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Chromosome aberration and lipid peroxidation in chromium-exposed workers

S. H. MAENG[1], H. W. CHUNG[4], K. J. KIM[1], B. M. LEE[2], Y. C. SHIN[3], S. J. KIM[1] and I. J. YU[1,2]*

[1] Center for Occupational Toxicology, Occupational Safety & Health Research Institute, Korea Occupational Safety & Health Agency, Daejeon 305-380, Korea
[2] Division of Toxicology, College of Pharmacy, Sungkyunkwan University, Suwon 440-746, Korea
[3] School of Occupational Safety and System Engineering, Inje University, Kimhae 621-749, Korea
[4] Department of Molecular Epidemiology, School of Public Health, Seoul National University, Seoul 110-460, Korea

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Chromosome aberration frequency and lipid peroxidation levels were analysed to investigate their efficacy as biological markers for monitoring the genotoxicity and oxidative damage in Korean chromium (Cr)-exposed workers. Fifty-one Cr-exposed workers and 31 age-matched controls in ten chrome-plating plants were sampled. The Cr level was measured in the workers' blood and urine, and in the ambient air at the workplaces. The conventional Giemsa staining method and fluorescence *in situ* hybridization (FISH) technique were used for chromosome aberration analysis. Spectrum green whole chromosome paint specific for chromosome 4 was used in the FISH procedure. As for lipid peroxidation, malondialdehyde (MDA) was measured in the blood plasma as thiobarbituric acid-reactive substances (TBARS). The blood Cr concentration was statistically correlated with both the frequency of chromatid exchange and the total frequency of chromosome/chromatid breaks and exchanges, as detected by the Giemsa staining. Meanwhile, the frequency of translocation, as detected by the FISH technique, was significantly higher in the Cr-exposed workers than in the controls and it correlated with the blood Cr concentration. Although the concentration of MDA, the metabolite of lipid peroxidation, in the exposed workers was higher than that in the controls, no statistically significant correlation between the MDA level and the blood or urine Cr levels was observed. Accordingly, the genotoxicity and oxidative damage (plasma lipid peroxidation) in the Korean Cr-exposed workers were consequential at quite low exposure levels, plus chromosome rearrangement, especially translocation, was clearly evident as a biological response marker for Cr exposure based on a significant positive correlation between the translocations detected by FISH and the Cr in the blood.

Keywords: chromatid exchange, fluorescence in situ hybridization (FISH), malondialdehyde (MDA), translocation.

Introduction

Exposure to hexavalent chromium [Cr(VI)] compounds is of concern to industries involved in the primary production of chromate, Cr plating, chromate pigment manufacture and the welding of stainless steel, as occupational exposure to Cr(VI) has been strongly associated with an increased incidence of human lung cancer (International Agency for Research on Cancer 1982, 1990). Various biological monitoring methods have been employed to monitor the internal

* Corresponding author: Il Je Yu, Center for Occupational Toxicology, 104-8 Munji-dong, Yuseong-gu, Daejeon 305-380, Korea. Tel: 82 42 863 8182; Fax: 82 42 863 8361; e-mail: u1670916@chollian.net

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exposure of workers to Cr(VI) based on analysing body fluids, such as whole blood, plasma and urine (Kortenkamp 1997), while the early biological effects of Cr(VI) have been monitored using cytogenetic endpoints, such as chromosome aberrations and sister-chromatid exchanges. Until now, exposure to Cr(VI) at levels below 50 $\mu g\,m^{-3}$ has been reliably verified by determining the Cr concentrations in the urine or plasma (Kortenkamp 1997). However, currently available biological monitoring techniques used to assess the internal dose and genotoxicity of Cr(VI) have not been clearly validated.

Since the great majority of chemicals and metals are non-radiomimetic, they produce genetic alterations far more effectively in the S-phase of the cell cycle (Preston and Hoffmann 2002), which means they induce mostly chromatid-type aberrations. However, recent research suggests that Cr, like a radiomimetic, can induce typical dicentrics and translocations in human lymphocytes and Chinese hamster ovary cells *in vitro* (Chung *et al*. 1999), which means it can produce genetic alterations with a similar effectiveness in all stages of the cell cycle. Chromosomal rearrangements, such as translocation and dicentrics, are important predictors of cancer risks, especially when caused by radiation exposure. Therefore, the ease of scoring translocation with the fluorescence *in situ* hybridization (FISH) technique has resulted in the recent emergence of 'chromosome painting' as an effective biodosimetry for environmental cancer risk agents (Lucas 1997, Fauth and Zankl 1999, Snigiryova *et al*. 1997, Puerto *et al*. 1999). Furthermore, the use of FISH with whole chromosome painting would seem to be more sensitive than conventional methods for detecting chromosomal injury resulting from occupational exposure to carcinogens (Sram *et al*. 2004).

Some of the important factors involved in determining the biological outcome of Cr exposure include the bioavailability, chemical speciation and solubility of Cr compounds, along with the intracellular reduction and interaction of Cr with DNA and oxidative processes (Singh *et al*. 1998).

Lipid peroxidation, the oxidative catabolism of polyunsaturated fatty acid, is widely accepted as a general mechanism for cellular injury and death. These deleterious effects are initiated by free radicals produced from polyunsaturated fatty acid during peroxide formation. Lipid peroxidation has been implicated in diverse pathological conditions, including cancer (Bagchi *et al*. 1995). Several studies have reported increased indices of lipid peroxidation in laboratory animals exposed to toxic amounts of Cr compounds (Kasprzak 1995), while lipid peroxidation has also been reported in workers exposed to Cr(VI), lead and manganese (Huang *et al*. 1999).

