sample numbers are 8 to 30 per group compared with the n = 3 to 5 commonly used in standard testing experiments and our replicates are within the range reported by Duez et al. (35). Measurements of percent tail DNA were determined to assess the extent of DNA damage. This parameter is the preferred measurement as it is linearly related to the DNA break frequency over a wide range of levels of damage (36). The unwounded version of the comet assay was carried out in parallel so levels of FPG-dependent oxidative damage (exceeding background levels of DNA strand breaks and labile site) could be determined.

Genotyping Analyses. Genomic DNA was isolated from whole blood (~1 mL) using a QiAamp blood spin column midi kit (Qagen, Crawley, United Kingdom), according to the manufacturer’s instructions. PCR genotyping reactions were carried out as previously reported to detect deletions in GSTM1 and GSTT1 (37), with the exception that GST and albumin (internal control) primers sequences were as detailed previously (38). In addition, previously reported RFLP-PCR methods were used to detect polymorphisms in NQO1 at
codon 609 (39), OGG1 at codon 326 (40), and XRCC1 at codon 194 (41).

**Plasma Preparation and Plasma Antioxidant Test.** Whole blood (500 μL; heparin anticoagulant) was centrifuged for 15 minutes at 1500 × g at room temperature. Plasma was stored at −70°C until analysis. Plasma antioxidant capacity was determined by measuring the ability of samples to inhibit superoxide-dependent phalasin fluorescence using a commercially available bioluminescence ABEL kit (Knight Scientific, Plymouth, United Kingdom) according to the manufacturer’s instructions. Briefly, bioluminescence was measured using a plate reader luminometer (Tecan Instruments, Reading, Berkshire, United Kingdom) and antioxidant capacity of each individual plasma sample was expressed as the percentage reduction in fluorescence emission compared with a negative control containing no plasma.

**Statistical Analyses.** All data were analyzed using SPSS version 10 statistical package (SPSS, Working, Surrey, United Kingdom). Differences between both mean and median tail DNA before and after treatment were calculated using a one-tailed paired Student’s t test as recommended by Duez et al. (35). In comparison of tail DNA values between individuals, the comet values were log transformed (mean values only) before analysis. If there were two genotypes in the group (GSTM1 and GSTT1) a one-way ANOVA test was used to analyze differences between the mean tail DNA with or without treatment. For NQO1, OGG1 and XRCC1 (three genotypes per group) the one-way ANOVA was accompanied by a post hoc Scheffe test. All correlations between comet tail DNA and plasma antioxidant capacity were determined by Pearson’s correlation coefficient test (2-tailed).

**Results**

We have previously shown that sodium dichromate induces detectable DNA strand breaks at concentrations as low as 100 μmol/L (34), which is relevant to potential human exposure. However, we emphasize that a higher concentration of 100 μmol/L for 1-hour exposure was most appropriate for the current study because of the need to optimize sensitivity in the search for interindividual differences. We were careful to ensure that this concentration did not cause cytotoxicity as assessed by ATP content and frequency of apoptosis over the period investigated (6, 34).

**Characteristics of the Study Population.** The mean age of volunteers was 34.8 ± 11.3 (SD, n = 72) years. Of the 72 subjects studied, 40 (55.6%) were female and 32 (44.4%) were male. Information gathered from the self-administered questionnaires revealed that 5.6% of the population were vegetarian, 23.6% were smokers, and 86.1% were Caucasian (Table 1).

<table>
<thead>
<tr>
<th>Table 1. General characteristics of the study population (mean age, 34.8 ± 11.3 years)</th>
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<tr>
<td>Gender</td>
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<td>Male</td>
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<td>Diet</td>
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<td>Non-Caucasian</td>
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A general linear univariate ANOVA model was constructed to identify potential confounding factors. Age, ethnicity, gender, alcohol consumption (in the previous week), cigarettes smoked per day on average, vegetable and fruit consumption (average number of servings consumed per day), and meat and fish consumption (average number of servings consumed per week) were analyzed as potential confounding factors. In the current study, none of these parameters was found to have a statistically significant effect on levels of DNA damage or plasma antioxidant status either before or after treatment (data not shown). Therefore, these factors were excluded from further analyses.

