

RESEARCH COMMUNICATION

Biomonitoring of Genotoxic Effects Among Shielded Manual Metal Arc Welders

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Abstract

Hexavalent chromium Cr (VI) used in shielded metal arc welding is widely recognized to act as a carcinogen, mutagen and teratogen. The carcinogenic potential of metals is a major issue in defining human health risk from exposure. Hence in the present investigation, 66 welders and 60 control subjects with similar mean ages, smoking prevalences and alcohol consumption were enrolled for DNA damage analysis of buccal cells by micronucleus (MN) and comet assay. Welders showed a significant increase in micronucleated cells compared to controls and a larger mean comet tail length. The current study thus suggested that chronic occupational exposure to Cr (VI) during welding could lead to increased level of DNA damage. Understanding the complexity of the relationships between exposure, basal DNA damage and MN frequencies requires larger scale studies and application of complementary biomarkers.

Keywords: Hexavalent chromium - welding - genotoxicity - micronucleus - comet assay

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Introduction

Metals are stable and persistent environmental contaminants. Welding processes produce gaseous and aerosol by-products that are composed of a complex mixture of metal oxides (McNeilly et al., 2004). The heaviest metal exposure occurs in the workplace among occupationally exposed groups. Various hazardous agents, such as fumes, gases, vapors, heat, noise and radiation, are produced during welding operations. The agents of greatest concern in industrial hygiene are fumes, and specifically, hexavalent chromium Cr (VI), which is a human carcinogen (ACGIH, 2002). Exposure to welding fumes and other particles is a considerable hygiene problem in stainless steel welding and related operations (Hewitt, 1996).

The International Agency for Research on Cancer (IARC) classified welding fumes as possibly carcinogenic to humans (group 2B) (IARC, 1990). The biological effects associated with welding fumes exposure are diverse and depend upon metal interactions and speciation. The concentration and solubility of Cr (VI) depends on the type of welding (Ulfvarson, 1981) Several studies showed that the total chromium concentration in welding fumes was higher in gas metal arc welding (GMAW) than in shielded metal arc welding (SMAW) but Cr (VI) concentrations were higher in SMAW than in GMAW (Voitkevich, 1995).

Together with inhalation and oral penetration, dermal contact is also a possible route of exposure of humans to Cr (VI). Another way of exposure of the digestive

tract is swallowing of Cr (VI) refluxed from airways via the muco-ciliary escalator in individuals exposed by inhalation. The respiratory tract is the main target for Cr (VI) carcinogenesis in individuals performing certain occupational activities, which involve exposures to levels in air that were several orders of magnitude higher than those found in the natural environment (US Department of Health and Human Services, 1993).

Studies have shown that welding fumes from stainless steel welding are mutagenic (Hedenstedt et al., 1977; Maxild et al., 1978). Fumes from welding of stainless steel contain Cr (VI) (Knudsen et al., 1992) and chromium is absorbed by the lungs and distributed to all organs (Stridsklev et al., 1993). Cr (VI) has mutagenic capability in somatic cells and germ cells (WHO working group, 1988) as well as mammalian assay systems.

Early identification of hazards is crucial to reduce exposure and carcinogenic risk. Therefore, the aim of our study was to investigate the genotoxic effects associated with occupational exposure of welders by Cr (VI) by analyzing DNA damage, using the alkaline single cell gel electrophoresis (SCGE) assay, also known as the Comet assay. To substantiate our results and to provide a cytogenetic parameter, the MN test was also carried out. MNs are thought to be biomarkers either of exposure to environmental mutagens and/or carcinogens or of genome instability, as this test allows the detection of both clastogenic and aneugenic agents (Schmid, 1975) and it has been successfully adopted for exfoliated cells (Stich et al., 1982).

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Materials and Methods

Selection of subjects

A total of 126 male individuals (66 welders and 60 controls) were analyzed in this study. All of them were employed in welding plants located at Coimbatore, South India. The welders had varying duration of exposure (5-20 years) and they were in the age group 24-51 years. All the welders were engaged in shielded manual metal arc welding. Welders were working with consumable stainless steel electrodes usually containing 20% chromium. The experimental group and controls were further branched as smokers (34 vs. 30), non-smokers (32 vs. 30) respectively. The control groups were selected from the general population with no history of occupational exposure to welding fumes or any known physical or chemical agent in the workplace, but belonged to the same age group and socio-economic status as the welders.