Accordingly, the present study evaluated chromosome aberrations or rearrangements in the peripheral lymphocytes and plasma lipid peroxidation in the blood plasma of Cr-exposed workers for their utility as biological response markers for Cr exposure.

Materials and methods

Study subjects

The study population included 51 chrome-plating and buffing workers and a reference group of 31 controls from industrial areas located in the Kyungnam and Choong-cheong regions of South Korea.

The Cr-exposed workers had been engaged in electroplating and buffing for between 1 month and 40 years. Chromic acid was the primary constituent in the plating baths. The age-matched controls were selected from office workers in the same regions. Written consent to participate in the study was obtained from all participants. The subjects were interviewed by questionnaire to obtain data that included job classification, medical history, work duration and general lifestyle items such as smoking and alcohol intake.

The general characteristics of the study participants, classified by plant, are shown in table 1. The mean age of the Cr-exposed workers was 34.1 (22–64) years and the range of work duration was between 1 month and 40 years. Forty-two of 51 Cr-exposed workers (82.4%) were smokers, and all the workers except nine regularly drank alcohol. Meanwhile, the mean age of the control subjects was 35.2 years, 18 (58.1%) were smokers and 28 (excluding three) regularly drank alcohol. All subjects were male. According to the questions on job classification and medical history, none of the participants had a history of liver disorder, renal dysfunction, heart disease, diabetes mellitus or cancerous diseases, and all were involved in plating, buffing and other tasks such as cleaning, packing and carrying work-pieces.

Sample collection

For air monitoring, the total Cr and Cr(VI) personal air samplers equipped with both a PVC and mixed cellulose ester membrane were used with a flow rate of 2–3 l min^{-1}. Blood was collected from the subjects into either a heparinized vacutainer for a chromosome aberration analysis, or an ethylene diamine tetra acetic acid (EDTA)-coated vacutainer to measure the malondialdehyde (MDA) level and Cr concentration. Spot urine specimens were collected immediately following the end of a work shift according to the American Conference of Governmental Industrial Hygienists' (2003) recommendations, and stored at $-20^{\circ}C$ until analysed for Cr.

Chromium in air, whole blood and urine

Analysis of total Cr and Cr(VI) was performed using the method of Shin and Paik (2000). The total Cr amount was analysed using a flame atomic absorption spectrophotometer (AAS, Model 300 Plus), while a flameless AAS Model 400 (both Varian Corp., Mulgrave, Australia) was used to determine low levels of total Cr. The Cr(VI) in the PVC filter was extracted using the method recommended by the National Institute for Occupational Safety and Health (1994). The Cr(VI) in the extraction solution was then separated and analysed using ion chromatography (Dionex ED-40) with an IonPac AS7 column (both Dionex Co., California, USA). The Cr in the whole blood and urine was analysed using the method of Nomiyama *et al*. (1980). After proper pretreatment of each sample, the Cr in the blood and urine was measured using a graphite furnace atomic absorption spectrophotometer (Analysis 100, Perkin-Elmer model, Conneticut, USA) at a wavelength of 357.9 nm. The creatinine concentrations were determined with an automated method based on the Jaffe reaction using an automatic analyser (Olympus AU400, Tokyo, Japan) to adjust for fluctuations in the urinary volume.

Chromosome preparation and staining

A total of 0.5 ml whole blood were added to 4.5 ml culture medium (RPMI 1640) containing foetal bovine serum (10%), L-glutamine (1.65 mM), antibiotics and phytohaemagglutinin (0.2 mg ml; all Gibco, New York, USA). The cultures were incubated for 50 h at $37^{\circ}C$ in a 5% carbon dioxide atmosphere following 2 h of colcemid (0.01 μg ml) treatment. The cells were fixed with Carnoy's fixatives, then air-dried preparations were made following standard procedures. All slides were coded and scored blind. Some of the slides were stained with a 4% Giemsa solution for 5 min and observed by light microscopy. The FISH procedure was conducted using Spectrum Green Whole Chromosome Paints (Vysis, Illinois, USA) specific for chromosome 4. A single whole chromosome-specific probe was selected for the FISH analysis as it considers the DNA contents in the whole genome, detects at least 10% of all exchanges and is cost effective. Plus, chromosome 4 was selected for the FISH analysis as the DNA content of chromosome 4 in the whole genome has been reported as 6.23% and it represents 11.7% of all exchanges that occur in cells (Mendelssohn *et al*. 1973). The hybridization procedure was carried out essentially according to the manufacturer's instructions. Thereafter, the unbound probe was washed off and the slides counterstained with propidium iodide (PI; Vysis). Scoring was performed using a fluorescence microscope equipped with a BA-2 filter (NIKON, Optiphot II, Tokyo, Japan). The nomenclature and conversion of metaphases were scored as whole-genome equivalents in the FISH according to the 'PAINT' (Protocol Aberration Identification and Nomenclature Terminology) system of Tucker *et al*. (1995). In FISH, only a fraction of all possible aberration events is scored because the analysis of the painted metaphases involves the identification of cells containing bicolour chromosomes. According to Tucker *et al*. (1995), the fraction of all translocations detected by hybridization (Fh) is determined from the amount of the genome that is painted, then compared with the fraction observed by G-banding (Fb) using the equation $Fh = 2Fs(1-Fs)Fb$, where Fs is the proportion of the painted genome. Two researchers were involved in observing the chromosome sample slides, and each scorer

Table 1. General characteristics and MDA levels of study population, plus chromium concentrations in environmental and biological media.