**Comet Assay Results.** We determined the coefficient of variation of the comet assay between different experiments using a cell line (mouse embryonic fibroblasts European Cell Culture Collection No. 99072801) with a range of concentrations of sodium dichromate. These values ranged from 14.1% to 26.1% without FPG and from 14.2% to 26.3% with FPG (n = 8 experiments in duplicate). Furthermore, we have shown previously that intraindividual variability of DNA strand breaks in human WBC over an 8- to 12-month period was of a relatively small coefficient of variation (24%) either with or without treatment of cells with sodium dichromate (34). A survey of previous studies indicates that these values compare favorably. Mean tail DNA was calculated for the population using WBC (with and without FPG incubation) to compare favorably. Mean tail DNA was calculated for the population using WBC (with and without FPG incubation) without treatment (control) and after treatment with 100 μmol/L sodium dichromate for 1 hour at 37°C (Fig. 1). This concentration of sodium dichromate does not reduce intracellular levels of ATP or induce apoptosis within the period of incubation as assessed by the terminal deoxynucleotidyl transferase–mediated nick end labeling assay (data not shown) indicating that DNA strand breaks observed were not secondary to toxicity.

The mean background level of DNA strand breaks in untreated WBC was 6.54 ± 0.16%. Background levels of DNA damage increased after FPG treatment to 13.45 ± 0.52%, indicating the presence of endogenous oxidative damage. After treatment with sodium dichromate there was a 1.6-fold increase in DNA strand breaks (mean, 10.14 ± 0.32%). A similar 1.5-fold increase was observed in levels of FPG-sensitive sites after sodium dichromate treatment (mean, 20.01 ± 0.71%). Analysis using a one-tailed paired Student’s t test (both using mean and median values) showed a statistically significant increase in tail DNA after treatment.
with sodium dichromate using both the conventional comet assay ($P = 0.0005$) and the FPG-modified comet assay ($P = 0.0005$). In addition, treatment with FPG significantly increased tail DNA levels in both control nuclei ($P = 0.0005$) and nuclei from CrVI-treated cells ($P = 0.0005$).

Interindividual ranges of DNA strand breaks in controls measured by the conventional and FPG-modified comet assay were 4.06% to 11.97% and 6.02% to 18.48%, respectively. After dichromate treatment, the range of DNA strand breaks was 7.19% to 25.84% in the conventional comet assay and 8.79% to 34.72% in the FPG-modified comet assay. These data indicate substantial interindividual variation in levels of both background and dichromate-mediated DNA strand breaks and oxidative DNA damage (Fig. 1). The mean level of FPG-dependent oxidative damage (FPG-dependent oxidative damage was calculated by subtracting the percent tail DNA value obtained by the conventional comet assay from the percent tail DNA value obtained after inclusion of FPG) was 6.91 ± 0.57%, and this was significantly increased ($P < 0.001$, one-tailed paired $t$ test) to 9.87 ± 0.66% after treatment with 100 μmol/L sodium dichromate for 1 hour at 37°C.

**DNA Strand Breaks in Relation to Genotype.** There was no statistically significant effect of any of the genotypes investigated on levels of frank DNA strand breaks as assessed by the conventional comet assay (data not shown). In addition, GSTM1, GSTT1, NQO1 (codon 187) and XRCC1 (codon 194) polymorphic genotypes had no statistically significant effect on levels of oxidative DNA damage as assessed by the FPG-modified comet assay in either controls or after treatment with sodium dichromate (summarized in Table 2).

Individually were analyzed for the OGG1 Cys326 polymorphism by PCR-RFLP (Fig. 2). No significant difference in control levels of tail DNA between Ser326/Ser326 and Ser326/Cys326 individuals (13.70 ± 4.49% versus 11.98 ± 3.22%, $P = 0.369$) or the Ser326/Ser326 and Cys326/Cys326 individuals (13.70 ± 4.49% versus 15.24 ± 5.64, $P = 0.655$) was detected as assessed by the FPG-modified comet assay. Furthermore, the difference between the mean tail DNA values in control (untreated) cells between the Ser326/Cys326 and Cys326/Cys326 individuals was also not statistically significant ($P = 0.219$). In contrast, after dichromate treatment, a role of codon 326 polymorphism in OGG1 was apparent. There was a statistically significant difference in mean tail DNA levels between Cys326/Cys326 homozygotes and both wild-type (26.56 ± 5.19% versus 19.61 ± 6.10%, $P = 0.008$) and Ser326/Cys326 individuals (26.56 ± 5.19% versus 18.12 ± 4.40, $P = 0.003$) as measured by the FPG-modified comet assay (Fig. 3). Wild-type and heterozygous individuals did not differ significantly ($P = 0.639$; Fig. 3).