The selection criteria for the subjects were based on a questionnaire according to the protocol published by the International Commission for Protection against Environmental Mutagens and Carcinogens (Carrano et al., 1988). The questionnaire covered standard demographic questions (age, genetic disorders, number of X-ray diagnoses, vaccinations, medication, smoking, alcohol, etc.) and occupational questions (years of exposure). We ensured that the welders and the controls did not markedly differ from each other except for occupational exposure. We also ensured that all the subjects had not been taking any medicines nor had they been exposed to any kind of radiation for 12 months before sampling.

The subjects who smoked >5 cigarettes/day at least for 1 year were considered as smokers and those who consumed >120gm of alcohol/day were considered as alcohol consumers in both groups. All subjects were informed of the objective of the study and gave their consent. The study was conducted in accordance with the principles for human experience as defined by the Helsinki Declaration.

Collection of specimens

Prior to buccal cell collection the exposed and control subjects were advised to rinse their mouth thoroughly with water to remove unwanted debris. Exfoliated buccal cells were obtained by gently rubbing the inside of both cheeks with an extra soft toothbrush for 1 min each. The participant then rinsed the mouth with 20 ml of 0.9% saline and expectorated into a 50 ml conical-based tube. The toothbrush was then rinsed in the tube and 30 ml saline was added before the cells were pelleted. The cells were washed with phosphate buffer saline (pH 7.4). The resulting cell suspension was divided into 2 parts; for Micronucleus (MN) assay and Comet assay.

Micronucleus assay: Cell suspension of 10 μ l was smeared on a microscopic slide and stained with 2% Giemsa (Sigma-Aldrich). Slides in triplicate were prepared for each subject. The micronuclei analysis was done using a light microscope, at \times 100 magnifications and with a \times 10 eye piece. Coded slides were used and 3000 cells from each individual were observed. The results were expressed as frequencies of micronucleated

cells in 100 cells. Only cells which were non-fragmented, non-accumulated, and non-overlaid and which had an untouched nucleus were examined.

Laboratory Analysis

Alkaline Comet Assay: The buccal cell suspension was centrifuged, the pellet obtained was mixed with 0.7% low melting agarose (LMA) and placed on fully frosted roughened slides previously coated with 1% normal melting point agarose. To the solidified agarose, a third layer of 0.1% LMA was applied and were immersed in freshly prepared ice cold lysis solution for 1 hour. The slides were then electrophoresed, neutralized, dried and stained with ethidium bromide.

A total of 100 randomly captured comets from each slide were examined at 400X magnification using an epifluorescence microscope (Zeiss) connected to an image analysis system. A computerized image analysis system acquires images, computes the integrated intensity profiles for each cell, estimates the comet cell components and then evaluates the range of derived parameters. To quantify the DNA damage, tail length (TL) was evaluated. Tail length (length of DNA migration) is related directly to the DNA fragment size and presented in micrometers which were calculated from the centre of the cell.

Statistical analysis

All statistical analyses were conducted using the SPSS for Windows statistical package, version 11.5 (IL, USA). Distribution of every variable obtained in this study did not depart significantly from normality and therefore parametric tests were considered adequate for the statistical analysis of these data. Samples were coded at the time of preparation and scoring. They were decoded before statistical analysis for comparison. Mean and standard deviation (SD) was calculated for each biomarker. The significance of the differences between controls and the welders end point means were analysed using students 't' test. Mean values and standard deviations were computed for the scores and the statistical significance ($P < 0.05$) of effects were determined.

Results

The demographic characteristics of the study subjects are presented in Table 1. The age, alcohol consumption and smoking status distributions were similar among exposed workers and controls. Among the smokers, the years of smoking and daily cigarette consumption were similar in the two groups.

The frequency of micronuclei (MN) was studied in 66 shielded manual metal arc welders and in 60 controls. Welders revealed a significant induction of MN when compared with controls. Individuals of the exposed as well as control groups with smoking habit and alcohol consumption showed an enhanced frequency of micronuclei when compared to non smokers and non alcoholics. Welders showed an increased MN frequency with an increase in duration of work ($P < 0.05$). A marginally significant correlation was observed between MN induction and duration of exposure (Table 2).

Table 1. Demographic Characteristics of Study Subjects

Characteristics		Sample Size (n=126)	Age (yrs)	Duration of Exposure (yrs)	No of Cigarettes per day	Frequency of Alcohol Consumption (gm/day)
Exposed (66)		66	37.3 ± 7.45	11.8 ± 4.65	-	-
Total	Yes	34 (51.5%)	35.7 ± 7.25	11.2 ± 4.38	14.8 ± 6.88	-
Smoking	No	32 (48.5 %)	39.0 ± 7.28	12.2 ± 4.79	-	-
Alcohol	Yes	29 (44.0 %)	38.5 ± 7.07	10.4 ± 4.61	-	233± 73.5
Consumption	No	37 (56.1%)	36.4 ± 7.61	12.9 ± 4.44	-	-
Controls (60)		60	38.7 ± 8.21	-	-	-
Total	Yes	30 (50.0%)	39.1 ± 8.85	-	15.7 ± 5.42	-
Smoking	No	30 (50.0%)	38.3 ± 7.64	-	-	-
Alcohol	Yes	28 (46.7%)	36.6 ± 7.48	-	-	274 ± 48.9
Consumption	No	32 (53.3%)	39.9 ± 8.03	-	-	-