						Geometric mean (ranges)			
Plants	n[a]	Number of smokers (%)[b]	Age (years ± SD)[c]	Work duration (months) (range)	MDA ($\mu mol\ l^{-1}$)	Total Cr ($\mu g\ m^{-3}$)	Cr (VI) ($\mu g\ m^{-3}$)	Cr in blood ($\mu g\ dl^{-1}$)	Cr in urine ($\mu g\ g^{-1}$ creatinine)
Control	31	18 (58.1)	35.2 ± 12.7	0	1.63 ± 1.44	0.058 (0.034–0.101)	0.035 (0.014–0.061)	0.17 (0.00–0.67)	3.39 (0.40–9.04)
A	6	6	37.2 ± 12.3	84.3 (5–228)	1.60 ± 1.38	40.20 (11.86–97.16)	26.32 (10.0–90.17)	3.99 (1.69–8.90)	20.08 (12.32–34.38)
B	8	6	40.0 ± 9.1	90.9 (1–252)	2.68 ± 1.58*	6.02 (0.95–29.28)	2.14 (0.27–7.32)	1.47 (0.26–8.99)	18.47 (10.10–31.39)
C	5	4	47.4	240.0 (12–480)	2.08 ± 1.10	60.08 (51.34–92.53)	45.36 (33.3–57.65)	1.06 (0.56–3.44)	20.93 (14.82–41.24)
D	1	1	27.0	24.0	2.04	11.07	2.15	0.73	16.2
E	2	1	39.0 ± 5.7	93.5 (48–139)	3.36 ± 1.17*	6.69 (6.09–7.35)	2.56 (2.08–3.14)	1.15 (0.36–1.19)	14.72 (9.71–22.31)
F	3	3	22.7 ± 3.2	12.0 (6–15)	3.29 ± 1.15*	1.71 (1.28–2.07)	1.07 (0.34–1.2)	0.37 (0.18–1.54)	18.74 (15.00–23.05)
G	6	4	36.2 ± 5.9	67.3 (1–252)	2.11 ± 1.15	1.47 (0.92–2.54)	0.84 (0.48–1.7)	0.47 (0.11–0.30)	9.61 (3.74–17.14)
H	8	6	42.0 ± 6.8	146.0 (60–360)	1.70 ± 1.15	85.01 (12.73–23.74)	6.14 (2.39–18.91)	0.56 (0.16–2.21)	14.67 (8.77–25.94)
I	4	3	42.3 ± 6.3	224.0 (144–372)	1.95 ± 1.29	4.08 (0.92–10.36)	1.07 (0.5–3.38)	0.26 (0.13–0.50)	3.36 (1.17–10.02)
J	8	8	29.6 ± 5.8	60.1 (1–125)	1.75 ± 1.20	26.43 (16.51–70.85)	1.89 (0.28–6.27)	0.80 (0.31–2.43)	7.21 (1.31–8.77)
All workers	51	42 (82.4)	34.1 ± 13.4	109.0 (1–480)	2.06 ± 1.38**	11.55 (0.28–523.74)	3.20 (0.27–90.17)	0.86** (0.11–8.99)	12.82** (0.66–8.74)

[a]Number of study population; [b]number of smokers includes daily smokers and subjects who stopped smoking less than 1 year before sampling; [c]ages are mean ± SD; *$p < 0.05$, multiple comparison versus the control (Dunn's method); **$p < 0.01$, Mann–Whitney rank sum test.

simultaneously observed duplicate sample slides. The two sets of data from the two scorers were pooled before statistical analysis.

Assay for malondialdehyde (MDA)

The level of MDA, an end-product of lipid peroxidation, in the blood plasma of the subjects was determined according to the TBARS (thiobarbituric acid-reactive substances) using the method of Erdincler *et al*. (1997). One sample volume was mixed thoroughly with 2 vols of a stock solution of 15% w/v trichloroacetic acid, 0.375% w/v thiobarbituric acid and 0.25 mol l^{-1} hydrochloric acid. The combined sample and stock solution was then heated for 30 min in a boiling water bath. After cooling, the flocculent precipitate was removed by centrifugation at 1000 *g* for 10 min. The absorbance of the sample was then determined at 535 nm and the TBARS concentration calculated using 1.56×10^5 M^{-1} cm^{-1} as the molar absorption coefficient.

Statistical analysis

All data was analysed using SPSS 10.0 to determine the mean (Student's *t*- or Mann–Whitney rank sum test), variance (ANOVA, multiple regression), and Kendall's τ for a statistical evaluation of the mean difference and relationship among the variables. Pearson's product moments were also analysed for any correlation between the exposure variables. The criterion for significance was $p < 0.05$.