![Figure 2. Representative agarose gel showing PCR-RFLP genotyping of codon 326 of OGG1 as described previously (39). As a positive control the identity of individuals of known genotype were confirmed by sequencing (data not shown).](image)

Interestingly, the same profile was observed when the data were analyzed as FPG-dependent oxidative DNA damage (Fig. 4). No significant difference in control levels of tail DNA between Ser326/Ser326 and Ser326/Cys326 individuals (6.99 ± 4.38% versus 5.71 ± 3.13%, $P = 0.560$) or the Ser326/Ser326 and Cys326/Cys326 individuals (6.99 ± 4.38% versus 9.09 ± 5.60%, $P = 0.442$) was detected. Furthermore, the difference between the mean control tail DNA levels between the Ser326/Cys326 and Cys326/Cys326 individuals was also not statistically significant ($P = 0.183$; Fig. 4). In contrast, after dichromate treatment, a role of codon 326 polymorphism in OGG1 was again apparent. There was a statistically significant difference in mean tail DNA levels between Cys326/Cys326 homozygotes and both wild-type (15.24 ± 6.59% versus 9.54 ± 5.48%, $P = 0.023$) and Ser326/Cys326 individuals (15.24 ± 6.59% versus 8.30 ± 3.88, $P = 0.011$) as measured by the FPG-dependent comet assay (Fig. 4). Wild-type and heterozygous individuals did not differ significantly ($P = 0.700$; Fig. 4).

**Correlation between DNA Strand Breaks and Plasma Antioxidant Capacity.** To investigate possible relationships

**Table 2. Mean levels of control and dichromate-induced FPG-dependent oxidative DNA damage in WBC and relationship to GST, NQO1, and XRCC genotype (n = 72)**

<table>
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<tr>
<th></th>
<th>Background oxidative damage</th>
<th>Posttreatment oxidative damage</th>
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<tr>
<td></td>
<td>TD (%)</td>
<td>$P$</td>
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<tr>
<td><strong>GSTM1</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Positive</td>
<td>36</td>
<td>6.77 ± 4.95</td>
</tr>
<tr>
<td>Negative</td>
<td>36</td>
<td>7.04 ± 3.60</td>
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<tr>
<td><strong>GSTT1</strong></td>
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<tr>
<td>Positive</td>
<td>58</td>
<td>6.90 ± 4.48</td>
</tr>
<tr>
<td>Negative</td>
<td>14</td>
<td>6.94 ± 3.63</td>
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<tr>
<td><strong>NQO1 (codon 609)</strong></td>
<td></td>
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<tr>
<td>Pro/Pro</td>
<td>47</td>
<td>6.90 ± 4.45</td>
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<tr>
<td>Pro/Ser or Ser/Ser</td>
<td></td>
<td>6.92 ± 4.09</td>
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<tr>
<td><strong>XRCC1 (codon 194)</strong></td>
<td></td>
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<tr>
<td>Arg/Arg</td>
<td>63</td>
<td>6.68 ± 4.16</td>
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<tr>
<td>Arg/Trp or Trp/Trp</td>
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<td>7.21 ± 5.48</td>
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NOTE: WBC were incubated with 100 μmol/L sodium dichromate for 1 hour at 37°C. Data were analyzed by a one-way ANOVA and post hoc Scheffe test. No significant correlations were found. FPG-dependent oxidative damage was calculated by subtracting the tail DNA percent value obtained by the conventional comet assay from the tail DNA percent value obtained after inclusion of FPG.