Values are represented as mean ± SD

Table 2. Micronucleus Frequency and DNA Mean Tail Length with Respect to Smoking, Alcohol and Work Duration in Study Subjects

Characteristics	N=126	MN (Mean ± SD)	Mean Comet Tail length (µm)
Controls Smoking Yes	30	3.81 ± 1.29	11.3 ± 3.14
(n=60) Status No	30	2.10 ± 0.76	7.3 ± 2.05
Alcohol Yes	28	3.15 ± 0.88	9.9 ± 1.33
Status No	32	2.05 ± 0.05	9.0 ± 1.88
Workers Smoking Yes	34	5.70 ± 1.08*	20.4 ± 4.45*
(n=66) Status No	32	4.06 ± 0.93*	16.5 ± 3.84*
Alcohol Yes	29	5.26 ± 0.04*	15.2 ± 2.83*
Status No	37	4.94 ± 0.83*	14.6 ± 2.12*
Years of ≤10	35	4.39 ± 0.83	17.3 ± 1.78
Exposure >10	31	6.75 ± 1.20	19.8 ± 1.28*

Values are represented as mean ± SD, *P values (<0.05) as compared to controls

Basal DNA damage (BDD) was studied in a total of 126 subjects using the Comet assay. The results of DNA damage are given in Table 2. In workers a significant increase (P < 0.05) in DNA mean tail length indicating BDD was observed when compared with controls. In exposed group, a significant difference was observed between smokers and non-smokers and between alcohol drinkers and never drinkers in relation to DNA migration (P < 0.05). DNA damage was further found to be significantly higher in subjects with a longer duration of work (P < 0.05).

Discussion

Exposure to Cr (VI) has been known for more than a century to be associated with induction of cancer in humans. Carcinogenicity requires massive exposures and is only encountered in well defined occupational settings. The overall evaluation was that Cr (VI) is carcinogenic to humans (Group 1), that is a human carcinogen (IARC, 1990). Several biomonitoring studies have reported mechanisms of metals-induced genotoxicity, mutagenicity and carcinogenicity including direct DNA damage (O'Brien et al., 2003), induction of DNA-protein crosslinks (Costa et al., 1993), chromosome aberrations (Jelmert et al., 1995) and micronuclei (MN) (Danadevi et al., 2004) by welding fumes.

The present investigation reports that welders under their particular conditions of exposure revealed a clear evidence of genotoxicity in buccal epithelial cells when

evaluated by Comet and MN assay. Previous investigations reporting genotoxic effects in welders using the MN test in epithelial cells are scanty. Benova et al., (2002) found double the frequency of buccal MN in Cr platers.

Moreover, most occupations involved exposures not only to Cr (VI) compounds but also to other recognized carcinogens, such as other metals, organic compounds and complex mixtures. It is thus important to discriminate the effects of confounding factors such as cigarette smoke and alcohol on genotoxic endpoints. Smokers usually show higher urinary chromium levels than non-smokers (González et al., 1991). The results of present cytogenetic analyses clearly showed that the combined exposure to cigarette smoke and Cr (VI) enhances the frequency of micronuclei and an increase in basal DNA damage in buccal epithelial cells of welders which is in line with our previous biomonitoring studies (Sellappa et al; 2010 a,b,c).

Alcohol use can increase the number of micronuclei (Dittberner et al., 1997). Likewise in the present study a minor increase in MN frequency and Comet tail length was observed between welders and controls with drinking habit that may indicate the existence of an influence of alcohol use on the micronuclei formation. Conflictingly our previous work on peripheral blood lymphocytes of welders revealed significant increase of MN and Comet tail length among welders with alcohol habit (Sellappa et al., 2010d).

Our findings indicate that exposure to welding fumes induce genotoxic effect in buccal epithelial cells, indicating a potential health risk for shielded manual metal arc welding workers. Therefore, to ensure maximum occupational safety, biomonitoring is of great value for assessing the risk for welding workers. Since DNA damage and cellular death are considered to be prime mechanisms during chemical carcinogenesis, these data may be relevant in risk assessment for protecting human health and preventing carcinogenesis.

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