Results

Cr concentration in air, blood and urine

The total Cr and Cr(VI) concentrations in the air at each plant are shown in table 1 and figure 1, and patterned as log-normal distributions in figure 2. The geometric means for the total Cr and Cr(VI) at all the plants were 11.55 μg m^{-3} (0.28–523.74 μg m^{-3}) and 3.20 μg m^{-3} (0.27–90.17 μg m^{-3}), respectively. One among the ten plants had an average concentration of Cr(VI) that exceeded the TLV (50 μg m^{-3}) of the ACGIH (2003) and Korean occupational exposure level

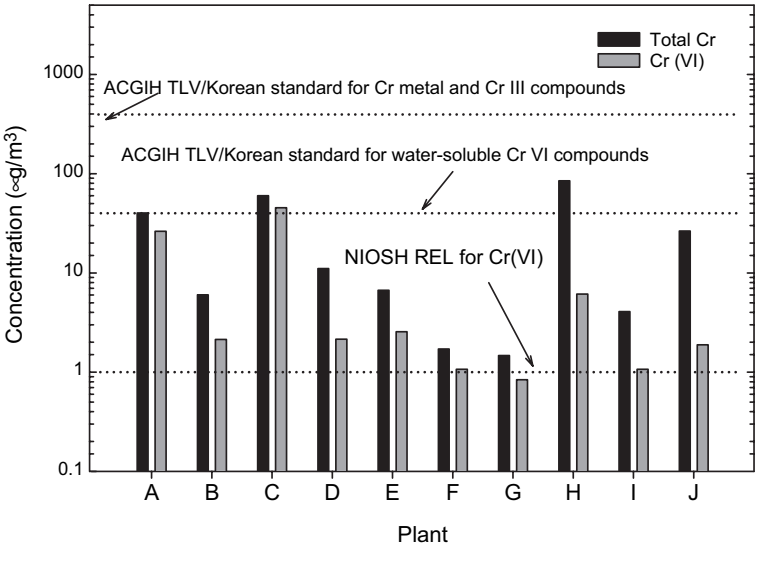

Figure 1. Average total chromium (Cr) and hexavalent chromium (Cr(VI)) concentrations in personal air samples according to plant. Cr III, trivalent chromium; ACGIH TLV, threshold limit (value) from the American Conference of Governmental Industrial Hygienists; NIOSH REL, exposure level recommended by the National Institute for Occupational Safety and Health.

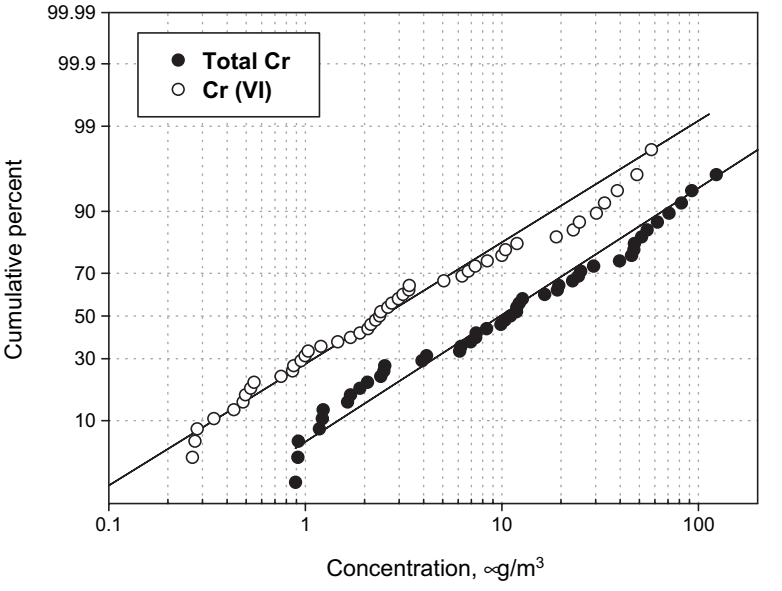

Figure 2. Distribution of total Cr and Cr(VI) concentrations in personal air samples.

(OEL), a standard of the Korean Ministry of Labor (1998), while the concentrations at eight plants exceeded the recommended value of 1 μg m^{-3} established by the National Institute for Occupational Safety and Health (1975).

The average Cr levels in the blood and urine from all the workers were 0.86 μg dl^{-1} (0.11–8.99 μg dl^{-1}) and 12.82 μg g^{-1} creatinine, respectively, both of which were significantly different when compared with the control values (0.17 and 3.39 μg g^{-1} creatinine, $p < 0.01$), plus the average concentration in the urine exceeded the biologic exposure index (BEI, 10 μg g^{-1} creatinine) of the ACGIH (table 1).

Chromosome aberration by Giemsa staining

Table 2 shows the frequency of chromosome aberration classified according to subjects' jobs. The mean frequency of chromosome aberration detected by conventional Giemsa staining was higher among all the Cr-exposed workers (1.00%, total frequency of aberration including gap; 0.72% excluding gap) than in the controls (0.64%, including gap, 0.36%, excluding gap), yet without statistical significance. The main types of aberration in the Cr-exposed workers were chromosome-type exchanges and chromatid-type breaks.

The frequency of chromosome aberration was somewhat higher in the smokers than in non-smokers in the control group as well as the exposed group, yet there was no statistical significance (table 3).

Despite a very low frequency of chromatid exchanges, the blood Cr concentration was statistically correlated with the chromatid-type exchanges ($p = 0.008$), and the sum of all types of aberration ($p = 0.037$) correlated with the blood Cr concentration based on a multiple regression analysis, where the effect of the smoking variable (number of cigarettes/day × sum of days smoking) was excluded.

Table 2. Frequency of structural chromosome aberration based on Giemsa staining according to plant.