Abbreviation: TD, tail DNA.
between antioxidant status and dichromate-induced DNA strand breaks, plasma antioxidant capacity was compared against levels of DNA strand breaks (measured using the FPG-modified comet assay). The mean plasma antioxidant capacity of the study population was 69.92 ± 10.50%. Age, ethnicity, gender, alcohol consumption (in the previous week), cigarettes smoked per day on average, vegetable and fruit consumption (average number of servings consumed per day), and meat and fish consumption (average number of servings consumed per week) were all analyzed as potential confounding factors. None of these parameters was found to have a statistically significant effect on plasma antioxidant status in the relatively small numbers of subjects in this study (data not shown). However, a statistically significant positive correlation between levels of FPG-dependent oxidative DNA damage and antioxidant capacity significant ($r = 0.253$, $P = 0.037$) was observed. When individuals were divided based on OGG1 genotype, wild-type and heterozygous individuals continued to display a positive trend ($r = 0.201$ and 0.403, respectively). In contrast, Cys$^{326}$ homozygote individuals displayed a negative trend ($r = -0.349$; Fig. 5A). When the data were analyzed as a ratio of oxidative DNA damage to plasma antioxidant capacity, Cys$^{326}$/Cys$^{326}$ individuals had a statistically significant ($P < 0.05$, one-way ANOVA followed by Tukey test) higher tail DNA percent/antioxidant capacity ratio than either heterozygous or wild-type individuals (Fig. 5B). There was no statistically significant difference ($P > 0.05$) between the Ser$^{326}$/Ser$^{326}$ and Ser$^{326}$/Cys$^{326}$ individuals (Fig. 5B).

**Discussion**

In the current study we observed 2.9- and 3.6-fold ranges in control levels of DNA strand breaks in WBC from the study population ($n = 72$), as measured by the conventional and FPG-modified comet assays, respectively. Previous studies have observed similar variable levels of DNA strand break in cells from human populations in the absence of any chemical insult (42–44). We have shown previously that intraindividual variability of DNA strand breaks over an 8- to 12-month period was of a relatively small coefficient of variation (24%) either with or without treatment of cells with sodium dichromate (34). After treatment with sodium dichromate (100 μmol/L; 1 hour) 3.1- and 3.9-fold ranges in levels of DNA strand breaks were observed as assessed by the conventional and FPG-modified comet assay, respectively. Possible causes of this variation include age, gender, diet, exercise, infection, sunlight, and smoking [reviewed by Møller et al. (45)] as well as genetic factors. Analysis using a general linear univariate ANOVA model showed that age, gender, ethnicity, alcohol consumption, vitamin intake, fruit, vegetable, meat and fish consumption, and smoking habit had no statistically significant effect on the measured DNA damage in either control or dichromate-induced DNA damage. In agreement with our findings, previous studies have also observed no significant association between these factors and levels of DNA damage (46–54).
Other potential sources of variability in response to dichromate treatment include interindividual differences in the intracellular reduction of chromium and the capacity to detoxify reactive intermediates. For example, large interindividual differences in the reduction of CrVI in plasma (55, 56) and blood (57) from human subjects and differences in chromium uptake in lymphoblastic cell lines derived from three different individuals (58) have been reported. In this study, potential differences in chromium uptake due to variability in reductive capacity of plasma will not contribute to the variability because in our experiments extracellular conditions were uniform. Variability could also be related to differences in DNA repair capacity. In support of this, Collins et al. (59) and Janssen et al. (60) observed a lack of repair activity on a DNA substrate containing 8-oxo-dG by a protein extract from homozygous OGG1 knockout mouse embryonic fibroblasts in a modified version of the comet assay. In contrast, mammalian cells overexpressing OGG1 repair 8-oxo-dG more rapidly after toxic insult with oxidants (69).

