Sister chromatid exchange analysis in lymphocytes of workers exposed to hexavalent chromium

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ABSTRACT To investigate the usefulness of sister chromatid exchange (SCE) analysis in lymphocytes as an indicator for mutagenic effects after *in vivo* exposure to hexavalent chromium (Cr), SCE frequency was analysed in lymphocytes of 44 Cr platers occupationally exposed to hexavalent Cr and 47 controls. Although urinary Cr analysis confirmed that the Cr platers were exposed to Cr, no effects of the exposure on SCE frequency were found. Smokers, both Cr platers and controls, had a significantly higher SCE frequency than non-smokers. These results suggest that SCE analysis in human lymphocytes is not a good indicator of possible mutagenic effects of exposure to hexavalent Cr.

Hexavalent chromium (Cr) is mutagenic in the Ames test and other tests *in vitro*^{1,2} and carcinogenic in experimental animals *in vivo*.³ Cr compounds are widely used in various industries and epidemiological studies have indicated a high incidence of lung cancer among workers occupationally exposed to hexavalent Cr compounds.⁴ If the hexavalent Cr is highly soluble, such as chromic acid in Cr plating, there seems to be an increased risk also for cancer in other organs.⁵

Since SCE analysis is a sensitive means of detecting DNA damage, many investigators have used SCE analysis in lymphocytes for monitoring human populations for exposure to environmental mutagens.⁶ The usefulness of SCE analysis in such monitoring studies, however, is obscure. For example, many studies have been published on the effects of smoking habits on SCE frequency in human lymphocytes⁷⁻⁹ because tobacco is one of the most common sources of mutagens in our daily living. The results in these studies, however, are contradictory.^{10,11} Although hexavalent Cr induces SCEs in human cultured cells *in vitro*,^{12,13} previous studies among workers exposed to Cr give contradictory results. Some investigators reported that lymphocytes of workers exposed to Cr in electroplating factories had a higher SCE frequency than those of controls,^{14,15} whereas others found no increase in SCE frequency in the lymphocytes of stainless steel welders exposed to Cr.^{16,17}

Thus to investigate the usefulness of SCE analysis in lymphocytes as an indicator for mutagenic effects after *in vivo* exposure to hexavalent Cr, SCE frequency was

analysed in lymphocytes of Cr platers occupationally exposed to hexavalent Cr in air¹⁸ and controls.

Material and methods

SUBJECTS

The subjects were 44 male Cr platers and 47 male controls. The Cr platers had been constantly engaged in hard Cr plating. There was a pungent odour near the baths. Some of the platers used both gloves and masks when working but others used only gloves. Their duration of employment was 0.5-30.7 years (range), averaging 13.8 ± 8.7 years (mean $\pm$ SD). Controls were various workers who had not been exposed to Cr compounds or any other harmful agents. To analyse the effects of exposure to Cr on SCEs independently from those of smoking habits, each subject was classified into "non-smokers" or "smokers." The subjects referred to as non-smokers had not smoked for at least the past two years. Each of the smokers smoked 5-60 cigarettes a day every day. Finally, we had four groups: 14 non-smoking Cr platers, 30 smoking Cr platers, 21 non-smoking controls, and 26 smoking controls. The data on age, sex, smoking habits, and employment of the subjects were based on personal interview.

SCE ANALYSIS

Venous blood was taken from each subject during working time on weekdays using heparinised vacutainer tubes. Whole blood (0.2 ml) was added within six hours of sampling to minimum essential medium (3 ml) supplemented with 10% fetal bovine

serum, 2% PHA-M, 13 $\mu\text{mol/l}$ 5-bromodeoxyuridine, 1.5×10^5 U/l penicillin G and 0.2 mmol/l streptomycin. Cultures were incubated in the dark for 68 hours at 37°C. After treatment with colcemid (0.3 $\mu\text{mol/l}$) for three hours at the end of culture, microscope slides were prepared by a conventional method and stained by the FPG technique.¹⁹ SCEs were counted in 25 cells containing 46 chromosomes in each preparation and the mean SCE frequency was calculated as SCEs per cell for each subject.

URINARY CR ANALYSIS

Urinary Cr was analysed as a measure of the degree of exposure. Urine was collected from each subject simultaneously with the blood sampling and was stored at -20°C before analysis. Cr was analysed by a direct flameless atomic absorption spectrophotometric method and the Cr concentration in urine was calculated by the standard addition method. Triplicate analyses were done and the mean was obtained in each sample. The coefficient of variation was 5.3% for ten measurements of the same sample. The lowest detectable level by the method was 0.04 $\mu\text{mol Cr/l}$ urine.

STATISTICAL ANALYSIS

Students *t* test and the linear correlation coefficient test

were used. Statistical significance denotes $p < 0.05$. All tests were two sided.

Results

No significant correlation was found between the SCE frequency of each subject and their age in either the Cr platers or the controls (figs 1 and 2).