			Structural aberrations (%)					Total	
				Chromatid type		Chromosome type			
Plant/group	n[a]	Number of cells observed	Gap	Break	Exchange	Break	Exchange	−Gap	+Gap
Control	31	3751	0.28	0.14	0.11	0.03	0.11	0.36	0.64
A	6	546	0.55	0.00	0.37	0.00	0.18	0.55	1.10
B	8	779	0.26	0.39	0.39	0.00	0.26	1.03	1.28
C	5	496	0.20	0.61	0.00	0.00	0.81	1.41	1.61
D	1	100	1.00	1.00	0.00	1.00	0.00	4.00	6.00
E	2	100	0.00	0.00	0.00	0.00	0.00	0.00	0.00
F	3	300	0.33	0.33	0.00	0.00	0.00	0.33	0.99
G	6	600	0.33	0.00	0.00	0.00	0.00	0.00	0.33
H	8	800	0.25	0.13	0.13	0.13	0.25	0.63	0.88
I	4	400	0.00	0.25	0.00	0.00	0.25	0.50	0.75
J	8	800	0.38	0.25	0.13	0.25	0.38	1.00	1.38
All exposed	51	5021	0.30	0.24	0.14	0.08	0.26	0.72	1.00

[a]Number studied.

Chromosome aberration by FISH

In the present study, multiple complicated chromosome rearrangements were found, particularly in the Cr-exposed workers. Although non-reciprocal translocations (figure 3) are easy to score, some exchanges are not easy to classify as symmetrical or asymmetrical. Therefore, the PAINT system was applied to describe and classify the chromosome rearrangements, and all types of translocation frequency were pooled (table 4). The frequency of chromosome rearrangement at workplace A was comparably high and the total frequency was statistically higher than that in the controls. There was little difference among the other workplaces studied. However, the numbers of translocation, insertion and acentric fragments per cell equivalent were statistically higher in all the exposed workers when compared with the controls. The frequency of aberrant cells in the exposed samples was also increased. When considering the smoking variable (table 5), the frequency of chromosome rearrangement and aberrant cells was generally higher in the smokers than in the non-smokers in both the exposed subjects and the controls.

Table 3. Frequency of Giemsa-stained chromosome aberration in smokers and non-smokers among study subjects.

				Structural aberrations (%)					Total	
					Chromatid type		Chromosome type			
Group		n[a]	Number of cells observed	Gap	Break	Exchange	Break	Exchange	−Gap	+Gap
Control	smoker	18	2173	0.23	0.09	0.14	0.05	0.14	0.41	0.64
	non-smoker	13	1578	0.32	0.19	0.06	0.00	0.06	0.32	0.63
Exposed	smoker	42	4143	0.3	0.22	0.14	0.10	0.19	0.65	0.84
	non-smoker	9	878	0.23	0.34	0.11	0.00	0.57	1.03	1.37

[a]Number studied.

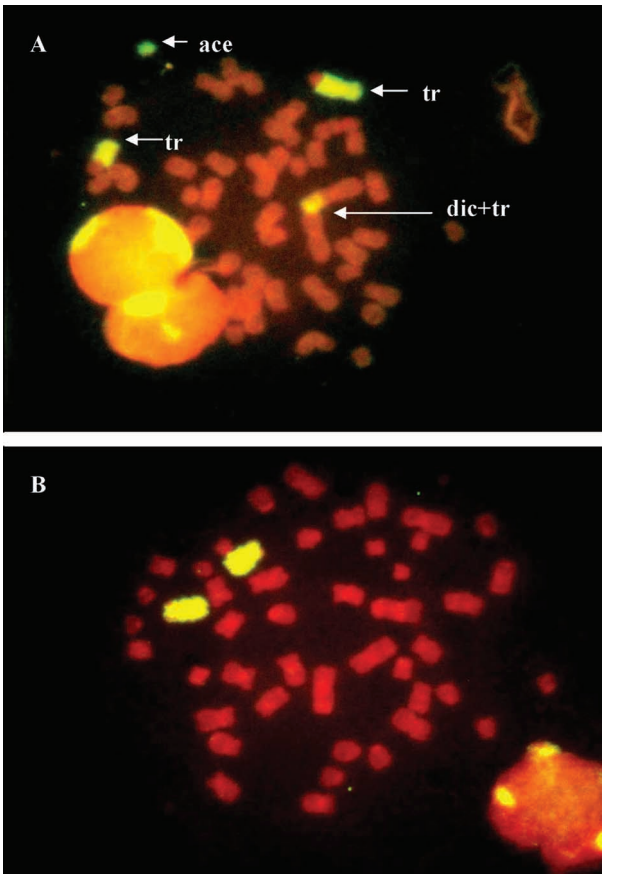

Figure 3. Chromosome aberrations (A) identified by FISH with a DNA probe for chromosome 4 in chromium-exposed workers compared with normal chromosome. (B) Ace, acentric fragment; tr, translocation, dic+tr, dicentric plus translocation.

Yet this tendency was marked among the exposed subjects, where the frequency of translocation and total chromosome rearrangement was significantly higher in the smokers among the exposed subjects than in the non-smokers ($p < 0.05$), while smoking had little effect on translocation in the control group.

Translocation was correlated with the blood Cr concentration ($p = 0.001$), and the sum of all types of aberration and colour junction correlated with the blood Cr ($p = 0.001$) and Cr(VI) ($p = 0.025$) when excluding the effects of confounders, smoking (number of cigarettes/day $\times$ sum of days smoking), and the duration of work.