The human OGG1 gene is located on chromosome 3p25-p26 and allelic deletions of this region occur frequently in a variety of human cancers (70) and this has been associated with elevated 8-oxo-dG levels in lung tumors (40). Analysis of the OGG1 gene in normal and tumor tissues has revealed somatic mutations and polymorphisms, such as the polymorphism at codon 326 that results in a Ser→Cys amino acid substitution in the protein (71, 72). The latter has been associated with an increased risk of orolaryngeal (73), lung (74), and esophageal (75) cancers. There is evidence that the Cys<sup>326</sup> polymorphism has a functional effect. For example, OGG1 Cys<sup>326</sup> protein was shown to have approximately seven times lower activity in a complementation assay of an E. coli mutant defective in the repair of 8-oxo-dG (76). In addition, the catalytic efficiency of excision for 8-oxo-dG from -irradiated DNA by OGG1-Ser<sup>326</sup> protein is twice that of OGG1 Cys<sup>326</sup> (77). Very recently, others have showed that the Cys<sup>326</sup>/Cys<sup>326</sup> OGG1 genotype represents a repair deficient phenotype compared with wild-type (78). In contrast, another study has indicated no difference in enzymic activity between the Ser<sup>326</sup> and Cys<sup>326</sup> variants of OGG1 in human peripheral blood lymphocytes (60). Our study, as outlined below, suggests that deficiency of the Cys<sup>326</sup> variant is evident only under conditions of oxidative stress. We found no statistically significant difference in the mean levels of background DNA damage between wild-type individuals and individuals with either one or two copies of the Cys<sup>326</sup> allele, as measured by the conventional or FPG-modified comet assay, was observed. Moreover, after dichromate treatment (100 µmol/L; 1 hour), there was no significant difference in mean levels of DNA strand breaks between the three groups as assessed by the conventional comet assay. In contrast, individuals homozygous for the Cys<sup>326</sup> allele had statistically significantly higher levels of DNA strand breaks after FPG incubation than either wild-type (P = 0.008) or
heterozygous (\(P = 0.003\)) individuals. Furthermore, the same trend was also observed when the data were analyzed as levels of FPG-dependent oxidative damage (\(P = 0.02\) and \(P = 0.01\), respectively). Although this association has been shown to be statistically significant, we recognize that a relatively small number (\(n = 8\)) of Cys\(^{326}\)/Cys\(^{326}\) OGG1 genotype individuals have been studied and these individuals all had an antioxidant capacity above 70%. We recognize that further analyses in a larger population and a wider range of antioxidant capacities are required for confirmation of our findings. In support of our observations a similar correlation between 8-oxo-dG in leukocytes and OGG1 genotype has been made by Tarng et al. (79) in a group of patients undergoing hemodialysis.

These data suggest a deficiency in the repair of sodium dichromate–induced but not background oxidative DNA damage in Cys\(^{326}\)/Cys\(^{326}\) individuals. No such deficiency was evident in heterozygotes compared with wild-type individuals. This suggests that a single copy of the Ser\(^{326}\) allele may represent sufficient functional reserve to repair the nature and extent of dichromate-induced oxidative damage in a manner indistinguishable from wild-type individuals. We hypothesize that the Cys\(^{326}\) amino acid in variant OGG1 is susceptible to thiol modification under conditions of oxidative stress so as to reduce the functional capacity of the enzyme. This would not contradict the observations of Janssen et al. (60) who reported no difference in the enzymic activities of purified OGG1 proteins containing either Ser\(^{326}\) or Cys\(^{326}\), but did not investigate the effect of oxidative stress. Interestingly, in a similar way, a recent study (80) has reported that activity of the DNA repair protein XPA is modulated by redox modification to a zinc finger motif present in its active site. In addition, reduced repair capacity of variant OGG1 protein only under conditions of oxidative stress would explain why no difference between background levels of oxidative DNA damage was observed between the three groups. Interestingly, a reduction in activity of FPG (the functional homologue of OGG1 in Escherichia coli) has already been shown as a result of oxidation of cysteine residues in the zinc finger region (81). In addition, OGG1 has been shown to be regulated by postranslational phosphorylation of an unidentified serine residue apparently by protein kinase C resulting in translocation of OGG1 from the chromatin and nuclear matrix to the condensed chromatin during mitosis (82). Ser\(^{326}\) is not thought to be a constituent of a potential protein kinase C consensus sequence in OGG1. It is possible, however, that phosphorylation of Ser\(^{326}\) by another kinase may also postranslationally regulate OGG1. Substitution of Ser\(^{326}\) with a cysteine residue may therefore potentially alter OGG1 activity and/or cellular location either by thiol oxidation or by avoidance of phosphorylation.