The mean SCE frequencies for non-smoking and smoking Cr-platers were 9.5 SCEs/cell and 10.7 SCEs/cell respectively, and those for corresponding non-smoking and smoking controls were 9.4 SCEs/cell and 10.7 SCEs/cell respectively (table). No differences between the mean SCE frequencies for Cr platers and corresponding controls could be shown. The mean SCE frequencies for smokers in both the Cr platers and the controls, however, were statistically higher than those for non-smokers.

The urinary Cr concentration of the platers ranged from 0.05 $\mu\text{mol/l}$ to 1.54 $\mu\text{mol/l}$ with a mean of 0.25 $\mu\text{mol/l}$; Cr was not detected in the urine of the controls. No significant correlation between SCE frequency of Cr platers and their urinary Cr concentration was observed in non-smoking or smoking Cr platers (fig 3).

Fig 1 Relation between SCE frequency and age of 44 Cr platers: ○ non-smokers, ● smokers.

Fig 2 Relation between SCE frequency and age of 47 controls: ○ non-smokers, ● smokers.

		Age (years)		Cigarettes smoked a day		Urinary Cr (μmol/l)		SCEs/cell		
No		Range	Mean ± SD	Range	Mean ± SD	Range	Mean ± SD	Range	Mean ± SD	
Cr platers	Non-smoking	14	20–65	34.5 ± 13.5	—	—	0.06–0.47	0.18 ± 0.13	7.6–12.1	9.5 ± 1.2
	Smoking	30	21–68	42.5 ± 12.5	10–40	20.0 ± 9.2	0.05–1.54	0.29 ± 0.31	8.1–13.7	10.7 ± 1.4
Controls	Non-smoking	21	21–72	37.2 ± 13.4	—	—	—	—	6.8–12.8	9.4 ± 1.6
	Smoking	26	25–62	36.4 ± 9.9	5–60	24.3 ± 12.9	—	—	7.5–14.3	10.7 ± 1.8

All subjects were male.

Fig 3 Relation between SCE frequency and urinary Cr concentration of 44 Cr platers: ○ non-smokers, ● smokers.

Discussion

The independency of SCE frequency in lymphocytes from the age of the donors in this study (figs 1 and 2) is consistent with the results of our previous report.²⁰ Thus we neglected the effects of age on SCE frequency in analysing the influence of exposure to Cr and smoking habits on SCE frequency.

Although the urinary Cr analysis suggests that the Cr platers were obviously exposed to Cr, the exposure had no effect on SCE frequency. The SCE frequency in human cultured lymphocytes was increased from 15.5 SCEs/cell to 20.4 SCEs/cell by exposure to 10^{-7} M $\text{Cr}_2\text{O}_7^{2-}$ in the culture medium,¹³ a concentration of $\text{Cr}_2\text{O}_7^{2-}$ equivalent of 0.20 $\mu\text{mol Cr/l}$. On the other hand, the urinary Cr concentration of the Cr platers ranged from 0.05 to 1.54 $\mu\text{mol/l}$ with a mean of 0.25 $\mu\text{mol/l}$. Since the concentration of substance in plasma is generally much lower than that in the urine,

the Cr concentration in plasma of the Cr platers may simply be too low to increase the SCE frequency. Moreover, hexavalent Cr is easily trapped by cells and after being trapped by cells, hexavalent Cr is easily reduced to trivalent Cr^{3+} ,^{21,22} which has little mutagenicity or SCE inducing ability.^{12,23} These findings suggest that inhaled hexavalent Cr cannot easily reach the lymphocytes in man. The exposure level of the Cr platers may be too low to give hexavalent Cr-exposure to lymphocytes.

Two previous SCE studies among workers exposed to $\text{Cr}^{14,15}$ indicate an increase of SCE frequency by Cr exposure, whereas two other studies were negative.¹⁶ Three of the four studies also analysed urinary Cr on workers; 2–32 $\mu\text{g Cr/g creatinine}$,¹⁵ 0.2–1.55 $\mu\text{mol Cr/l urine}$,¹⁶ and 5–155 $\mu\text{mol Cr/mol creatinine}$ ¹⁷; the urinary Cr concentration in our Cr platers was 0.05–1.54 $\mu\text{mol Cr/l urine}$. There is no major difference between these values and suggest that the degree of exposure to Cr was similar in all the studies. These results, however, were contradictory and seem to depend on the effects of smoking on SCEs. Since smoking is probably one of the most effective factors influencing SCE frequency in human lymphocytes, we have to analyse effects of exposure to Cr on SCEs independently from those of smoking habits. Only two previous SCE studies in workers exposed to Cr have analysed effects of smoking habits on SCEs.^{16,17} These studies, like ours, indicate that exposure to Cr does not influence SCE frequency but that smoking increased SCE frequency. Two other SCE studies in workers exposed to Cr with positive results have no analysis of smoking.^{14,15} The results in the studies are possibly confounded by smoking.

The results in this study suggest that SCE frequency in human lymphocytes is not a good indicator for the mutagenic effects of in vivo exposure to hexavalent Cr. Further population analysis studies are required before SCE frequency can be used as a mutagenic indicator in human populations.

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