Malondialdehyde levels

The concentration of malondialdehyde (MDA) in the blood plasma from the workers and controls is shown in table 1. The mean MDA concentration for all the exposed workers (2.06 $\mu mol\, l^{-1}$) was significantly higher ($p < 0.01$) than that for the controls (1.63 $\mu mol\, l^{-1}$). The correlations between the MDA levels and the total Cr in the air, Cr (VI) in the air, Cr in the blood and urine, and other general

Table 4. Chromosome rearrangement identified by FISH probe for chromosome 4.

					Number of aberrants observed (number per cell equivalent)						
Group	n[a]	Number of cells scored	Number of cell equivalents	Number of aberrant cells (%)	Translocation	Dicentric	Insertion	Acentric fragment	Ring	Total[b]	Colour junction
Controls	31	30524	3571.31	16 (0.05)	18 (0.00504)	2 (0.00056)	3 (0.00084)	3 (0.00084)	0	26 (0.00728)	22 (0.00616)
A	6	6508	761.44	32 (0.49)	39 (0.05122)	2 (0.00263)	12 (0.01576)	11 (0.01445)*	0	64 (0.08405)*	69 (0.09062)*
B	8	7671	897.51	21 (0.27)	18 (0.02006)	0	8 (0.00891)	8 (0.00891)	0	34 (0.03788)	34 (0.03788)
C	5	5094	596.00	20 (0.39)	26 (0.04362)	3 (0.00503)	2 (0.00336)	5 (0.00839)	1 (0.00168)	36 (0.06040)	35 (0.05873)
D	1	1369	160.17	7 (0.51)	4 (0.02497)	2 (0.01249)	2 (0.01249)	1 (0.00624)	0	10 (0.06243)	11 (0.06868)
E	2	1530	179.01	4 (0.26)	2 (0.01117)	0	0	1 (0.00559)	0	5 (0.02793)	6 (0.03352)
F	3	3823	447.29	16 (0.42)	13 (0.02906)	2 (0.00447)	5 (0.0112)	2 (0.00447)	0	22 (0.04918)	25 (0.05589)
G	6	5295	619.52	17 (0.32)	14 (0.02260)	1 (0.00161)	2 (0.00323)	9 (0.01453)	0	26 (0.04197)	21 (0.03390)
H	8	7778	910.03	30 (0.39)	38 (0.04176)	1 (0.00110)	6 (0.00659)	7 (0.00769)	0	52 (0.05714)	51 (0.05604)
I	4	3824	447.41	13 (0.34)	17 (0.03800)	0	3 (0.00671)	4 (0.00894)	0	24 (0.05364)	23 (0.05141)
J	8	6598	771.97	24 (0.36)	28 (0.03627)	2 (0.00259)	5 (0.00648)	6 (0.00777)	1 (0.00130)	42 (0.05441)	42 (0.05441)
All exposed	51	49490	5790.33	184 (0.37)**	199 (0.03437)**	13 (0.00225)	47 (0.00812)**	54 (0.00933)**	2 (0.00035)	315 (0.05440)**	317 (0.05475)**

[a]Number studied. *$p < 0.05$; multiple comparisons versus the control (Dunn's method); **$p < 0.01$, controls versus all exposed, Mann–Whitney rank sum test.
[b]Total of the preceding five columns, from 'translocation' to 'ring'.

Table 5. Chromosome rearrangement identified by FISH probe for chromosome 4 with smoking variable.

Group		n[a]	Number of cells scored	Number of cell equivalents	Number of aberrant cells (%)	Number of aberrants observed (number per cell equivalents)						
						Translocation	Dicentric	Insertion	Acentric fragment	Ring	Total	Colour junction
Controls	smoker	18	17460	2042.82	10*** (0.06)	11 (0.00538)	2 (0.00098)	2 (0.00098)	3 (0.00147)	0	18 (0.00881)	17 (0.00832)
	non-smoker	13	13064	1528.49	6 (0.05)	7 (0.00458)	0	1 (0.00065)	0	0	8 (0.00523)	9 (0.00589)
Exposed	smoker	42	40778	4771.03	165**** (0.40)	179** (0.03752)	12 (0.00252)	40 (0.00838)	49 (0.01027)	2 (0.00042)	282** (0.05911)	282 (0.05911)
	non-smoker	9	8712	1019.30	19 (0.22)	20 (0.01962)	1 (0.00098)	7 (0.00687)	5 (0.00491)	0	33 (0.03238)	35 (0.003434)

[a]Number studied; *p <0.01, All control versus all exposed, Mann–Whitney rank sum test; **p <0.05, smokers versus non-smokers, Mann–Whitney rank sum test, ***p <0.01, smokers versus non-smokers, Mann–Whitney rank sum test; ****p <0.01, smoker versus non-smokers, Student's t-test.

[b]Total of the preceding five columns, from 'translocation' to 'ring'.

variables were analysed by multiple regression, yet the MDA level in the blood plasma was not statistically correlated with any other variables.

Discussion

The total Cr and Cr(VI) concentrations in the personal air samples both exhibited log-normal distributions, even though the fraction of Cr(VI) in the total Cr level varied between 10 and 80% according to the plant. Although no correlation was confirmed between the total Cr level and other internal dose marker levels, the Cr(VI) level in the air was correlated with the whole blood Cr level ($r^2 = 0.42$, $p < 0.01$) as well as the urinary Cr level ($r^2 = 0.51$, $p < 0.01$) when the data from all the exposed subjects were pooled in a statistical analysis (Pearson's correlation), plus a correlation was also found between the urinary and blood Cr levels ($r^2 = 0.35$, $p < 0.05$).