As with levels of DNA damage, large interindividual variations in levels of plasma antioxidant capacity (mean, \(69.2 \pm 10.5\%\); range, 27.8-91.6%) were observed. The relationship between plasma antioxidant capacity and WBC intracellular redox status is complex. There is evidence of a positive correlation between intracellular and plasma (extracellular) levels of glutathione, ascorbate, and vitamin E (83-85). Furthermore, intracellular levels of both glutathione and ascorbate are negatively correlated with 8-oxo-dG levels in lymphocytes (86). In addition, plasma levels of antioxidants including ascorbate, \(\beta\)-carotenoids, lycopenes, and vitamin E are also negatively correlated with levels of DNA oxidation in human lymphocytes (87-90). Therefore, plasma antioxidant capacity is likely to be an indicator of WBC intracellular redox status and may be related to the ability to activate sodium dichromate within the cell by the reductive antioxidants GSH and ascorbate. In this study we observed a statistically significant (\(P = 0.037\)) positive correlation between sodium dichromate–induced FPG-dependent oxidative DNA damage and plasma antioxidant capacity. When the population was analyzed on the basis of OGG1 genotype, Ser\(^{326}\)/Ser\(^{326}\) and Ser\(^{326}\)/Cys\(^{326}\) individuals continued to display a positive correlation (\(r = 0.201\) and 0.403, respectively). In contrast, for Cys\(^{326}\)/Cys\(^{326}\) individuals a negative correlation (\(r = -0.349\)) was observed.

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**Figure 6.** Proposed relationship between OGG1 genotype, plasma antioxidant capacity, and levels of dichromate-induced oxidative DNA damage. See text for explanation and discussion.
Further analysis revealed that Cys<sup>326</sup>/Cys<sup>326</sup> individuals had a statistically significant (P < 0.05, one-way ANOVA followed by Tukey test) higher tail DNA percent/antioxidant capacity ratio than either heterozygous or wild-type individuals. These data suggest that Cys<sup>326</sup>/Cys<sup>326</sup> individuals have a functionally different relationship between plasma antioxidant capacity and sodium dichromate–induced oxidative DNA damage than either heterozygous or wild-type individuals. Despite the relatively low power of this study for the Cys<sup>326</sup>/Cys<sup>326</sup> genotype, the finding of a statistically significant difference between these and Ser<sup>326</sup>/ Ser<sup>326</sup> individuals emphasizes the biological importance of this polymorphism in the context of responsiveness to chromium-mediated oxidative DNA damage.

Although the basis of this difference remains to be determined, we hypothesize that in wild-type and heterozygous individuals the major determinant of DNA damage by dichromate is reductive activation by GSH (as shown in Fig. 6), which correlates with plasma antioxidant activity. In contrast, although THH still plays a role in sodium dichromate activation in Cys<sup>326</sup>/Cys<sup>326</sup> individuals, impaired DNA repair capacity of variant OGG1 protein under conditions of oxidative stress is proposed to become rate limiting through thiol oxidation and enzyme inactivation. Therefore, Cys<sup>326</sup>/Cys<sup>326</sup> individuals with a relatively low antioxidant (and GSH) capacity are expected to have higher levels of dichromate-induced DNA oxidation despite less activation because of lower cellular reductants. We propose that the reason why the codon 326 Cys residue in OGG1 impairs function is due to higher chances of thiol modification of this residue under conditions of high oxidative stress. As antioxidant levels increase, thiol modification becomes less prevalent and the Cys residue no longer impairs OGG1 function and therefore no difference is found between these two proteins in their ability to repair DNA damage (see Fig. 6).

In summary, the results of this study suggest that the Cys<sup>326</sup>/Cys<sup>326</sup> OGG1 genotype is responsible for a substantial proportion of the interindividual variation in response to dichromate-induced oxidative DNA damage. We hypothesize that this is a result of reduced repair activity of variant OGG1 protein (conferring by the Cys<sup>326</sup> residue) under conditions of cellular oxidative stress and that the Cys<sup>326</sup>/Cys<sup>326</sup> OGG1 genotype represents a phenotype that is deficient in the repair of sodium dichromate-induced oxidative DNA damage. Consequently, OGG1 genotype is a candidate biomarker of susceptibility for CrVI-induced lung cancer in occupationally exposed individuals.

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Interindividual Variability in Response to Sodium Dichromate–Induced Oxidative DNA Damage: Role of the Ser326Cys Polymorphism in the DNA-Repair Protein of 8-Oxo-7,8-Dihydro-2′'-Deoxyguanosine DNA Glycosylase 1

Amanda J. Lee, Nikolas J. Hodges and James K. Chipman


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