Moreover, while the chromatid-type break frequency and total aberration frequency were correlated with the blood Cr level, this was limited as an index of Cr exposure owing to the extremely low frequency in this study population. The effect of smoking on the structural chromosome aberrations was not statistically significant.

According to PAINT nomenclature, the whole chromosome painting of human chromosome 4 using the FISH method could effectively detect certain types of damage (e.g. translocation and insertion) resulting from Cr exposure, which were not otherwise discernible with conventional Giemsa staining methods. As such, the data presented here show a statistical increase in translocation, insertion and acentric fragments per cell in the exposed workers. The effect of smoking on chromosome rearrangement was also significant in the Cr-exposed subjects, while not in the controls, suggesting that smoking has some kind of additive effect to Cr exposure on the induction of chromosome rearrangement. This result is in line with Wu *et al*. (2000), who observed similar synergistic effects using a different cytogenetic endpoint, sister chromatid exchanges, in Cr workers. However, no such effect was found in Danadevi *et al*. (2004) at the level of DNA damage when assessed by a comet assay, or by Zhitkovitch *et al*. (1996) at the level of DNA–protein cross-links. These contrasting results might have been because the DNA single-strand breaks, alkali-labile sites and cross-links detected by a comet assay are somewhat different parameters reflecting chromosome rearrangement than sister chromatid exchanges or translocation. In addition, the present study population based on smoking pack years was smaller than that used by Danadevi *et al*. (2004) to demonstrate a relationship between smoking pack years and chromosome rearrangement. However, when using a multivariate regression statistically to control for possible confounders, such as smoking and work duration, the frequency of translocation was significantly correlated with the blood Cr level, indicating that 'translocation' can be used as a biological marker for Cr exposure from the environment.

Although urinary Cr can be regarded as a reliable marker of internal Cr exposure and is sufficiently sensitive for biological monitoring at exposure levels well below occupational limits, only Cr in erythrocytes is diagnostic for internal

exposure to Cr(VI), because the chromate anion, unlike Cr(III) complexes, can cross the cell membranes of red blood cells via anion carrier proteins (Kortenkamp 1997). However, in a previous study among stainless-steel welders, airborne Cr (VI) at levels of around 100 $\mu g\ m^{-3}$ did not give rise to elevated concentrations in the red blood cells of about 50% of the exposed workers (Angerer *et al*. 1987). The correlations between urinary and plasma Cr levels are excellent, so that one monitoring method can be conveniently substituted for the other (Angerer *et al*. 1987, Stridsklev *et al*. 1993). Whereas urinary Cr concentrations reflect the absorption of Cr primarily over the last 1–2 days, intracellular Cr concentrations reflect the burden of Cr(VI), because only Cr(VI) penetrates red blood cells (Barceloux 1999). Plasma Cr levels also reflect recent exposure to both Cr(VI) and trivalent Cr (Cr(III)). Cr studies in patients with acute dichromate (VI) poisoning found that the concentration of Cr(VI) in the erythrocytes exceeded the plasma Cr concentration by four- to six-fold, implying that the whole blood Cr was approximately two to three times higher than the plasma Cr (Wood *et al*. 1990). The toxicokinetic properties, such as solubility and adsorption, of various Cr compounds are different. In most cases, hexavalent compounds are more easily absorbed though cells and tissues compared with trivalent compounds, in part because of the relatively greater ease with which hexavalent Cr compounds traverse biological membranes (Barceloux 1999). In the present study, only the whole blood Cr level was useful in monitoring the genotoxic effects in Cr(VI)-exposed workers, although correlations were found between the urinary and blood Cr levels. Thus, the whole blood Cr levels provided a more accurate reflection of the Cr(VI) exposure that induced genotoxic effects than the urinary Cr level, as expected.

For chromosome 4, which comprises 6.23% of the genome, 11.7% of all exchanges are detectable. Thus, the total number of metaphases scored for each dose is multiplied by the appropriate fraction, 11.7, to yield the number of metaphase-equivalents, which is the number of cells necessary to be scored by G-banding to yield the corresponding amount of information (Mendelssohn *et al*. 1973).

In the present study, the ratio of the translocation frequency to the dicentric frequency was about 9:1. Theoretically, this ratio of chromosome exchange should be 1:1 (Finnon *et al*. 1995), yet the translocation frequency induced by radiation and radiomimetic chemicals, such as bleomycin, has been reported as three or four times higher than the dicentric frequency (Tucker *et al*. 1993, Natarajan *et al*. 1994). In an *in vitro* study, about 15% of cells carrying chromatid exchanges would be expected to produce descendents with translocation (Marshall and Obe 1998). No mechanism is yet known that favours a symmetrical rearrangement over an asymmetrical one between two broken chromosomes. Natarajan *et al*. (1992) reported that inhibitors of DNA repair had a different effect on the yield of dicentrics than on the yield of translocations, indicating that the mispair process leading to dicentrics and translocations may be different. In the present study, the higher frequency of translocations than dicentrics in the Cr-exposed workers may have been related to the fact that the translocations were based on both chromatid- and chromosome-type exchanges, which are detectable by conventional chromosome analysis, and the failure of the FISH method to detect dicentrics.

Despite many previous reports about the genotoxic effects of Cr(VI) compounds, their action mechanism has not been clarified and is assumed to be complex (Vaglenov *et al*. 1999, De Flora 2000). Several authors have also reported on the clastogenic and aneugenic activity of Cr compounds (De Flora *et al*. 1990, Jelmet *et al*. 1992, Knudsen *et al*. 1992, Vaglenov *et al*. 1999). A cytokinesis-block micronucleus assay (MN) is considered as an efficient tool for assessing both chromosome breaks and whole chromosome loss (Fenech *et al*. 1994), while the FISH method using a DNA pancentromeric probe facilitates the study of clastogenic or aneugenic events in peripheral lymphocytes and buccal cells (Surralles *et al*. 1997, Benova *et al*. 2002). Benova *et al*. suggested that at least part of the MN in Cr(VI)-exposed workers originated from excision-repairable DNA lesions when they studied the influence of AraC, an inhibitor of excision-repair, to detect whether the MN in the lymphocytes of Cr-exposed workers originated from chromosome or chromatid breaks. In addition, Koshi *et al*. (1984) and Knudsen *et al*. (1992) observed a statistically significant increase of dicentrics, translocations, minutes and rings among manual metal arc stainless-steel welders. Thus, Cr(VI) is recognized as one of the major hazardous materials in stainless-steel welding. The FISH method using a whole chromosome probe, namely 'chromosome painting', has already been used as a form of biodosimetry for radiation exposure, since increasing levels of translocation and dicentrics have been measured in radiation-exposed population as compared with controls (Lucas 1997, Snigiryova *et al*. 1997, Fauth and Zankl 1999, Puerto *et al*. 1999). Furthermore, from the current findings, the FISH method using a whole chromosome probe can also be an effective tool for assessing chromosome rearrangement, like translocations in a Cr(VI)-exposed population, as Cr(VI) has both non-radiomimetic and radiomimetic (namely chemical and physical) properties that can produce genetic alteration. Kortenkamp (1997) suggested that given the toxicokinetics of inhaled Cr(VI) and dynamics of lymphocyte traffic, blood lymphocytes are inappropriate for monitoring the biologically effective dose and early biologic effects resulting from exposure to Cr(VI) at levels below 50 $\mu g\, m^{-3}$, the current exposure limit in many industrialized countries, and at higher levels. However, effect monitoring using lymphocytes may well be useful, and certainly the current results indicated that a chromosome rearrangement analysis of blood lymphocytes using FISH was useful for monitoring the early biological effects arising from Cr(VI) exposure at quite low exposure levels.

Bagchi *et al*. (1997) reported on the induction of lipid peroxidation, which has been implicated in diverse pathological conditions including cancer (Halliwell and Gutteridge 1984), and single-strand breakage based on the chronic administration of sodium dichromate Cr(VI) to rats. The possible role of oxidative damage in metal-induced carcinogenesis has also been reported (Kasprzak 1995). Several data suggest a correlation between oxidative-induced promutagenic DNA alterations and the presence of nickel and Cr compounds, and Cr-related cell damage has been implicated (Zhitkovitch *et al*. 1998). Yet, negative results have been reported from the same assays on human volunteers and experimental animals (Kuykendall *et al*. 1996, Mirsalis *et al*. 1996). Huang *et al*. (1999) suggested evidence of an index of oxidative damage from Cr exposure based on significant positive correlations

between MDA and Cr in both the blood and urine. Meanwhile, Elis *et al*. (2001) reported no significant difference between the control and exposed groups with regard to lipid peroxidation, plasma lipid susceptibility to oxidation, the total plasma antioxidant status, and serum paraoxonase. Therefore, the question still remains as to whether humans exposed to Cr(VI) in the workplace are exposed to levels high enough to acquire and maintain oxidative damage, thereby leading to an increased risk of cancer. In the present study, despite the statistical increase in the MDA level in the exposed group compared with the control group, no correlations between MDA and Cr were observed in either the blood or the urine sampled during working hours. Even 35 workers with urinary Cr levels above the biologic exposure index limit ($>10.0\ \mu g\ g^{-1}$ creatinine) exhibited no correlation between their MDA level and an internal dose marker, such as the blood Cr or urinary Cr level. Examination of Cr(VI)-exposed Sprague–Dawley rats has indicated that the DNA oxidative damage in the lung tissues caused by Cr(VI) inhalation is induced by the generation of reactive oxygen species only during the earlier phase of exposure, and these levels return to the control levels in the latter exposure period (Maeng *et al*. 2003). The present study also revealed the importance of considering the sampling time or final exposure hours for oxidative damage, such as plasma lipid peroxidation from Cr exposure. As such, plasma lipid oxidation could not be confirmed as a reliable marker for monitoring the biological response in Cr-exposed workers. Therefore, a further investigation with a larger study population considering the sampling time would seem necessary to confirm a correlation between MDA and other exposure variables. Determining plasma lipid peroxidation (MDA) by measuring the TBARS with a spectrophotometer to is known to be simple and time-saving yet not a sensitive assay when compared with methods that use high-performance liquid chromatography or immunohistochemistry (Huang *et al*. 1999, Zhang *et al*. 2002). Therefore, further studies on the association of Cr with plasma lipid peroxidation are warranted.

Conclusions

The study showed that the frequency of several types of chromosome aberration and the level of lipid peroxidation were significantly higher in Cr-exposed workers when compared with control subjects. As such, the genotoxicity and oxidative damage (plasma lipid peroxidation) in the Korean Cr-exposed workers were consequential, and a significant positive correlation between the translocations detected by FISH and the Cr in the blood indicated that chromosome rearrangement, especially translocation, may be an effective biological response marker for Cr exposure.

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